ISSN: 2456-1878

International Journal of Environment Agriculture and Biotechnology

(IJEAB)

An open access International Journal

AI PUBLICATION

Vol.- 2 | Issue - 1 | Jan - Feb , 2017

editor@ijeab.com | http://www.ijeab.com/

DOI: 10.22161/ijeab/2.1

Editorial Board

Denis Magnus Ken Amara
Department of Soil Science, School of Agriculture, Njala University, Private Mail Bag, Freetown, Sierra Leone.

Dr.SubhaGanguly
Associate Professor , Department of Veterinary Microbiology, Arawali Veterinary College, Sikar, India

Shoib A. Baba
Indian institute of integrative medicine, Sanatnagar, Srinagar, India.

Vol-2, Issue-1, January - February 2017

http://dx.doi.org/10.22161/ijeab/2.1.1 *ISSN: 2456-1878*

Effect of nitrogen fertilizer on different attributes of gladiolus (*Gladiolus grandiflorous* L.) cv. American Beauty

Kuldip Kumar, C. N. Singh, V. S. Beniwal, Rohit Pinder, Ravinder Singh Poonia

Abstract*— *An experiment was conducted to evaluate the effect of nitrogen fertilizer on growth, flowering and vase life of gladiolus (Gladiolus grandiflorous L.) cv. American Beauty at the farm of the Department of Horticulture, C.C.R (P.G.) College, Muzaffarnagar (Uttar Pradesh). The treatments comprised of four levels of nitrogen (0, 40, 60, 80 kg/acre) in a randomized complete block design with factorial concept and replicated four times. The results revealed that minimum days taken for spike initiation (86.89 days), days taken for first flowering (99.37 days) were observed under control treatment N_0 whereas, maximum plant height (49.21cm), spike length (127.17 cm), rachis length (61.31 cm), number of florets per spike (18.00) and vase life (11.73 days) was found with N_2 (60 kg/acre Nitrogen).

The result shows that using 60 kg/acre nitrogen can improve the growth and yield of gladiolus cv. American Beauty like vegetative, flowering and vase life attributes. Hence, this optimum nitrogen level can be recommended for the commercial cultivation of gladiolus.

Keywords*— *Nitrogen, spike, rachis, florets, vase life, gladiolus.

I. INTRODUCTION

Gladiolus (*Gladiolus grandiflorus* L.), Iridaceae family, is also known as "Queen of bulbous flowers", which is valued for its beautiful flower spikes. Generally called "sword lily" due to foliage shape belong to family and originated from South Africa, is a prominent bulbous cut flower plant (Sharma *et al.*, 2013). Its cultivation is getting popular for its beautiful flowering spikes due to more vase life as a cut-flower. Its magnificent inflorescence with variety of colors and number of pretty florets has made it attractive for diversified use in the garden. It is an important cut-flower in both domestic and international market. Nutrient status of the plants can be a pointer to the response of plant to the fertilization and internal content of the nutrients determine the fertilizer requirements. Nitrogen applied as fertilizer is the main source used to meet the requirements of plant growth (Polara *et al.*, 2014). The nutrients such as nitrogen play a major role in the growth and development of plants. Nitrogen is an essential macro element that improves the chemical and biological properties of soil and thereby stimulates the production of higher yield in plants. It should be emphasized that to increase plant quality and productivity nutrients need to be available from the soil during a plant's growth period. Nitrogen fertilizer is one of the important factors in canopy formation that its deficiency leads to a decrease of photosynthesis. This objective can be achieved through balanced and judicious application of plant nutrients.

II. MATERIALS AND METHODS

An experiment was conducted to determine the "Effect of nitrogen fertilizer on different attributes of gladiolus (*Gladiolus grandiflorous* L.) cv. American Beauty" at the farm of the Department of Horticulture, C.C.R (P.G.) College, Muzaffarnagar (Uttar Pradesh) for two years and the data were pooled. The experiment was laid out in randomized complete block design with four replications and different levels of Nitrogen fertilizer.

The half-dose of Nitrogen along with full dose of phosphorous and potassium was given in the form of basal dose which was thoroughly mixed in experimental plots before planting. Remaining half-dose of nitrogen applied at 30 days after transplanting having four levels of nitrogen (0, 40, 60, 80 kg/acre) and the size of the plots was 4 m^2 (2m x 2m). Freshly harvested spikes were kept in vase containing 2% sucrose solution at room temperature to calculate the longevity of spikes.

The variety adopted was American beauty, which shows good performance in Muzzafarnagar (U.P.) conditions.

Soil samples were taken and were analyzed and pH of soil was done according to Piper (1966) and available nitrogen in soil. (Subhiah and Asija, 1956)

Chemical characteristics of experimental soil (Horticulture farm, Department of Horticulture, C.C.R (P.G.) College, Muzaffarnagar)

Chemical analysis:

Percentage	Chemical composition of soil
0.03	Nitrogen
0.10	Phosphorous
1.05	Potassium
0.49	Organic carbon

http://dx.doi.org/10.22161/ijeab/2.1.1 *ISSN: 2456-1878*

The crop was raised by using standard cultural practices. A basal dose of well rotten FYM was uniformly mixed in the soil. Corms were treated with 0.2% Bavistin solution for half an hour and were dried under shade for few minutes. Corms were then planted 5 cm deep and 25 cm apart, with row-to-row distance of 40 cm.

The observations were recorded for traits like plant height, days taken for spike initiation, days taken for first flowering, spike length, rachis length, number of florets per spike and vase life and were statistically analyzed as per the procedure outlined by applying the technique of analysis of variance (ANOVA) as suggested by Panse and Sukhatme (1967). All the statistical analysis was carried out by using OPSTAT statistical software.

III. RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads:

1. **Plant height**

   It is evident from the table that plant height was influenced by different levels of nitrogen fertigation. However, taller plants (49.21cm) were observed in 60 kg/acre nitrogen (N_2) which was significantly at par with N_3 whereas, shortest plants were observed in N_0 (43.75cm) treatment. This might be due to the reason as the nitrogen flow into the plants cause the better growth and stimulate the auxillary buds resulting in more flowers stalk height. Similar results were reported by Singh *et al.* (2000).

2. **Days taken for spike initiation**

   Perusal of data presented in the table shows that spike emergence was early in the plants with N_0 treatment (86.89days) and was significantly superior to all the other treatments whereas, maximum days (95.59days) were taken for spike emergence under N_3. Chanda *et al.* (2000) reported that increase the doses of nitrogen resulted delayed the emergence of spike and nitrogen promotes vegetative growth in gladiolus.

3. **Days taken for first flowering**

   Comparison of different fertigation levels shows significant results in comparison to days taken for first flowering. Early flowering (99.37days) was shown in N_0 treatment whereas, maximum duration (104.84days) were taken under 80 kg/acre nitrogen (N_3). Increasing levels of nitrogen were marked to delay the heading significantly and thereby prolonged the duration of flowering.

Table: Effect of different levels of nitrogen on attributes of gladiolus cv. American Beauty

Vase Life (days)	Number of florets per spike	Rachis Length (cm)	Spike Length (cm)	First Flowering (days)	Spike Initiation (days)	Plant Height (cm)	Treatments Nitrogen kg/acre
9.09	14.27	46.43	100.81	99.37	86.89	43.75	**N_0**
10.59	16.39	52.95	118.46	101.99	92.17	46.28	**N_1**
11.73	18.00	61.31	127.17	103.49	93.75	49.21	**N_2**
11.61	17.53	56.55	124.03	104.84	95.59	47.44	**N_3**
0.38	0.54	0.51	0.53	1.87	1.86	2.19	**CD at 0.05%**

Where N_0 - (0 kg/acre), N_1- (40 kg/acre), N_2- (60 kg/acre), N_3- (80 kg/acre)

4. **Spike length**

   Data presented in the table indicates that variation in spike length among different levels of nitrogen fertigation was found to be highest (127.17cm) under 60 kg/acre nitrogen (N_2) whereas, lowest spike length (100.81cm) was observed under control (N_0). It is well established that the nitrogen is one of the major essential elements, which regulates the cell and tissue functions of the plant being essential part of the nucleic acid, mitochondria and cytoplasmic contents of the cells. These results indicates that wherever nitrogen, whether or not in combination with P and K or both, was added into the soil has showed increase in the spike length (S.J. Butt, 2005).

5. **Rachis length**

   Significant variations were observed w.r.t rachis length of the plants. Highest length (61.31cm) was observed in plants having N_2 treatment whereas, lowest rachis length (46.43cm) was observed in the plants under control (N_0). This might be due to greater uptake of nutrients into the plants system which involved in cell division, cell elongation as well as protein synthesis which ultimately enhanced the rachis length. Similar results were found by Kumar *et al.* (2003) in China aster and Lehri *et al.* (2011) in Gladiolus.

6. **Number of florets per spike**
   Maximum number of florets per spike (18) was found in the plants having 60 kg/acre nitrogen (N_2) treatment which was at par with N_3 treatment whereas, minimum number of florets (14.27) per spike was observed under control (N_0). Similar results were founded by Kumar *et al.* (2003) in China aster, Lehri *et al.* (2011), Singh and Bijimol (2003) in Gladiolus.
7. **Vase life**
   Maximum longevity of spike (11.73days) was observed in the plants grown under N_2 treatment which was at par with N_3 treatment whereas, minimum vase life of spikes (9.09days) was observed under N_0 treatment. Maximum vase life might be due to the accumulation and delay in degeneration of carbohydrates and proteins in the plants.

IV. CONCLUSION

Different levels of N-fertigation affect the different attributes of gladiolus cv. American beauty. Cultivar under the study showed better performance at comparatively medium fertigation level i.e 60 kg/acre nitrogen (N_2) than, lowest and highest levels. It may also concluded that excessive use of nitrogen beyond a certain limit is not only wasteful but also results in adverse effects on both plant and soil structure.

Hence, medium fertigation level i.e. 60 kg/acre nitrogen is recommended for general cultivation of gladiolus cv. American Beauty for this region.

REFRENCES

[1] Butt, S.J. 2005. Effect of N, P, K on some flower quality and corm yield characteristics of gladiolus. *Journal of Tekirdag Agricultural faculty*. **2**(3): 212-214.

[2] Chanda, S., Barma, G. and Roy chowdhury, N. 2000. Influence of different level of nitrogen, phosphorus and potassium on growth and flowering of gladiolus. *The Horti. J*. **13**(1): 76-86.

[3] Kumar, J., Chauhan, S.S. and Singh, D.V. 2003. Response of N and P fertilization on China aster. *J. Orna. Hort*. **6**(1): 82.

[4] Lehri, S.M., Kurd, A.A., Rind, M.A. and Bangulzai, N.A. 2011. The response of *Gladiolus tristis* L. to N and P2O5 fertilizes. *Sarhad J. Agric*. **27**(2): 185-188.

[5] Panse, V.G., and Sukhatme, P.V. 1967. Statistical Methods for Agricultural Workers, *I.C.A.R., Pub.* New Delhi **pp.** 336.

[6] Piper, C.S. 1966. Soil and Plant Analysis. *Hans Publications*, Bombay **pp.** 368.

[7] Polara, N.D., Gajipara, N.N. and Barad, A.V. 2014. Effect of nitrogen and phosphorus on nutrient content and uptake in different varieties of African Marigold (*Taget eserecta* L.). *The Bioscan.* **9**(1): 115-119.

[8] Sharma, Jyoti, Gupta, A.K., Kumar Chandan and Gautam, R.K.S. 2013. Influence of zinc, calcium and boron on vegetative and flowering parameters of gladiolus cv. Aldebran. *The Bioscan.* **8**(4): 1153-1158.

[9] Singh, A.K. and Bijimol, G. 2003. Effect of spacing and nitrogen on gladiolus. *J. Ornam. Horti*. **6**(1): 73-75.

[10] Singh, A.K. and Singh, S. 2000. Effect of spacing and zinc on growth and corm production in gladiolus (*Gladiolus grandiflorous* L.) raised from cormels. *The Hort, J.* **13**(1): 87-92.

[11] Subhiah, B.V., and Asija, G.L. 1956. A rapid procedure for determination of available N in soil. *Curr. Sci* **25**: 259-260.

http://dx.doi.org/10.22161/ijeab/2.1.2 *ISSN: 2456-1878*

Effect of Different Stages of Umbel Picking on Seed Quality Parameters, Yield and Economics of Fennel

Hans Raj, Neeraj Pawar, Rajender Singh, Kuldip Kumar, K.K. Thakral, Rohit Pinder, Ravinder Singh Poonia

Krishi Vigyan Kendra, Rohtak, India

***Abstract*—** *The fennel seeds with uniform size green colour and optimum fiber content are preferred by customer and have high demand in national as well as International Market with premium price. For this purpose, the present investigation was carried out at the Vegetable Research Farm, Department of Vegetable Science and Seed Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar to find out the optimum stage at which umbel/seed should be harvested for maximum acceptable fiber content in seed. For this purpose seeds of all order umbels were harvested at 25,30,35,40,45,50,55 and 60 days after anthesis. The fiber content of seed irrespective of umbel order was found optimum when seeds/umbels were harvested at 30 and 40 days after anthesis but maximum net return was obtained at 40 days after anthesis stage. In case of other quality parameters i.e. test weight, germination percentage, seedling length; vigour index and yield were increased with each 5 days delay in harvesting after 40 days of anthesis.*

***Keywords*— Seed Quality, fennel seeds, vigour index, anthesis.**

I. INTRODUCTION

Fennel is one of the four most important seed spices which are grown on sizeable area. It is cultivated throughout the temperate and sub-tropical region of the world for its aromatic seeds, which are used as culinary spices. Fennel is cultivated as a garden or home yard crop throughout India up to altitude of 1825 m. In India, it is mainly cultivated in the state of Gujarat and Rajasthan and to some extent in Uttar Pradesh, Bihar, Madhya Pradesh, Punjab and Haryana. The fennel seed contains 0.7 to 6.0% pale yellow aromatic essential oil which has its use in cosmetics and medicines. The main constituent of oil in fennel is anethole (anise camphor) (50-70%). Green seeds with uniform size and free from chemical residues, bioagents or physical impurities and optimum fiber content are preferred by customer and have high demand in national as well as International Market with premium price.

II. MATERIALS AND METHODS

The present investigation was carried out at the Vegetable Research Farm, Department of Vegetable Science and Seed Science and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar during 2003-2004. The area is situated at 29.10^{o} N latitude and 75.46^{o} E longitude at an elevation of 215.2 meters above the mean sea level where temperature below freezing point accompanied by frost during winter is common and average rainfall in this area is about 350-400 mm per annum which is unevenly distributed. Most of the rainfall is received during July to September along with few showers of cyclonic rains during winter and spring months. The soil of experimental field was low in organic carbon, medium in available phosphorus and high in potash with slightly alkaline in reaction. The seed of fennel variety HF-33 (Hisar Swarup) was sown in 3.0 x 2.4m plot size on 15th October. As per recommendation half dose of nitrogen i.e. 25 kg N/ha and full dose of phosphorus i.e. 25 kg P_2O_5/ha was applied at the time of sowing and remaining half dose of nitrogen was top-dressed in two equal split doses at one month interval. To find out the stage at which the umbel should be harvested/picked for better quality, twenty five umbels of each (main, primary and secondary) order umbel were tagged as and when flowers opened. The umbels were harvested as per the treatments i.e. 25, 30, 35, 40, 45, 50, 55, and 60 days after anthesis and these eight treatments were replicated thrice in randomized block design. Weeding, thinning and irrigation operations were carried out as and when required.

Fiber content was estimated by the method suggested by Gupta *et al.* (1992) and calculated with the following formula.

http://dx.doi.org/10.22161/ijeab/2.1.2 *ISSN: 2456-1878*

$$\text{Crude Fiber (\%)} = \frac{\text{Weight of crude fiber (D – E)}}{\text{Original weight of sample (B-A)}} \times 100$$

Where;

A= Weight of thimble
B= Weight of thimble and sample
D= Weight after drying
E= Weight after ashing.

The benefit cost ratio was calculated by the following formula:

$$\text{Benefit cost ratio} = \frac{\text{Gross return}}{\text{Total cost of production}}$$

III. RESULTS AND DISCUSSION

1. Test weight and Germination percentage

The test weight was same at 55 and 60 days (8.10, 6.70 and 4.78g in main, primary and secondary umbels, respectively) after anthesis which was statistically at par with 50 days in all order umbels. With each delay in harvesting, there was significant improvement in germination up to 60 days after anthesis in all the orders of umbels except from 50 to 55 days after anthesis for main order and 55 to 60 days after anthesis for secondary umbels. Maximum seed germination was recorded in main umbel seeds (94.6%) followed by 88.0 and 82.3 per cent in primary and secondary umbel seeds (Table 1).

2. Seedling length and Vigour index

The significant increase in seedling length was observed with each delay in harvesting up to 60 days after anthesis in main umbel seeds and upto 55 days after anthesis in case of primary and secondary umbel seeds (Table 2).

The vigour index was found zero at 25 and 30 days after anthesis there was no seed germination but after that with each 5 days delay in umbel picking/ harvesting it increased significantly up to 60 days after anthesis. Vigour index was highest at 60 days after anthesis and it was 1191.9, 941.6 and 798.3 in main, primary and secondary umbel seed, respectively. Test weight of seed from main, primary and secondary umbels was significantly influenced by stage of picking of umbel because seeds which were harvested early after anthesis was immature, under developed and under sized. They might have less stored food material, resulted in less or poor germination due to under developed embryo. However, seeds were bold which were harvested at later stage of umbel development. Such seeds showed better seed germination percentage even as compared with the standard. Bhati (1990) also reported increased test weight of seeds harvested at full grown seed turning yellow stage than half length seed in fennel. Seed harvested at 25 and 30 days after anthesis did not germinated at all. Late harvested seeds were significantly more vigorous as explained earlier.

3. Fiber content

The fiber content around 24 per cent is considered good for chewing fennel. When the main umbels were picked up 30 days after anthesis, the fiber content was 20.75 per cent which was statistically at par with 35 and 40 days. As regards, primary umbels (18.52, 19.15 and 19.17%) and in secondary umbels (19.10, 21.73 and 24.00%) optimum fiber content was found at 30, 35 and 40 days of anthesis (Table 3). After 40 days of anthesis, the crude fiber was higher than optimum rendering it unfit for chewing purpose. These results are in conformity with the results of Tiwari and Agarwal, 2004.

4. Seed yield (q/ha) and Economics

The seed yield as influenced by stage of umbels picking are presented in Table 3. The data revealed that seed yield increased with each delay in harvesting up to 55 days after anthesis but improvement in seed yield was significant up to 50 days after anthesis. However, the seed yield for chewing purpose depends upon fiber content of seed, for this purpose 30 to 40 days of anthesis was found optimum.

The economics of the different treatments as influenced by stage of umbels picking are presented in Table 4. The results clearly indicated that net return was highest when the umbels were harvested at 40 days after anthesis i.e. Rs. 67,379/ha with the benefit cost ratio of 4.57 followed by umbels picking at 35 days after anthesis. Umbels picked at 25 days after anthesis resulted into net loss of Rs. 18871 per hectare. The seeds/umbels harvested at 30 days after anthesis, yielded less, however, seeds were of good quality. When the umbels were harvested at 35 and 40 days after anthesis, the seed yield of 14.12 and 17.25 q/ha was obtained with acceptable fiber content and good attractive colour. After this stage, the seed lost their desirability of required colour and the fiber content i.e. 24 per cent (Tiwari and Agarwal, 2004). Higher net return as well as benefit cost ratio at 35 and 40 days stage was due to higher price of chewing fennel and also due to comparative good seed yield. Bhati (1990) indicated that maximum seed yield was obtained with umbel picking at full length green seed (12.26 q/ha) followed by full grown turning yellow seed (12.13 q/ha) and half-length seed (9.30 q/ha) whereas, umbel picking of half-length seed gave maximum net profit.

IV. CONCLUSION

It is concluded from the present investigation that for obtaining better chewing quality fennel with optimum fiber content the umbel/seed should be harvested/picked 30-40days after anthesis but maximum net return Rs. 67,379 and benefit cost ratio 4.57 was obtained when umbels/seeds were harvested at 40 days after anthesis stage. Other seed quality parameters i.e. test weight, germination percentage, seedling length, vigour index and yield was found better at later stage of umbel harvesting i.e. 50,55 and 60 days after anthesis.

REFERENCES

[1] Gupta, P.C., Khatta, V.K. and Mandal, A.B., 1992. Analytical Techniques in Animal Nutrition. *Directorate of Publication*, CCSHAU, Hisar. pp. 23-24.

[2] Bhati, D.S., 1990. Effect of stage of umbel picking and nitrogen fertilization on fennel (*Foeniculum vulgare*). *Indian J. Agron.* **35**(4): 375-379.

[3] Randhawa, G.S. and Mahey, R.K., 1985. Research and development of essential oils in Punjab. *Indian Perfumer*. **29**: 99-104.

[4] Chubey, B.B., Dorrell, D.G. and Marshall, H.H., 1976. Essential oils. *Res. Canada, Agric.* **21**(4): 15-16.

[5] Tiwari, R.S. and Agarwal, A., 2004. Production Technology of Spices. *International Book Distributing Co*., Lucknow. pp. 284-295.

Table.1: Effect of stage of umbels picking on test weight and per cent seed germination in fennel

Seed germination (%)			Test weight (g)			
Secondary umbel	**Primary umbel**	**Main umbel**	**Secondary umbel**	**Primary umbel**	**Main umbel**	**Treatments**
			Days after anthesis			
0.0	0.0	0.0	2.10	2.76	3.50	25 days
0.0	0.0	0.0	2.95	3.52	4.32	30 days
29.6	32.6	28.3	3.40	4.70	5.90	35 days
37.6	50.3	43.6	4.05	5.98	7.35	40 days
61.3	62.6	65.6	4.50	6.35	7.70	45 days
70.0	74.3	80.3	4.70	6.50	7.98	50 days
79.3	84.3	86.0	4.78	6.70	8.10	55 days
82.3	88.0	94.6	4.78	6.70	8.10	60 days
5.6	3.1	6.5	0.21	0.25	0.31	C.D. at 5%

Table.2: Effect of stage of umbels picking on seedling length and vigour index-I in fennel

Vigour Index			Seedling length (cm)			
Secondary umbel	**Primary umbel**	**Main umbel**	**Secondary umbel**	**Primary umbel**	**Main umbel**	**Treatments**
			Days after anthesis			
0.0	0.0	0.0	0.0	0.0	0.0	25 days
0.0	0.0	0.0	0.0	0.0	0.0	30 days
139.1	166.2	192.6	4.7	5.1	6.8	35 days
206.8	291.7	335.7	5.5	5.8	7.7	40 days
367.8	431.9	557.6	6.0	6.9	8.5	45 days
490.0	609.2	778.9	7.0	8.2	9.7	50 days
761.3	885.1	997.7	9.6	10.5	11.6	55 days
798.3	941.6	1191.9	9.7	10.7	12.6	60 days
27.7	15.4	8.37	0.4	0.3	0.6	C.D. at 5%

http://dx.doi.org/10.22161/ijeab/2.1.2 *ISSN: 2456-1878*

Table.3: Effect of stage of umbels picking on seed yield (q/ha) and fiber content (%) in fennel

Fiber content (%)			Seed yield (q/ha)	Treatments
Secondary umbel	**Primary umbel**	**Main umbel**		
Days after anthesis				
12.13	12.18	14.70	8.25	25 days
19.10	18.52	20.75	10.12	30 days
21.73	19.15	21.17	14.12	35 days
24.00	19.17	22.44	17.25	40 days
31.06	24.33	25.65	19.12	45 days
33.91	29.34	29.52	19.50	50 days
34.20	34.36	31.60	19.87	55 days
34.57	35.01	33.62	19.87	60 days
3.35	2.43	2.51	0.72	C.D. at 5%

Table.4: Effect of stage of umbels picking on economics of fennel seed crop

Benefit cost ratio	Net returns (Rs./ha)	Cost of production (Rs./ha)	Gross income (Rs./ ha)	Selling price of seed (Rs./ kg)	Seed yield (q/ha)	Treatments
		Days after anthesis				
0	-18871	18871	0	0	8.25	25
1.60	11489	18871	30360	30	10.12	30
3.74	51729	18871	70600	50	14.12	35
4.57	67379	18871	86250	50	17.25	40
3.04	38489	18871	57360	30	19.12	45
2.58	29879	18871	48750	25	19.50	50
2.10	20869	18871	39740	20	19.87	55

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

Design of vacuum impregnation chamber for soaking of *Gulabjamun* in sugar syrup and optimization of wall thickness by Finite Element Analysis (FEA)

Maheshkumar G.[1] , Menon Rekha Ravindra[2]

[1]Asst Professor of Dairy Engineering, Dairy Science College, Hebbal, Bengaluru, India
[2]Senior Scientist, SRS-NDRI Bengaluru, India

Abstract**—** *The application of vacuum impregnation technique for soaking of Gulabjamoon in sugar solution was conceptualized and the equipment was designed and developed. Vacuum impregnation unit (VIU) was operated under vacuum and hence the design of its wall thickness was of critical consideration. VIU facilitated rapid soaking of Gulabjamun in sugar syrup under full vacuum in cyclic process. VIU is cylindrical in geometry, designed to work at 65-80 ºC at 5 kPa pressure (vacuum) on the inside and was exposed to atmospheric pressure on the outside. This leads to compressive forces acting on inside of the cylinder wall. The shell thickness will have direct bearing on stresses developed. There will be implosion (due to compressive forces) of VIU when Von Mises stress generated is more than yield stress of stainless steel (205 MPa). Wall thickness of cylinder of VIU was optimized by Finite Element Analysis (FEA) by modeling and simulation using Pro/ENGINNER. ANSYS-14 was used for analysis of Von Mises stress, deformation and factor of safety. The wall thickness of shell was analyzed by hyper tetrahedron meshing. To validate, design software developed by ASME was used for shell thickness determination. The model prediction was shown to be in good agreement with the analytical calculation. The FEA resulted in Von Mises Stress of 135.79 Mpa, deformation of 1.55 mm and factor of safety of 1.5. VIU was fabricated as per FDA C-GMP standards from 4.00 mm thick AISI-316 SS material. The working drawings were developed and actual fabrication was carried out adopting the prescribed sanitary standards. The unit was subjected to various safety tests and it successfully passed out all of them. Satisfactory production of Gulabjamoon was carried out in the newly designed and developed equipment resulting in a product of excellent quality confirming validity of the design.*

Keywords—Vacuum impregnation unit (VIU), ANSYS, PRO/ENGINEER.

I. INTRODUCTION

Vacuum impregnation is a new process to be adopted in dairy industry for faster impregnation of sugar syrup into *Gulabjamun* under pulsed full vacuum condition. VIU was to be fabricated as per FDA C-GMP standards from AISI-316 SS. It is cylindrical in geometry designed to work at 65 ºC to 80 ºC and 5kPa pressure (vacuum).

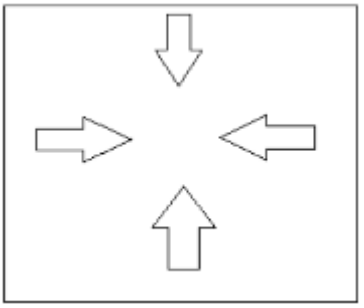


Fig.1: Forces acting inside vacuum impregnation unit (Buckling

VIU is exposed to atmospheric pressure on the outside which leads to compressive forces acting on the inside of the vessel that results in buckling when wall thickness is lower than the critical value (Fig 1). The mechanical strength of the VIU as determined by its thickness of the metal sheet (wall thickness) used in its fabrication is the critical design parameter. There will be implosion due to compressive forces when stress developed is more than yield stress of SS-316, the material used for fabrication of VIU. The following design steps elaborate the procedures adopted for FEA and optimization of shell thickness of VIU.

II. MATERIALS AND METHODS

VIU is a cylindrical vessel with hemi spherical cover on bottom side and flat circular plate on top side. These covers are joined to the cylindrical chamber by welding. VIU was designed to work in full vacuum of 5kPa and the

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

outside operating pressure being 101kPa (NTP). Following design data (Hauviller1993). viz., composition of material, mechanical properties, dimensional drawing, boundary conditions required for FEA for stress analysis are shown in Table 1 & 2 . Though AISI 316 was used for the VIU in this study, for comparison AISI 304 SS is also given in these Tables. The size of the unit was designed based on capacity of processing and the major dimensions are shown in Fig 2 which also describes the other constructional features of VIU.

Table.1: Composition of stainless steel

Element	***Unit***	***AISI-304 SS***	***AISI-316 SS***
Carbon	*% max*	*0.08*	*0.08*
Manganese	*% max*	*2.00*	*2.00*
Phosphorus	*% max*	*0.045*	*0.045*
Sulphur	*% max*	*0.030*	*0.030*
Silicon	*% max*	*1.00*	*1.00*
Chromium	*% max*	*18-20*	*18-20*
Nickel	*% max*	*8-10.5*	*10-14*
Molybdenum	*% max*	-	*2-3*
Source: www.sail.co.in			

Stainless Steel AISI 316 is the preferred metal for food processing equipment that comes in direct contact with the food providing an excellent corrosion resistance due to its alloying components of high nickel and molybdenum (Table 1). Important mechanical properties of AISI 316 are summarized in Table 2

Table.2: Mechanical Properties of AISI-316 SS

	Properties	**Stainless steel grade**		
A	Mechanical properties	Unit	AISI-304	AISI-316
	Ultimate tensile strength (UTS)	Mpa	515	515
	Yield stress	Mpa	205	205
	Young Modulus of elasticity	Mpa	$1.93x10^5$	$1.93x10^5$
	Density	Kg/m^3	8006	8006
	Poisson ratio	-	0.27-0.30	0.27-0.3
	Hardness	HR_B	92	95
B	**Thermal properties**			
	Coefficient thermal expansion	$10^{-6}/^{\circ}C$	19.8	19.8
	Specific Heat	j/kg K	500	500
	Thermal conductivity	w/mK	16.2	16.2
	Source: www.sail.co.in			

The design boundary conditions required for stress analysis by FEA are given in the Table 3. The size of the VIU, diameter and height, were arrived at based on the desired processing capacity as given in the following Table 3. The normal physical conditions required during syrup impregnation are also shown in the Table 2 . A wall thickness of 4.0 mm was considered based on the design equation of ASME, 2011. The operating vacuum inside the cylinder was assumed to be at 5000 Pa fand for the analysis. The inside temperature was assumed to be at 80C and the Table 2 describes all other parameters.

Table.3: Design Data and boundary conditions

Description	**Unit**	**Value**
Material of construction	SS	AISI-316 SS
Inner diameter of chamber	mm	400
Length of vacuum changer	mm	750

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

Wall thickness	mm	4
Operating pressure (inside)	kPa	5
Operating pressure (outside)	kPa	101.325
Operating Temperature inside	º C	80
Operating Temperature outside	º C	25-30
Fixed support	VIU was permanently mounted on SS frame.	

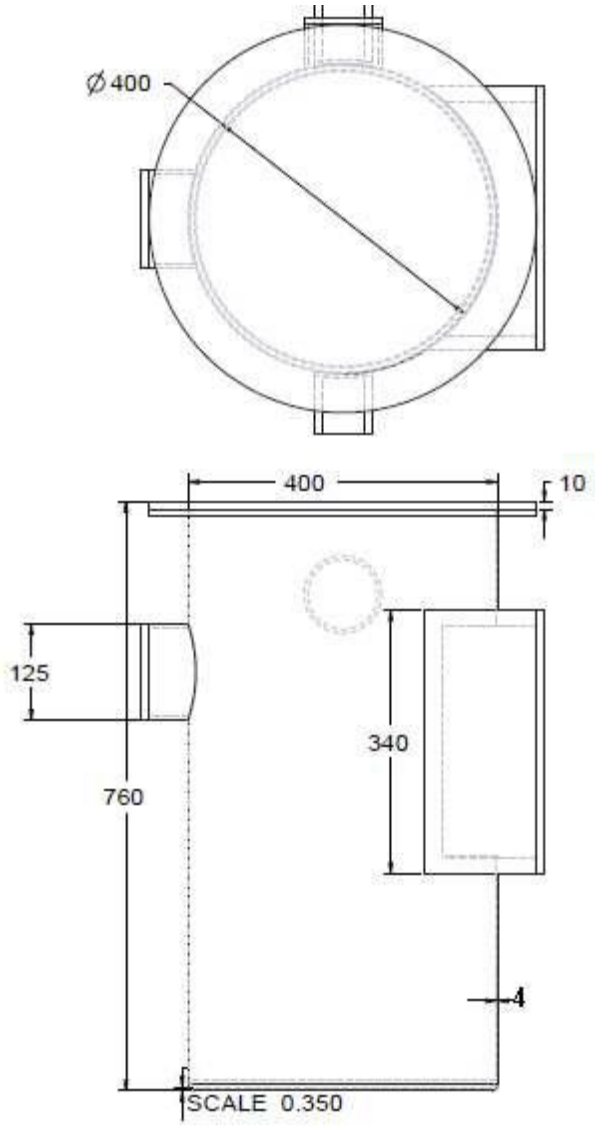


Fig.2: Dimensional drawing of Vacuum imprenation unit (all dimension are in mm)

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

2.1 3-D Model Generations

The 3-D model of the VIU **was** developed using Pro/ENGINEER software (Tickoo & maini , 2009) The stress (FEA) analysis using ANSYS-14.(ANSYS, 2007) The 3-D modeling procedure ,cycle and steps are explained in Fig. 2 and 3.

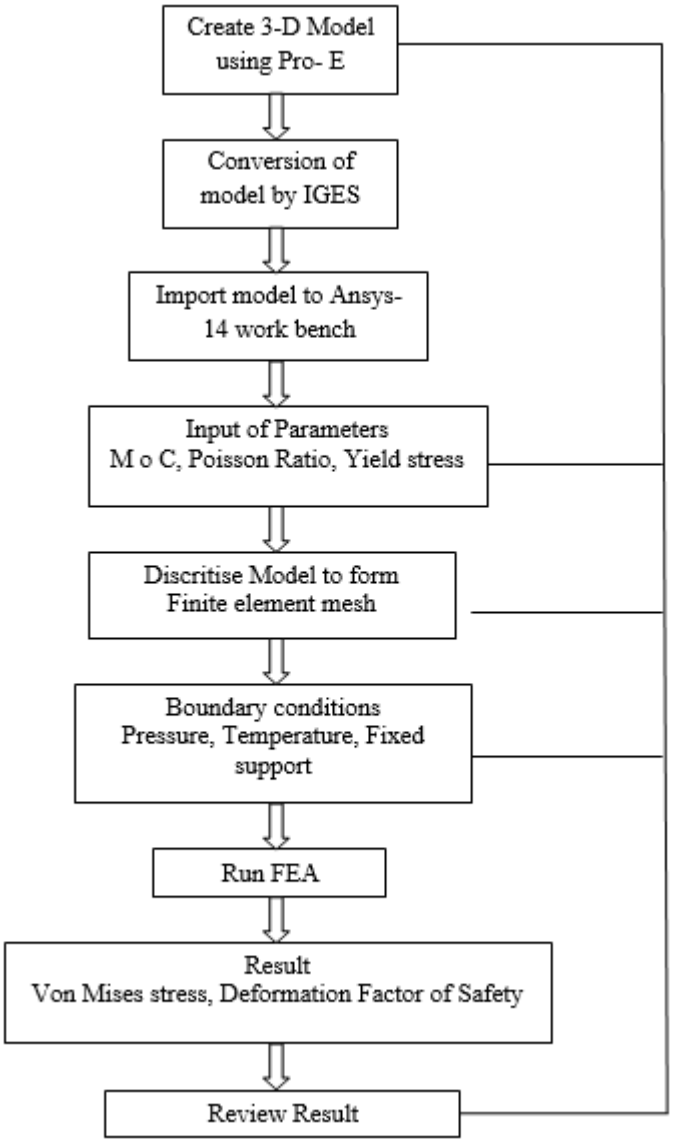


Fig. 3: Stress analysis cycle

2.2 Thermal stress analysis cycle (FEA)

In order to optimize the wall thickness of VIU, the stress analysis was performed using design software (PRO-ENGINEER and ANSYS-14) by following the procedures as detailed by Kraan *et al.*, 2004; Gajjar *et al.*, 2011; Chand *et al.*, 2012.; Abdhul 2013. The stress analysis cycle is shown in Fig 3.

2.3. 3-D modeling for Stress analysis (FEA)

The 3-D modeling of the VIU **was** performed using Pro/ENGINEER soft ware. The assembly 3-D model of equipment was saved in IGES-(Initial Graphics Exchange Specification) format to import to ANSYS-14 workbench for stress analysis. The operating parameters, material properties and boundary conditions were fed to Anysys-14 work bend for stress analysis. The stress (FEA) analysis procedure and steps are explained in Table 4

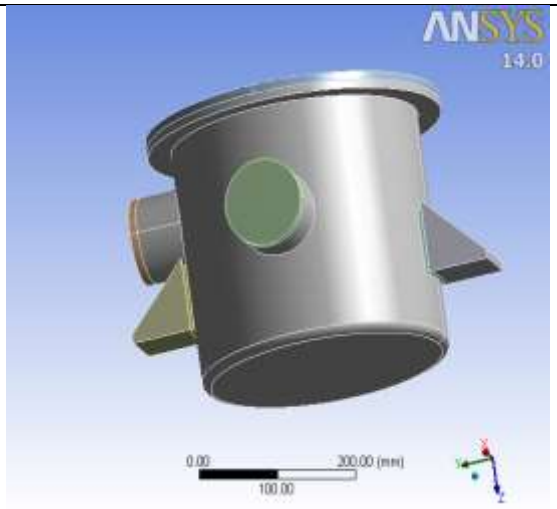
http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

Table.4: Modeling and Finite Element analysis using Pro-E and ANSYS-14

i. 3-D model Generation using –PRO-E

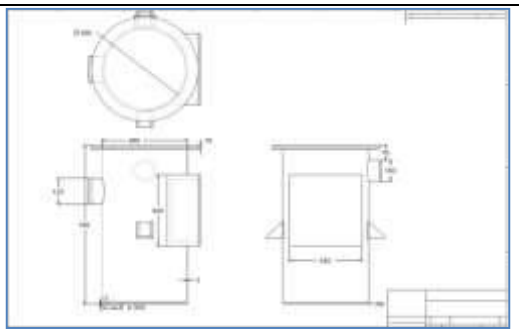
The 3-D modeling of the Vacuum impregnation unit was done in Pro/ENGINEER soft ware. The assembly 3-D model of equipment was saved in IGES-(Initial Graphics Exchange Specification**)** format to import to Ansys-14 workbench for stress analysis.

Moc - AISI-316 SS
Diameter - 400 mm
Length - 750 mm
Wall thickness - 4 mm



3-D Model of VIU

ii. Dimensional Drawing of Equipment



Dimensions of VIU in mm

iii Defining FEA model

Model was defined by feeding

a. Mechanical properties of AISI-316 Stainless steel
b. Wall thickness 4 mm

stainless steel > Constants

Density	8.e-006 kg mm^-3
Thermal Conductivity	1.62e-002 W mm^-1 C^-1

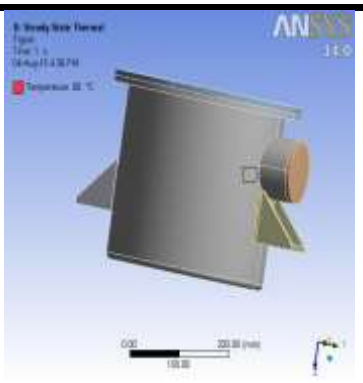
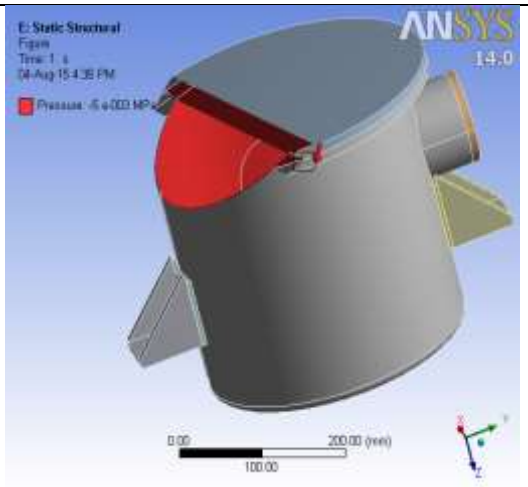
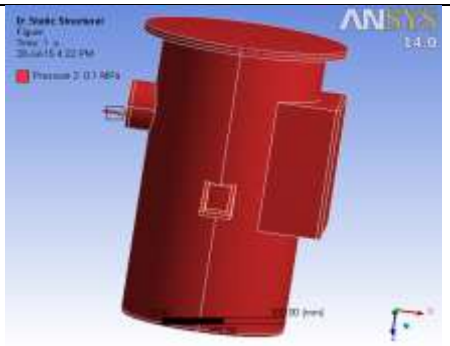
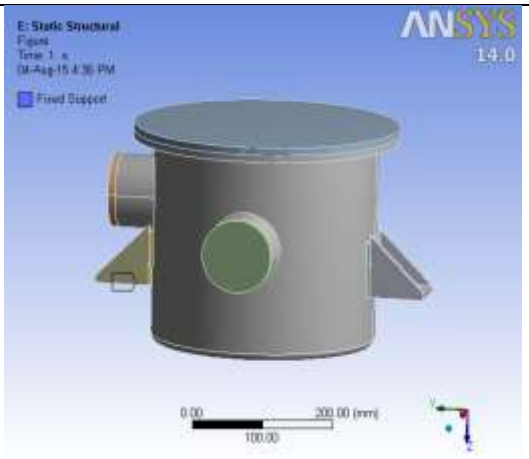
stainless steel > Isotropic Elasticity

Temperature C	Young's Modulus MPa	Poisson's Ratio	Bulk Modulus MPa	Shear Modulus MPa
	1.93e+005	0.28	1.4621e+005	75391

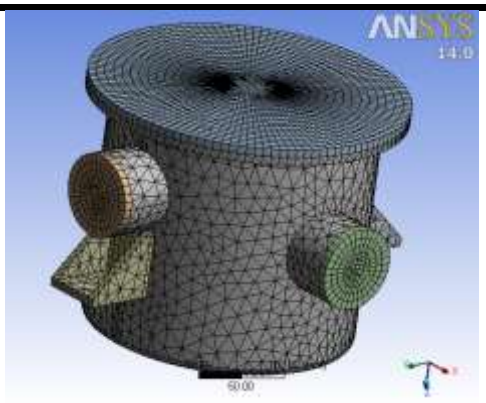
stainless steel > Tensile Yield Strength

Tensile Yield Strength MPa
205

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

iv. Defining Boundary conditions Inside temperature 80°C Outside temperature 30°C	 Inside tempararue 80C
Inside – Pressure (full vacuum) 5	 Inside operating pressure 5kpa
Outside – Pressure 101kPa (NTP)	 Outside pressure NTP
Fixed support - by Pedestal to skid of equipment	 Model was mounted SS Skid by bolting

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

v. Mesh Generation Meshing was done using tetrahedron mesh. In this tetrahedron meshing method the component was divided into small triangles which give number of nodes and elements of the component to be analyzed. The meshing was done by varying mesh sixe from 20,18,16,14, and 12mm. Due to change in density of the meshing it resulted in variation of number of nodes and element of meshed component Type: Hexahedron mesh Element size -12 mm No. of nodes - 81157 No. of Elements -30513	 Tetrahedron Hyper meshing
vi. Run Finite Element Analysis to determine	a. Von Mises stress – Kpa b. Deformation- mm c. Factor of safety
vii. Review Results	Compare with yield stress of Stainless steel (205MPa)
viii. Rerun stress analysis, if yield stress of material is less then Von-Mises steel	Changes the wall thickness and meshing

2.4. Validation of Shell thickness

To validate wall thickness determined by ANSYS-14, shell thickness of VIU was calculated by ASME design equation 1 (ASME 2011). ASME approved design soft ware performed the design procedures and calculations.

The shell thickness calculation was to determine the wall thickness of the cylinder under vacuum without holes, nozzles etc. This calculation does not take into account the extra stress around holes for nozzles and is therefore a basic strength calculation. Calculation codes are as per ASME (ASME 2010) norms.

Wall thickness is calculated by using

$$t = \frac{P*R}{2SE-0.6P} \qquad \text{Eq. 1}$$

where,

t is the cylinder wall thickness in corroded condition (m),

P is the design pressure (MPa),

R is the cylinder inside radius in corroded conditioning (m),

S is the maximum allowable stress at design temperature (MPa) and

E is the joint efficiency in fraction.

3.4.1 Procedure to run software programme for calculation of shell thickness (ASME 2011)

The calculation also requires the user to enter dimensions of model, pressure, operating temperature, yield stress value and density of stainless steel etc. using data (Table 1-3 and Fig 2)

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

Table.5: Data input for thickness calculation

Type of shell		Cylinder	
Design pressure	P	0.2	N/mm^2 (= 1 MPa = 10 Bar)
Design temperature	T	80	^0C
Material description	-	AISI-316	
Select yield stress and specific gravity from material database			
Yield stress, design temp.	S	205	N/mm^2
Specific gravity	ρ	8006	kg/m^3
Outside diameter	D_o	408	mm
Length tangent to tangent	L	750	mm (If not a sphere)
Nominal wall thickness	t	4	mm
Corrosion allowance	Ca	1	mm
Tolerance	tol	1.03	mm
Joint efficiency	E	0.25	-
Semi angle at apex cone	α	0	degree (For cone only)
Design Code	-	ASME	(ASME, Dutch R., PED)
		Calculate	

III. RESULTS AND DISCUSSION

Results of the FEA analysis for the optimization of wall thickness of VIU are shown in Fig 4. The results of the stress analysis are presented in terms of Von Mises (equivalent) stress and, deformation and factor of safety below (Figs. 4-6).

3.1 **Stress analysis of the Vacuum impregnation unit**

The general view of the stress analysis is given in Fig. 4. It depicts a magnified picture of the highest and lowest peak stress regions. The red circle and two yellow color circles at the bottom show the regions where the highest (peak) compressive stresses are generated which are much less than yield stress of SS-316 (Fig 4 & 5). The peak stresses were seen only at bottom of the chamber (red & yellow color).

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

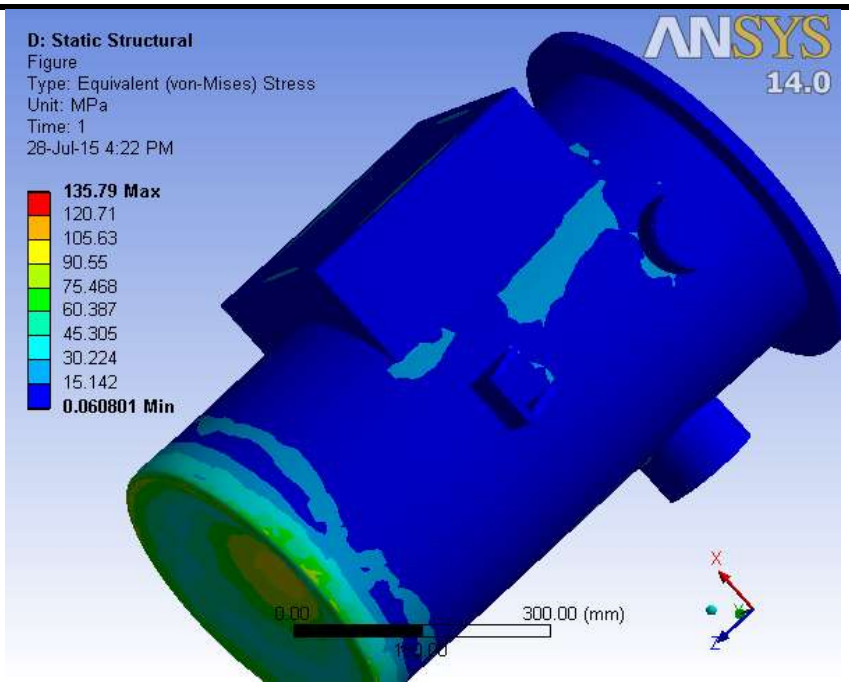


Fig.4: Max Van Mises Stress is 135.79 MPa

3.2 Deformations from the stress analysis

To complete the analysis, deformation generated from the stress analysis is presented in Fig. 5. The maximum total displacement was found to be 1.55 mm, noticed at the bottom of VIU.

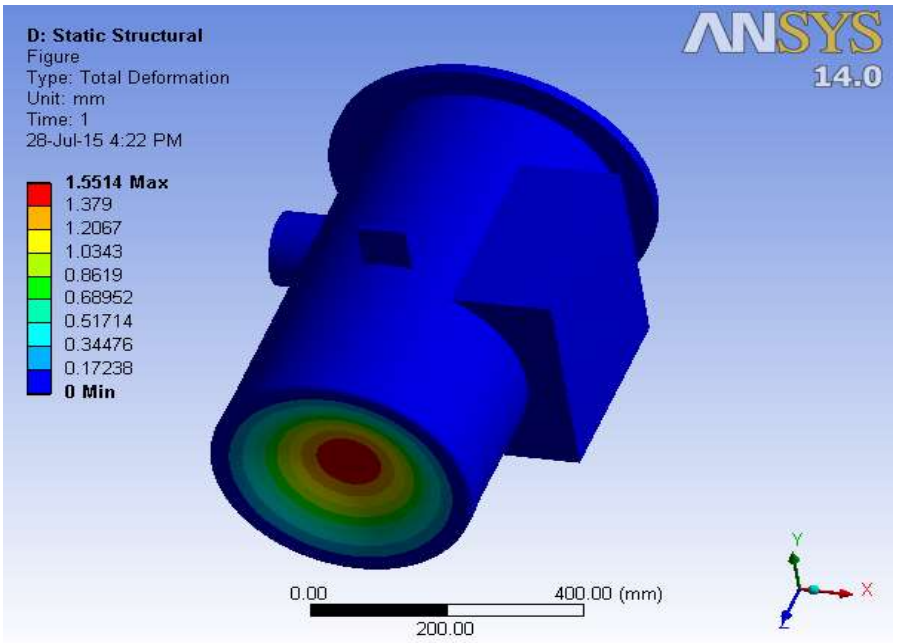


Fig.5: View of the total max. Deformation (1.55mm)

3.3 Factor of Safety

It is evident from the result of stress analysis (Fig 6) the minimum factor of safety obtained was 1.51 which is indicated in yellow color at the bottom of chamber. The highest factor of safety value (15) is shown in blue color. The vessel had experienced maximum stress at its bottom only.

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

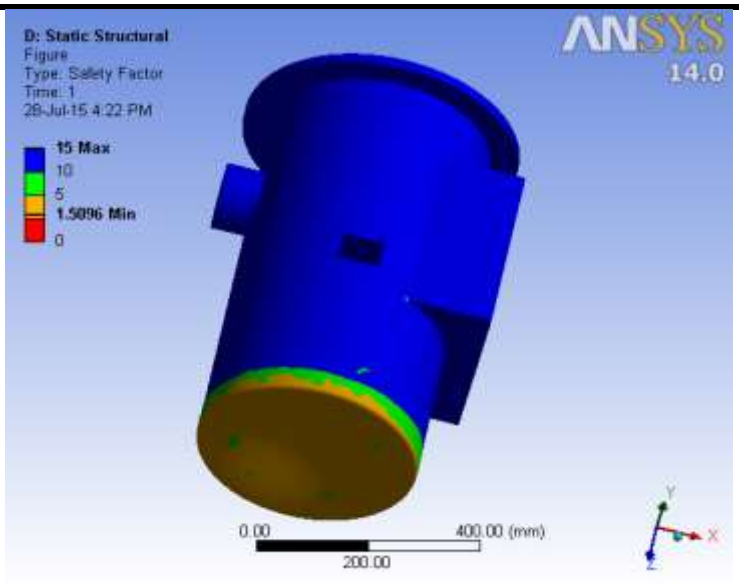


Fig 6 Min Factor of safety is 1.5

Further, to validate the FEA results, wall thickness of VIU was determined by using ASME approved design software program as described below.

3.4 Determination of shell wall thickness by using ASME approved design equation software.

Calculations were performed to support the design and structural analysis by FEA.To validate wall thickness of the shell, design data were fed to ASME design equation based soft ware. The results of thickness analysis results shown Table 5. The wall thickness obtained from the ASME calculation was 2.82 mm.

Table.5: Result of Analysis of shell thickness

Wall thickness calculation of Cylinder
according ASME

Allowable stress	$S =$	$205 =$	205.00 N/mm^2
Corroded thickness	$t_c = t - Ca - tol =$	$4 - 1 - 1.03 =$	1.97 mm
Cylinder:			
Corroded inside radius	$R = \frac{D_o}{2} - t_c =$	$\frac{408}{2} - 1.97 =$	202.03 mm
Required wall thickness	$t_r = \frac{P * R}{S*E - 0.6*P} =$	$\frac{0.2 * 202.03}{205*0.25 - 0.6*0.2} =$	0.79 mm
Nominal required thickness	$t_m = t_r + Ca + tol =$	$0.790 + 1 + 1.03 =$	2.82 mm
Max. Allowable Working Press.	$MAWP = \frac{S * E * t_c}{R + 0.6 * t_c} =$	$\frac{205 * 0.25 * 1.97}{202.03 + 0.6 * 1.97} =$	0.50 N/mm^2
Thickness analysis, $t > t_m$?	t = 4 mm is OK		
Weight			30.48 kg
Enclosed volume			0.094 m^3

http://dx.doi.org/10.22161/ijeab/2.1.3 *ISSN: 2456-1878*

Above calculation does not take into account the extra stress around holes for nozzles. VIU consists of loading door, sight glass, flanges etc. They create abrupt changes in cross section and lead to stress concentration and reduce the strength of material. To overcome this 4 mm wall thickness was considered for fabrication.

Discussion

The Von Mises criterion states that failure occurs when the energy of distortion reaches yield stress (implosion in vacuum vessels). The maximum Von Mises stress obtained from stress analysis was 135.79 Mpa (Table 5), which was highest stress generated at the bottom of vacuum chamber (red color). The VIU was fabricated using 4.0 mm thick AISI-316 SS material. The peak stresses in the model was 135.79 MPa which was much below the yield strength (205 MPa) for SS-316. The Stresses generated due to high vacuum (5 kPa) are within the acceptable limits. The maximum deformation 1.55 mm which was generated at the bottom as indicated in red color circle which is very small (Fig. 4.2). Factor of safety obtained was 1.51 (Fig 4.3). It implies that the vacuum vessel can with stand compressive load up to 205 MPa. The analysis clearly showed that the stress were generated only at the bottom. The structural strength could be greatly increased by providing cross flat stiffeners. Inclusion of stiffeners would further reduce Van Mises stresses by limiting the deformation and increasing factor of safety.

The results of this analysis and simulation confirmed the correctness of the procedures and also in confirmation with ASME design procedures.

The working drawings of VIU were developed and actual fabrication was carried out adopting the prescribed sanitary standards. The unit was subjected to various safety tests and it successfully passed out all of them. Satisfactory production of *Gulabjamoon* was carried out in the newly designed and developed equipment resulting in a product of excellent quality confirming validity of the successful design.

IV. SUMMARY AND CONCLUSION

- Designed and fabricated t a VIU from 4 mm thick AISI-316 to operate at 80° C under 5kPa Vacuum based on FEA, simulation and ASME procedures.
- The VIU unit was safe from implosion as the generated stress (135MPa) was lesser than yield stess of AISI-316 SS (205MPa).
- The shell wall thickness of 4 mm assured a safe design considering Von Mises creterian.
- Maximum Van misess stess was concetrated only at the bottom of the VIU.
- Von Moises stresses developed at the critical section of the VIU could be reduced by providing a reinforcement in the form of a stiffener made of SS flat at the bottom of the vessel by staggered welding. It is suggested a stiffener made of 25x6 mm flat would suffice to enhance the strength of the unit and explore an opportunity to use a 3 mm thick material instead of 4.0 mm thick which would greatly reduce material cost.

REFERENCES

[1] Abdul Shaikh, and P.T.Nitnaware (2013). Finite Element Analysis & Thickness Optimization of Vacuum Chamber for Electron Microscopy Applications. International Journal of Modern Engineering Research (IJMER). 3 (3), 666-1671

[2] ANSYS (2007) Release 11.0 documentation for ANSYS, Swanson Analysis System Inc., User's Manual, 2007

[3] ASME (2011). An International Code - 2010 ASME Boiler & Pressure Vessel Code Section VIII Rules for Construction of Pressure Vessels - Division 2.

[4] Chand Gopi , A. A.V.N.L. Sharma, G. Vijay Kumar and A. Srividya (2012). Thermal analysis of shell and tube heat exchanger using mat lab and floefd software, international Journal of Research in Engineering and Technology, 1(3), 276-281.

[5] Gajjar, V.Y., Jaiveshkumar , Gandhi D. (2011) Design analysis of an Automotive Vacuum suspended power brake using pro/MECHANICA , International journal of Tech and Engineering systems, 2(3): 301-3050

[6] Hauviller, C. (1993). Design of vacuum chambers for experimental regions of colliding beam machines, IEEE 1993 Particle Accelerator Conference.

[7] Kraan, M.J., J. Buskop, M. Doets and C. Snippe (2004). Structural analysis of the vacuum vessel for the lhcb vertex locator (velo) ,National Institute for Nuclear Physics and High Energy Physics,Kruislaan 409 1098 SJ Amsterdam The Netherlands NIKHEF Reference no.: MT-VELO 04-1 EDMS no: 432626; 1-24.

[8] Maheshkumar G (2016) Design and development of microcontroller based sub-baric thermal processor for manufacture of fried and soaked dairy products . Ph D thesis submitted to NDRI Karnal, India

[9] Tickoo sham,. maini Depak (2009). Pro/ENGINEER , Wildfire Instructor 4.0 for designers ISBN1-932709-19CADCIm Technologies USA

[10] http://www.ansys.com/Products/Structures/ANSYS-Mechanical

[11] www.sail.co.in

http://dx.doi.org/10.22161/ijeab/2.1.4 *ISSN: 2456-1878*

Response of Pea (*Pisum sativum*) to Sugar Industry Effluent Treatment

Kamlesh

Department of Energy & Environmental Sciences, Chaudhary Devi Lal University, Sirsa, India

Abstract*—Sugar mills play a major role in polluting the soil and water bodies by discharging a large amount of waste water as effluent. The present research work has been carried out to assess the impact of sugar mill effluent applied in different dilutions i.e 25%, 50%, 75% and 100% along with control on pea (Pisum sativum) seeds to investigate their effect on seed germination and growth parameters such as germination percentage, shoot length, root length, seedling vigour, plant fresh weight and dry weight by pot culture. The low effluent pH (4.3), Total dissolved solids (TDS, 1990 mg/L), Biochemical oxygen demand (BOD, 850 mg/L) and Chemical oxygen demand (COD, 2920 mg/L) indicated the high inorganic and organic content with an acidic load. The present study was carried out with the aim that sugar mill effluent shows positive or negative effect on plant growth. The result indicated that sugar mill effluent did not show any inhibitory effect on germination percentages and germination values at lower concentration in the seeds tested.*

Keywords*— *Sugar mill effluent, Pisum sativum, seed germination, seedling vigour.

I. INTRODUCTION

Industrial activities generate a wide variety of waste effluents which are generally discharged into water courses. Among the effluent discharging industries such as fertilizer, pulp and paper, textile, sugar mills, tanneries, distilleries etc, sugar mills play a major role in polluting the water bodies because it contains large quantities of chemical elements. India is the second largest producer of sugar in the world after Brazil with 550 sugar mills and 220 million tons cane per year and total sugar production 13.5 million tons per year (**Kaur *et al.*, 2010)**. Sugar industry effluent contains several organic and inorganic contents in different concentrations. The effluents also alter the physico-chemical characteristics, and flora and fauna of receiving water bodies. Use of sugar mill effluents for agricultural purposes is a highly warranted utility of water pollutants proposition. The continuous use of such type of effluents harmfully affects the crops when used for irrigation. The effluent not only affects the plant growth but also deteriorate the soil properties when used for irrigation (**Maliwal *et al*., 2004**).

Use of industrial waste water for irrigation purposes has emerged an important way to utilize its nutrients and removal of its pollution load by growing tolerant plant species. Efforts have been made by different workers to determine the effect of sugar mill effluent on seed germination of various crops such as paddy (**Samuel and Muthukkaruppan, 2011**), *Vigna angularis, Vigna cylindrical* and *Sorghum cernum* (**Doke *et al.*, 2011**), *Brassica campestris* (**Beg *et al.*, 2010**), wheat, barley, pea, black gram and mustard (**Nath *et al.*, 2007**), rice (**Rath *et al.*, 2013**), fenugreek (**Kamlesh and Kidwai, 2016**), Black gram (**Vaithiyanathan *et al.*, 2014; Elayaraj, 2014**), cow pea (**Mycin, 2014**) and *Raphanus sativus* (**Vijayaragavan *et al.*, 2011**). Plant responses to sugar mill effluent has been studied and reviewed extensively by **(Vaithinathan *et al.*, 2014; Elayaraj 2014; Kamlesh, 2016).**

The present study was conducted to evaluate the impact of different concentrations of sugar mill effluent on seed germination and other growth parameters of Pea (*Pisum sativum*).

II. MATERIALS AND METHODS

An effluent sample was collected from the outlet of the sugar mill situated at Bhali, Rohtak (Haryana co-op. sugar mills ltd), Haryana, India during the month of November. Effluent sample was collected in precleaned, sterilized plastic container and were stored at 4^{o}C for physico-chemical analysis. The methodology of APHA (2010) was followed for physico-chemical analysis of the collected effluent. The seeds of pea (*Pisum sativum*) were procured from certified local supplier and were surface sterilized by following the method of Aery (2010) prior to germination studies. Different concentrations of effluent (25%, 50%, 75% and 100%) were prepared by using distilled water along with control in triplicate. Ten healthy seeds were sowed in each pot of different concentrations to study the response of test plant. Germination percentage was recorded

after 7 days. Growth of the root and shoot length were measured after 14 days with the help of meter scale. Fresh and dry weight of test plants was determined on a digital balance.

Statistical analysis was done by using SPSS software ver. 20. Data were analyzed for mean, standard deviation and one way analysis of variance. ANOVA is used to compare these data between treated seedlings and control seedlings. P values less than 0.05 was considered to be significant.

Table.1: Physico-chemical analysis of effluent

S.No.	Parameters	Sugar Effluent
1	Colour	Dark brown
2	Odour	Decaying smell
3	pH	4.3
4	Temperature	34ºC
5	EC (µS)	3.2
6	TS (mg/l)	2400
7	TDS (mg/l)	1990
8	TSS (mg/l)	410
9	COD (mg/l)	2920
10	BOD (mg/l)	850
11	Total hardness (mg/l)	210
12	Total alkalinity (mg/l)	320
13	Chloride (mg/l)	190

III. RESULTS AND DISCUSSION

The physico-chemical analysis of sugar mill effluent is given in Table-1.

The characterization of the effluent revealed that it is dark brown in colour which could be due to presence of melanoidin which is the product of sugar amine condensation and decaying smell due to presence of indole and sulphure compounds **(Rath *et al.*, 2010).** According to previous studies pH plays a significant role in toxicity **(Truman *et. al.*, 1986, Martin, 1987**). The pH of the effluent was found acidic in nature having pH of 4.3. The acidic nature of sugar effluent might be due to the use of sulphuric acid and phosphoric acid during sugarcane juice clarification **(Memon *et al.*, 2006, Ayyasamy *et al.*, 2008)**. The value of total solids, total dissolved solids and total suspended solids were 2400 mg/l, 1990 mg/l and 410 mg/l respectively. The effluent had higher BOD, 850 Mg/l and COD, 2920 mg/l. The higher concentration of BOD and COD indicated the higher concentration of organic and inorganic substances in the effluent. Further the values of total hardness (TH), total alkalinity (TA) and chloride were 210 mg/l, 320 mg/l and 190 mg/l respectively. The presence of high amount of COD, BOD, suspended solids, total hardness were also recorded by **Baruah *et al.*, 1996**; **Medhi *et al.*, 2008.**

The observation made on the effect of sugar mill effluent on growth parameters of pea (*Pisum sativum*) are presented in **Tables (2-7)**. The results clearly indicate that at lower concentrations test plant responded in a better way in comparison of control showing the supply of essential nutrients needed for plant growth and metabolism. The higher concentration of effluent decreased the germination studies parameters. Similar observations were obtained by **Singh and Mishra (1987), Verma and Verma (1995), Lakshmi and Sundaramoorthy (2000), Rajesh *et al.*, (2013)** and **Vaithiyanathan *et al.*, (2014)** while studying the effect of various industrial effluent on some agricultural crops. The increase in germination study parameters at lower concentration may be due to presence of growth promoting nature of nutrients present in the diluted effluent **(Sahai *et al.*, 1979).** The reduction in growth parameters at higher concentrations of effluent may be due to the presence of excess amount of elements present in the effluent which inhibit the seed germination and growth by interfering the metabolic activities **(Biradar *et al.*, 1989; Srivastava *et al.*, 1988; Verma and Verma, 1995; Kaushik *et al.*, 2004).**

Table.2: Effect of different concentrations of effluent on germination percentages

S. No.	Concentration (%)	Germination (%)	Reduction /Increase (%)
1	Control	56.7±5.77	-
2	25	70±10.00	23.45
3	50	76.7±5.77*	35.27
4	75	53.3±5.77	-6.0
5	100	43.3±5.77	-23.63

* Indicates- significant

Table.3: Effect of different concentrations of effluent on seedling vigour

S. No.	Concentration (%)	Vigour	Reduction /Increase (%)
1	Control	1338.3±323.8	-
2	25	1401.3±191.5	4.71
3	50	1667.7±72.6	24.61
4	75	952.6±47.9	-28.82
5	100	678.3±31.9*	-49.32

* Indicates- significant

Table.4: Effect of different concentrations of effluent on shoot length (cm)

S. No.	Concentration (%)	Shoot length	Reduction (%)
1	Control	10.2±0.20*	-
2	25	9.3±0.15*	8.82
3	50	9.6±0.26*	5.88
4	75	7.8±0.10*	23.53
5	100	7.3±0.11*	28.43

* Indicates- significant

Table.5: Effect of different concentrations of effluent on root length (cm)

S. No.	Concentration (%)	Root length	Reduction /Increase (%)
1	Control	10.9±0.06	-
2	25	10.7±0.10	-1.83
3	50	11.5±0.23*	5.50
4	75	10.8±0.15	-0.92
5	100	9.2±0.06*	-15.6

* Indicates- significant

Table.6: Effect of different concentrations of effluent on fresh weight (mg)

S. No.	Concentration (%)	Fresh weight	Reduction /Increase (%)
1	Control	0.21±0.01	-
2	25	0.23±0.01	9.52
3	50	0.18±0.01	-14.28
4	75	0.14±0.02*	-33.33
5	100	0.11±0.00*	-47.62

* Indicates- significant

Table.7: Effect of different concentrations of effluent on dry weight (mg)

S. No.	Concentration (%)	Dry weight	Reduction /Increase (%)
1	Control	0.05±0.01*	-
2	25	0.07±0.00	40
3	50	0.03±0.01	-40
4	75	0.02±0.00*	-60
5	100	0.01±0.00*	-80

* Indicates- significant

IV. CONCLUSION

In the present study sugar mill effluent was studied to know its effect on initial growth parameters such as germination percentage, seedling vigour, shoot length, root length, fresh and dry weight of pea (*Pisum sativum*). From the present work, it is concluded that lower concentration (25%-50%) of sugar mill effluent promoted the growth while higher concentration inhibited the seedling growth.

REFERENCES

[1] Aery, N.C. (2010). Manual of Environmental Analysis. Anne Books pvt.Ltd.

[2] American Public Health Association (2010).Standard methods for examination of water and waste water, APHA, Washington, DC.pp17.

[3] Ayyasamy P. M., R. Yasodha, S. Rajakumar, P. Lakshmanaperumalsamy, P.K.S.M. Rahman, S. Lee., (2008). *Bulletin of Environment Contamination and Toxicology* 81,449-454.

[4] Baruah, B.K., Baruah, D. and Das, M. (1996). Study on the effect of paper mill effluent on the water quality of receiving wetland. *Poll. Res*. 15(4), 389-393.

[5] Beg, M.Z., Kumar, J. and Khan, A.J. (2010). A study on the effect of sugar industry effluent on *Brassica campestris* L.var. Varuna type-59. *Indian J. Sci. Res*. 1(1), 31-37.

[6] Biradar, B.B., Mahadevappa, M. and Shrinivasamurthy, C.A., (1989). Influence of industrial effluent on seed germination and seedling growth of some selected plants. *Progress in Pollution Research*. Pro. Nat. Young Scientists. Seminar Environ. Pollut. University of Agricultural Science, Bangalore, 85-89.

[7] Doke, K.M., Ejazuddin, M. K, Joseph, R., Asif, S. (2011). Physico-chemical analysis of sugar industry effluent and its effect on seed germination of *vigna angularis, vigna cylindrical* and *sorghum cernum*. *Annuls of Env. Sc*., vol.5, 7-11.

[8] Elayaraj, B. (2014). Physico-chemical analysis of sugar factory effluent stress on seedling growth of black gram (Vignamungo (L.)Hepper) varieties. *International Letters of Natural Sciences*. ISSN 2300-9675.12(1)85-93.

[9] Kamlesh, (2016). A review on soil irrigation effect of sugar mill effluent on seed germination. *International Journal of Environment, Agriculture and Biotechnology (IJEAB)*, 1(4), *ISSN: 2456-1878*., 868-872.

[10] Kamlesh, and Kidwai, M.K. (2016). Effect of sugar mill effluent on response of Fenugreek (*Trigonella foenum-graecum*) varieties. *International Journal of Environment, Agriculture and Biotechnology (IJEAB)*, 1(4), *ISSN: 2456-1878*.,. 1(4), 844-852.

[11] Kaur, A., Vats, S., Rekhi, S., Bhardwaj, A., Goel, J., Tanwar, R.S. and Gaur, K.K. (2010). Physico-chemical analysis of the industrial effluents and their impact on the soil microflora. *Procedia Environmental Sciences*, 595–599.

[12] Kaushik, P., Garg, V. K. and Bhupinder, S., (2004). Effect of textile effluents on growth performance of wheat cultivars. *Bioresource Technology*, 96, 1189-1193.

[13] Lakshmi, S. and Sundaramoorthy, P. (2000). Effect of sugar mill effluent on germination, seedling growth and biochemical changes in ragi (*Elusine corocana* Gaertn). *Indian J. Environ. Ecoplan.*, 3(3): 501-506.

[14] Maliwal, G.L., Patel, K.P. and Patel, M.N. (2004). Pollution Resident.231-269.

[15] Martin, R.B. (1987). Transferin binding of Aluminium and iron. *Clinical Chem*. 33: 405- 407.

[16] Medhi, U.J., Talukdar, A.K. and Deka, S. (2008). Effect of pulp and paper mill effluent on seed germination and seedling growth of Mustard (*Brassica campestris*), Pea (*Pisumsativam*) and Rice (*Oryzasatival*) seeds. *Poll Res*. 27(3): 437-442.

[17] Memon A. R., S. A. Soomro, A. K. Ansari (2006) *J. App. Env. Sci.* 1, 152-157.

[18] Mycin, T.R. (2014). Use of sugar mill effluent for irrigation: An evaluative study on the response of germination and growth of cow pea *Vigna unguiculata* (L.) Walp. *Asia Pacific Journal of Research.* 1(XVIV), 44-55.

[19] Nath, K., Dharam, S. and Yogesh, K. S. (2007). Combinational effects of distillery and sugar factory effluents in crop plants. *J. of Env. Biol*. 28(3),577-582.

[20] Rajesh, M., Jayakumar, K., Sathees Kannan, T.M. and Sankar Ganesh, K. (2013). Influence of sugar mill effluent on seed germination and seedling growth of Cluster Bean *(Cyamopsis tetragonaloba)*. *International J. Environ. And Bioenergy*, 7(2): 54-62.

[21] Rath, P., Biswal, P.K. and Misra, M.K. (2013). Effect of sugar factory distillery spent wash on germination and seedling growth of rice (*Oryza sativa* L). *IJSID*.ISSN:2249-5347, 3(1),191-201.

[22] Rath P., G. Pradhan, M. K. Mishra (2010). *Journal of Phytology* 2(5), 33-39.

[23] Samuel, S. and Muthukkaruppan, S.M. (2011).Physico-chemical analysis of sugar mill effluent, contaminated soil and its effect on seed germination of paddy (*Oryza sativa* L.).*International journal of pharmaceutical and biological archives* ; 2(5): 1469-1472.

[24] Sahai, R., Agarwal, N. and Khosla, N. (1979). Effect of fertilizer factory effluent on seed germination, seedling growth and chlorophyll content of *Phaseolus Linn. Trop. Ecol.*, 20(2): 155-165.

[25] Srivastava N., Neelum and Sahai, R. (1988). Ecophysiological response of certain crop plants of Eastern Uttar Pradesh to industrial effluents. *Int. J. Ecol. Environ. Sci.*, 14: 229-248.

[26] Singh, K.K. and Mishra, L.C. (1987). Effect of fertilizer factory effluent on soil and crop productivity. *Water, Air and Soil Pollution*, 33: 309-320.

[27] Truman, R.A., Humphreys, F.R., Ryan, P.I. (1986). Effect of varying solution ratios of aluminium to calcium and magnesium on the uptake of phosphorous by *Pinus radiate. Plant Sci.* 96: 109-133.

[28] Vaithiyanathan, T., Soundari, M. and Sundaramoorthy, P. (2014). Response of black gram (vigna mungo L.) to sugar mill effluent treatment. *International Journal of Research in Botany*. 4(1), 15-18.

[29] Verma, A. and Verma, A. P. (1995). Effect of tannery effluent on seed germination and chlorophyll content in *Phaseolus radiates* L. *J. Industrial Pollution Control*, 11(1): 63-66.

[30] Vijayaragavan, M.,Prabhahar, C., Sureshkumar, J., Natarajan, A., Vijayarengan, P. and Sharavanan, S. (2011). Soil irrigation effect of sugar mill effluent on changes of growth and biochemical contents of *Raphanussativus* L. *Current Botany*, 2(7):09-13.

http://dx.doi.org/10.22161/ijeab/2.1.5 *ISSN: 2456-1878*

Production Function Analysis of Member Dairy Cooperative Society for Milch Cow in District Etawah (U.P.)

Dr. Ashish Chandra, Dr Arun Bhadauria

Asstt professor, Amity Business School, Amity University Lucknow Campus, Lucknow, U.P. India

Abstract— *This study covered Cobb douglas production function, Tukey and Kramer analysis on members dairy cooperative society for milch cow in district Etawah of U.P. In study researchers have taken post- stratified into Landless, Marginal, small, medium and large herd size categories. The study effect of various factors of production in (Rs.) like Feeding cost included (dry fodder + green fodder), expenditure of concentrate included (grain + khali + mineral material and chunni / choker) and miscellaneous expenses included (labor charge and fixed cost) on milk produced by the cow of dairy cooperative society members in annual in different categories of farmers. Further, the researchers have found out the comparative analysis of all the categories of dairy cooperative society members. At last Tukey and Kramer test was applied on all the category of dairy cooperatives society members in milch cow to get into the depth of the problem under investigation. This study is helpful to find out the elasticity of different factors of milk production by means of comparative analysis in all categories of members dairy cooperative society in milch Cow by Cob douglas production function analysis.*

Keywords— Elasticity of fodder, Elasticity of concentrate, Elasticity of miscellaneous, Return to scale, Classification Code: Agriculture Management.

I. INTRODUCTION

As per an assessment made by the Planning Commission Report-2012, the domestic demand for the milk by 2020-21 is expected to be 172.20 million tons. India would have sufficient production to meet such demand. The international body on the farm sector in its latest 'Food Outlook' report also estimates global milk production in 2020 grow by 2% to 772 million tones.

India ranks first in milk production, accounting for 18.5 % of world production. India ranks first in milk production, accounting for 18.5 % of world production, achieving an annual output of 146.3 million tones during 2014-15 as compared to 137.69 million tonnes during 2013-14 recording a growth of 6.26 %. Whereas, the Food and Agriculture Organization (FAO) has reported a 3.1 % increase in world milk production from 765 million tones in 2013 to 789 million tones in 2014.

The per capita availability of milk in India has increased from 176 grams per day in 1990-91 to 322 grams per day by 2014-15. It is more than the world average of 294 grams per day during 2013. This represents a sustained growth in availability of milk and milk products for the growing population Dairying has become an important secondary source of income for millions of rural households engaged in agriculture. The success of the dairy industry has resulted from the integrated co-operative system of milk collection, transportation, processing and distribution, conversion of the same to milk powder and products, to minimize seasonal impact on suppliers and buyers, retail distribution of milk and milk products, sharing of profits with the farmer, which are ploughed back to enhance productivity and needs to be emulated by other farm produce/producers.

India's milk production rise by 4% i.e., 127.9 million tonnes in 2011-12 and per capita availability was 291 gms/day while in 2010-11 milk production was 121.8 million tones and per capita availability was 281 gms/day.*.

In domestic market demand of milk and dairy products is increasing very high but the production processing facilities of milk in India is not up-to the mark.

The study analyzed various factors of production in (Rs.) like Feeding cost included (dry fodder + green fodder), expenditure of Concentrate included (grain + khali + mineral material and chunni / choker) and miscellaneous expenses included (labor charge and fixed cost) on milk produced by the cow of dairy cooperative society members in annual in different categories of farmers i.e, landless, marginal, small, medium and large on the basis of land

holding capacity. Analyses of Cobb Douglas production function, researchers find out elasticity of fodder, concentrate and miscellaneous factors of milk production. Further, the researchers have indentified percentage of data variation on different category members of dairy cooperative society. At last Tukey and Kramer test was applied on all the category of dairy cooperatives society members in milch cow to get into the depth of the problem under investigation. This study is helpful to find out the comparative analysis in all categories of members dairy cooperative society in milch Cow.

"Etawah" in Uttar Pradesh is famous for its Bhadawari breeds of buffalo and Jamunapari breed of goats. The said breed of buffalo were also known for consuming less fodder relative to production of high fat content milk. However, all the milch animals such as buffalo, cow and goats are grazed in the ravines and the forest area between Jamuna and Chambal rivers of Etawah district of U.P. The numbers of milch livestock of Etawah district during 2012 were reported as total number of female adult cows 1, 10,825 total number of adult females' buffaloes 92065 and total female adult goats were 2, 41, 61.

The trend shows that very soon Etawah district will get an important place in the future, map of "milk Grid" of India Influenced milk yields. The test for efficiency of resource use revealed that there was inefficiency in the use concentrates. Profit maximization I~equires that the marginal value product of an input be equated to the price. If this condition is fulfilled in the study area with respect to concentrates, the average milk YleLo per animal per year would increase by 73% above the current levels. An important conclusion of the study is that there could be substantial in milk output and consequently gains in farm profits if the amount of concentrates fed to the animals is increased above the cur-r-errt level s. It is recommended that:- (i) effot'ts be intensified to educate the benefits of inct~eased feeding of concentt'ates to the (i i) animals, constraints which contribute to the unavailability of concentt'ates when farmers need them be removed, (iii) farmers be educated on how they can the excess animal feeds which is p r-ociuc ed the wet season to feed the animals during the (iv) be and educated on how best season, they can utilize the farm by-products while ~hey are of high nutrition value to feed the animals.

Prajneshu,(2008), the set of Cobb-Douglas production functions is usually fitted by first linear zing the models through logarithmic transformation and then applying the method of least squares. However, this procedure is valid by producing on an average of 2.801 lakh liters per day during 1986-87 which was increased to 3.83 lakh litres per day during 2006-07 and 5.20 lakh liters per day during 2011-12, and further increased by 6.81 lakh liters per day during 2014-15. There were 3020 cooperative milk producers- societies during 1986-87, increased to 4272 during 20011-12 and 4576 during 2014-15.

II. REVIEW OF LITERATURE

Murithi, Festus Meme,(2002), study was motivated by the need to find means of increasing milk supply in Kenya in order to meet an expected rise in demand. The study was concerned with the efficiency of resource use in smallholder milk production. The major objective of the study was to determine whether tnere are possibilities of increasing milk production through re-allocation of the resources used in milk production~ The problems encountered by farmers involved in milk production were also examined. The data used in the study were collected from 60 smallholders who are members of five Dairy Co-operative Societies which are affiliated to the Meru Central Farmers Co-operative Union. A Cobb-Douglas milk production function was fitted using the inputs used in milk production. The results showed that concentrates significantly

only when the underlying assumption of multiplicative error-terms is justified. Unfortunately, this assumption is rarely satisfied in practice and accordingly, the results obtained are of doubtful nature. Further, nonlinear estimation procedures generally yield parameter estimates exhibiting extremely high correlations, implying thereby that the parameters are not estimated independently. In this paper, use of expected-value parameters has been highlighted and the advantages of their use have also been discussed. Finally, the developed methodology has been illustrated by applying it to the wheat yield time-series data of Punjab.

Venkatesh P. and Sangeetha V.,(2011), a study was conducted to examine the cost structure and resource use efficiency of dairy farms in the Madurai district of Tamil Nadu. The dairy farmers were selected by using multi stage random sampling technique. Tabular analysis and Cobb-Douglas production function were used in this study. Total costs per lactation per animal estimated were of the order of Rs.12776.09, Rs 11791.20 and Rs.12079.28 and returns per rupee of investment 0.78, 1.08 and 0.95 respectively on small, large and pooled farms. Feed cost was the higher input cost in dairy farming (61.6%). The cost of production milk per litre was less in case of large farms (Rs. 4.62)

compared to small farms (Rs. 5.39). Results indicated the inverse relationship with the size and the herd of the total costs, due to economies of scale. Functional analysis showed barring human labour on small farms all the selected input variables such as green fodder, dry fodder, concentrates and health care were positive and significant impact on the production of milk indicating the potentiality of their further use.

Meena G. L. et.al.,(2012), study was undertaken in Alwar District of Rajasthan with the objectives to examine the input-output relationships and assess the resource use efficiency in milk production. The study covered 75 cooperative member milk producers and 75 non-cooperative member milk producers. The results of Cobb-Douglas production function revealed that concentrate had positive and significant influence on returns from buffalo milk across all the household categories for both the member and non-member groups. Green fodder and dry fodder were also influenced the returns from milk significantly across all the household categories for both the member and non-member groups with the sole exception of large category of non-member group. D_1 (winter) and D_2 (Rainy) dummy variables were found to be positive and statistically significant. The results of Chow's test clearly revealed that the production functions between member and non-member groups differed significantly. The results of the resource use efficiency revealed that green fodder was over-utilized in small and medium categories for both the member and non-member groups, dry fodder was over-utilized by medium category of member group, concentrate was over-utilized by only medium category of member group and by small & medium categories of non-member group while it was under-utilized by large category of non-member group and labour was over-utilized by only small category of member group.

Singh, K. M. et. al., (2012), Dairy farming has emerged as an important source of livelihood, particularly on small holder households. The efficient management of dairy cooperative system has facilitated milk production and marketing in Bihar. An attempt was made to analyze the milk contribution to dairy co-operative, producers' share in consumer rupee and cost of milk production in Bihar. Per litre cost of milk production varied from Rs. 10.12 for crossbred cows to 13.90 and Rs. 13.57 for buffalo and local cows, respectively, which are higher than price paid by co-operatives for standard milk (fat-6% and SNF-21%). Herd size and type of milch animal along with parity had significant influence on cost of milk production. Production cost is likely to decrease with increase in size of unit and in production of crossbred cows in herd. More than two-third of milk produced by co-operative members is marketed through dairy co-operatives in Bihar. The producers' share in consumer rupee is about 58% for all categories of herd since all are marketing their milk through co-operatives only. Dairy farmers should also be advised for meeting the requirements of feed by providing desired nutrients through feeding of green fodder which not only reduces intake of concentrates but also helps in reducing the cost of production. Treatment of dry fodder with urea helps in improving its nutritive value, and such technologies may be popularized to make feeding balanced and cost effective.

Crispen D. et.al.(2014), study looked at the operational challenges to smallholder dairy farming. Focus in this study was specifically on Mayfield Small Scale Dairy Settlement Scheme in Chipinge District of Zimbabwe. The study made use of interviews, questionnaires, observations and project reports in collecting both qualitative and quantitative research data. Semi-structured questionnaires were administered to a sample of 75 farmers randomly selected from a total population of 345 family farmers on the dairy settlement scheme. In addition, 24 key informants were conveniently sampled for interviews from among the scheme's management, farmer committee leaders and extension staff. The study noted that while dairy operations at the settlement scheme managed to yield notable benefits to the farmers, there were a number of operational challenges working against full commercialization of production at the scheme. These problems bordered on lack of access to capital, poor production and marketing infrastructure, weak extension support, insecure land tenure, lack of farmer involvement in production planning and poor social relations between farmers and management on the one hand and among the farmers themselves, on the other hand. The study recommends that these problems be addressed, not only at Mayfield Dairy Settlement Scheme, but also elsewhere, if smallholder dairy operations are to serve as real tools for rural transformation in Zimbabwe and other less developed countries.

Carla, D. (2014), studied that women play an important role in the economic and social development of societies, but they are often denied equal opportunities because of socially embedded gender inequalities. This research looks at the potential of dairy cooperatives for women‟s empowerment in South India. Dairy production is of great importance for rural economy in India and women contribute significantly to this activity. The Women Empowerment in Agriculture Index developed by the

http://dx.doi.org/10.22161/ijeab/2.1.5 *ISSN: 2456-1878*

International Food Policy Research Institute was adapted and applied as a research tool. Using a snowball sampling technique, structured interviews were conducted with women involved in four different dairy cooperatives (29) and women selling at the private market (29). The results of the study indicate that there are economic benefits for women participating in dairy cooperatives. However, the outcomes for women s empowerment are ambiguous. Only in some domains women in dairy cooperatives rank their empowerment status higher compared to non-members. The results point to the fact that economic gains provided by cooperatives may not always lead to greater empowerment for women. Moreover, the analysis indicates that women in mixed-gender cooperatives experience greater decision-making power compared to women in single-gender cooperatives. This study suggests that additional measures supporting women‟s role in dairy cooperatives and a more participatory management are required in order to enhance gender equality.

III. RESEARCH METHDOLOGY

District Etawah milk producers' cooperative union was purposively selected from the state of Uttar Pradesh. Exhaustive lists of all the milk producers' cooperative societies in Etawah district milk producers' cooperative union were prepared. Researchers have selected randomly 150 non member of dairy cooperative society & 150 members of dairy cooperative society from 10 Villages of 2 blocks selected in district Etawah. All the milk producing household members and non members were classified into five categories, viz., Landless, Marginal, Small, Medium and Large farmers on the basis of land holding capability. Thus, in all, 300 households were interviewed during the year 2008-09. The primary data were collected to help of well structured pre-tested schedule by the personal inquiry method. The data collected were subjected to tabular analysis in order to study the comparative economics of milk production. Cobb-Douglas type Production Functional analysis was applied on cow milk production with three variables like-fodder, concentrate and miscellaneous of different categories landless, marginal , small, medium and large member farmers of dairy cooperative society.

The study effect of various factors of production in (Rs.) in case of milk cooperative societies members in annual in different categories.

$$y = a x_1^{b_1} x_2^{b_2} x_3^{b_3} \quad \ldots\ldots (1)$$

$$\log y = \log a + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 \quad \ldots(2)$$

Where

- Y = Production of milk in (Rs.)
- X_1 = Feeding cost included (dry fodder + green fodder)
- X_2 = Expenditure of Concentrate included (grain + khali + mineral material and chunni / choker)
- X_3 = Miscellaneous expenses included a labor charge and fixed cost.
- b_i = Respective elasticity's of milk production
- a = constant

Having estimated the cost of milk production, it is desirable to ascertain the reliability of these fodder costs, concentrate cost and miscellaneous expanses estimates. The most commonly used "t" test was applied to ascertain whether the cost of milk is significantly different from zero or not at some specified probability level.

"t" cal=b_j / standard error of b_j..

If calculated "t" value is greater than the table value of "t" at a specified probability level and "n-k-1" degree freedom, bj is said to be statistically significant.

IV. REASEARCH AND FINDINGS

Table.1: COBB DOUGLAS PRODUCTION FUNCTION ANALYSIS OF MEMBERS DAIRY COOPERATIVE SOCIETY (COW)

Category of farmers	Elasticity Fodder	Elasticity of Concentrate	Elasticity of Miscellaneous	R^2 value
Land less	3.600*	2.471	1.073	78.81
"t" test value	2.14	0.27	0.02	
S.E value	(0.2598)	(1.4449)	(1.5081)	
Marginal	23.84*	-3.558*	1.0769	84.74
"t" test value	11.53	-2.48	0.19	
S.E value	(0.1195)	(0.22275)	(0.16856)	
Small	18.6680*	150.1412*	-328.62	91.51

http://dx.doi.org/10.22161/ijeab/2.1.5 *ISSN: 2456-1878*

"t" test value	6.25	1.87	-2.18	
S.E value	(0.203263)	(1.161996)	(1.153742)	
Medium	3.910	1.035	1.037	43.44
"t" test value	1.45	0.07	0.07	
S.E value	(0.4070)	(0.2165)	(0.2280)	
Large	11.708*	-12752.6	9223.16	87.18
"t" test value	2.67	-0.91	0.93	
S.E value	(0.40066)	(4.5120)	(4.2695)	

The analysis has revealed that Landless member farmers of dairy cooperative society failed to give sufficient Concentrate and Miscellaneous inputs to the milch cow but Fodder could provide significant effect result on cow milk production and further medium category only fodder and concentrate could provide a significant effect on milk production and next small member farmers were doing same as marginal farmers and in large member farmers, only fodder could provide a significant effect on milk production of cow but in medium members farmers of dairy cooperative society none of the variables like Fodder, Concentrate and miscellaneous inputs could provide a significant effect result on cow milk production. The analysis further revealed that 78.81, 84.74, 91.51, 43.44 and 87.18 % of the variation was explained by three input variables in land less, marginal, small, medium and large member farmers of dairy cooperative society respectively. Moreover, all the variables in this category remained the same.

Summary of all categories of Members of Dairy Cooperative Society for Milch Cow

One-way Analysis of Price by Category

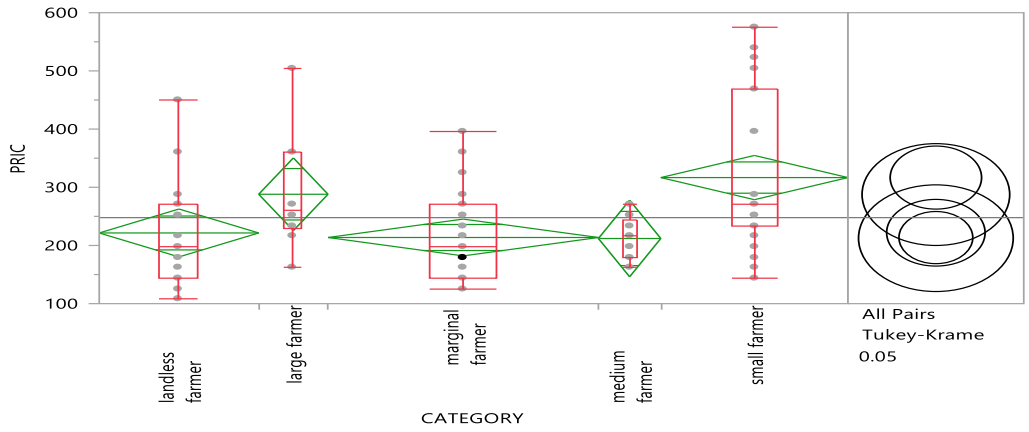


Table.2: Tukey-Kramer Analysis

Level			Mean
small farmer	A		316.66667
large farmer	A	B	288.00000
landless farmer		B	221.47826
marginal farmer		B	213.69231
medium farmer	A	B	212.00000

Table.3: Ordered Differences Report

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
small farmer	medium farmer	104.6667	38.33406	-1.775	211.1086	0.0563
small farmer	marginal farmer	102.9744	24.93415	33.740	172.2088	0.0007*
small farmer	landless farmer	95.1884	28.26024	16.718	173.6584	0.0092*
large farmer	medium farmer	76.0000	45.76066	-51.063	203.0633	0.4626
large farmer	marginal farmer	74.3077	35.30226	-23.716	172.3312	0.2259
large farmer	landless farmer	66.5217	37.72505	-38.229	171.2726	0.4005
small farmer	large farmer	28.6667	36.86852	-73.706	131.0392	0.9365
landless farmer	medium farmer	9.4783	39.15854	-99.253	118.2095	0.9992
landless farmer	marginal farmer	7.7860	26.18402	-64.919	80.4909	0.9983
marginal farmer	medium farmer	1.6923	36.83017	-100.574	103.9584	1.0000

Summary of all categories of Members of Dairy Cooperative Society for Milch Cow:

The analysis are revealed that in table no 2 & 3, mean of small farmers was observed Rs. 316.667 followed by large farmers Rs. 288.00, Landless Rs. 221.48, Marginal farmers Rs. 213.69 and least for Medium farmers Rs. 212.00. This indicated fact that small farmer interestedness in milch animals especially in cow is the highest.

Tukey test was applied to get into the depth of the problem under investigation. This indicated that there is no significant statistical difference between small large and medium member farmers for milch cow. Further, there is no difference between Large, Landless Marginal and farmers.

Further indicated the fact that P value for Small and Large farmer, Small and Landless were observed significant at 5 % level of Probability (.0007 and .0092) respectively.

Table.4: Return to Scale for the Dairy Cooperative Society Members (Cow):

S.N.	Category	β_1	β_2	β_3	Total $\beta_1 + \beta_2 + \beta_3$	Return to Scale ≥1
1	Landless	3.600	2.471	1.073	7.144	**≥1**
2	Marginal	23.84	-3.558	1.0769	21.358	**≥1**
3	Small	18.6680	150.1412	-328.62	-159.810	≤1
4	Medium	3.910	1.035	1.037	5.982	**≥1**
5	Large	11.708	-12752.6	9223.16	-3.17.76	≤1

β_1= Elasticity of Fodder

β_2= Elasticity of Concentrate

β_3= Elasticity of Miscellaneous expanses

The above table no 4 reveal that Elasticity of milk production for all the five categories of member of dairy cooperative society in cow namely Landless, marginal, small, medium and large farmers. The last column indicates their economies of scale. Their respective value were observed 7.144, 21.3589, -159.8108, 5.982 and -3.17.76 respectively. Out of these five categories, namely small and large farmers were observed had decreasing return to scale with a value of -159.8108 and -3.17.76 respectively.

The remaining three categories, i.e., landless, marginal and medium exhibited increasing return to scale with the value of 7.144, 21.3589 and 5.982 respectively. The analysis further reveals that return to scale was the highest for marginal farmers followed by landless and medium member farmers of dairy cooperative society.

It will here mention that the policy makers and planners engaged in dairy enterprise should concentrate all the above two categories small and large member farmers in case of a cow should be given proper attention to enhance milk production in the area under jurisdiction.

V. CONCLUSION

The results of the study revealed that Landless member farmers of dairy cooperative society failed to give sufficient Concentrate and Miscellaneous inputs to the milch cow but Fodder could provide significant effect result on cow milk production and further medium category only fodder and concentrate could provide a significant effect on milk

production and next small farmers were doing same as marginal farmers and in large member farmers, only fodder could provide a significant effect on milk production of cow but in medium members farmers, none of the variables like Fodder, Concentrate and miscellaneous inputs could provide a significant effect result on cow milk production. Analysis of Tukey test indicated that there is no significant statistical difference between small, large and medium member farmers and no difference between Large, Landless and Marginal farmers.

Analysis of Elasticity of milk production for all the five categories of member of dairy cooperative society in cow namely Landless, marginal, small, medium and large farmers. Out of these five categories that return to scale was the highest for marginal farmers followed by landless and medium member farmers.

It will here mention that the policy makers and planners engaged in dairy enterprise should concentrate all the above two categories small and large member farmers in case of a cow should be given proper attention to enhance milk production in the area under jurisdiction.

REFERENCES

[1] http://planningcommission.nic.in/reports/genrep/ar_eng1112.pdf
[2] Murithi, Festus Meme.2002 Efficiency of resource use in smallholder milk production: the case of Meru central dairy farmers, Kenya, http://erepository.uonbi.ac.ke:8080/xmlui/handle/123456789/21808
[3] Meena G. L.*et.al*, 2012. Milk Production Function And Resource Use Efficiency In Alwar District of Rajasthan, *INTERNATIONAL JOURNAL OF SCIENTIFIC & TECHNOLOGY RESEARCH VOLUME* 1, ISSUE 8, 115-119
[4] Prajneshu.2008. Fitting of Cobb-Douglas Production Functions: Revisited, *Agricultural Economics Research Review*, Vol. 21 , 289-292.
[5] Venkatesh P. and V. Sangeetha.2011. Milk Production and Resource Use Efficiency in Madurai District of Tamil Nadu: An Economic Analysis, *Journal of Community Mobilization and Sustainable Development* Vol. 6(1), 025-030.
[6] Deoghare, P.R. and N.K. Bhattacharya. 1994. Economics of buffalo milk production in Mathura district of Uttar Pradesh. *Indian Journal of Animal Science*, **64**(12): 1368- 1372
[7] Baruah, D.K.; A.B. Sarker and N.N. Bora. 1996. A study of economics of milk production in Assam. *Indian Journal of Dairy Science*, **49**(1): 17-22.
[8] Economic survey 2015-16: http://www.news18.com/news/business/economic-survey-2015-16-india-ranks-first-in-milk-production-accounting-for-18-5-per-cent-of-world-production-1208209.html 11:37 AM 23rd Nov, 2016.

http://dx.doi.org/10.22161/ijeab/2.1.6 *ISSN: 2456-1878*

A Study on the Removal Characteristics of Nickel Ion from Wastewater by Low-Cost Nano Adsorbent

R Sivakumar, S Arivoli*, V Marimuthu

*PG and Research Department of Chemistry, Thiru Vi Ka Government Arts College, Thiruvarur, Tamilnadu, India.

Abstract—*The work aims at adsorption studies of nickel from aqueous solution onto activated nano carbon prepared from Syringodium Isoetifolium Leaves, by acid treatment was tested for its efficiency in removing nickel ion. The process parameters studied include agitation time, initial nickel ion concentration, adsorbent dose, pH and temperature. The adsorption followed second order reaction equation and the rate is mainly controlled by intra-particle diffusion. The equilibrium adsorption data were correlated with Langmuir, Freundlich, Temkin, Dubinin-Radushkevich, Hurkins-Jura, Halsay, Redlich-Peterson, Jovanovich and BET isotherm models. The adsorption capacity (Q_m) obtained from the Langmuir isotherm plot at an initial pH of 6.5 and at 30, 40, 50, 60 ± 0.5 ^{0}C. The influence of pH on nickel ions removal was significant and the adsorption was increased with increase in temperature. A portion of the nickel ion was recovered from the spent ASI-NC using 0.1M HCl.*

Keywords—Activated Syringodium Isoetifolium Leaves Nano Carbon (ASI-NC), Adsorption isotherm, Equilibrium, Intra-particle diffusion. Nickel ion, Thermodynamic parameters.

I. INTRODUCTION

Heavy metal pollution of water and water bodies is a serious environmental problem that affects the quality of water. The consequences are decreasing water supply, increase in cost of purification, eutrophication of water bodies and decrease in aquatic production [1]. In order to tackle the menace poise by heavy metal pollution of water, several options have been adopted. These include oxidation and reduction, chemical precipitation, filtration, electrochemical treatment, ion exchange, membrane separation, reverse osmosis, adsorption, evaporation and electrolysis [2]. However, adsorption has been proven to be one of the best options available for the removal of heavy metals from aqueous solution [3]. In view of the above, several researches have been conducted using various materials as adsorbents [4]. However, some of these adsorbents also contain other toxicants; some are expensive and are characterized with limited surface area for adsorption.

A search of literature revealed that fruit stone has been used for adsorption of some heavy metals from aqueous solution but literature is scanty on the use of activated carbon produced from fruit stone for the adsorption of nickel ions from aqueous solution. Therefore, the objective of the present study is to investigate the possibility of using ASI-NC.

II. MATERIALS AND METHODS

2.1. Adsorbent

The *Syringodium Isoetifolium* Leaves collected from East Coastal area of Nagapattinam district was Carbonized with concentrated Sulphuric Acid and washed with water and activated around 1000°C in a muffle furnace for 5 hrs the it was taken out, ground well to fine powder and stored in a vacuum desiccators.

Syringodium Isoetifolium

2.2. Chemicals

All chemicals used of high purity commercially available Analar grade. 1000 mg/L of stock solution of nickel was prepared by dissolving accurately weighed 4.4786 gram of nickel sulphate in 1000 ml distilled water. All experimental solutions were prepared by diluting the stock solution to the required concentration. The pH of each experimental solution was adjusted to the required initial pH value using dilute HCl (or) NaOH before mixing the adsorbent. The concentration of residual nickel ion was determined with atomic absorption spectrophotometer (Perkin Elemer 2380).

http://dx.doi.org/10.22161/ijeab/2.1.4 *ISSN: 2456-1878*

2.3. Batch experiments

The effect of various parameters on the removal of nickel ion onto ASI-NC was studied batch adsorption experiments were conducted at (30-60°C). For each experimental run, 50 ml of nickel solution of known initial concentration and pH were taken in a 250 ml plugged conical flask. A 25 mg adsorbent dose is added to the solution and mixture was shaken at constant agitation speed (150 rpm) sample were withdrawn at appropriate time intervals (10-60 min) and the adsorbent was separated by filtration. The residual solutions were analyzed to determine the nickel ion concentration.

The effect of dosage of adsorbent on the removal of nickel ion was measured by contacting 50 ml of 50 mg/L of nickel ion solution with 25 mg of ASI-NC till equilibrium was attained. Adsorption equilibrium isotherm is studied using 25 mg of ASI-NC dosage per 50 ml of nickel ion solution. The initial concentration were ranged from (10 to 50 mg/L) in all sets of experiments. The plugged conical flask was shaken at a speed of 150 rpm for 60 minutes. Then the solution was separated from the mixture and analyzed for nickel ion concentration. The adsorption capacity was calculated by using a mass equilibrium equation as follows:

$$q_e = (C_0 - C_e)\, V/M \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, C_0 and C_e being the initial nickel concentration (mg/L) and equilibrium concentration, respectively V is the experimental volume of nickel ion solution expressed in liters [L] and M is the adsorbent mass expressed in grams [g]. The nickel ion ions percentage can be calculated as follows:

$$\%R = (C_0 - C_t) \times 100/C_0 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The effect of pH on the rate of adsorption was investigated using nickel concentration of 20 mg/L constant ASI-NC dosage. The pH values were adjusted with dilute HCl and NaOH solution. The adsorbent-adsorbate mixture was shaken at room temperature using agitation speed (150 rpm) for 60 minutes. Then the concentration of nickel in solution was determined.

III. RESULTS AND DISCUSSION

3.1 Effect of agitation time and initial Nickel ion concentration

The kinetics of adsorption of nickel ion by ASI-NC is shown in Fig. 1 with smooth and single plots indicating monolayer adsorption of nickel ions on the ASI-NC. The removal of nickel ions increased with the lapse time and attains equilibrium in 60 min for 50 mg/ L. With increase in nickel ions concentration from 10 to 50 mg/L, the amount of nickel ions adsorbed increased while the percent removal decreased, indicating that the nickel ions removal by adsorption on ASI-NC concentration dependent.

3.2 Effect of ASI-NC mass

The amount of nickel ion adsorption increased with the increase in ASI-NC dose and reached a maximum value after a particular dose shown in Fig. 2 and taken an initial nickel ions concentration of 20 mg/L, complete nickel ions removal was obtained at a maximum ASI-NC dose of 125 mg. The increase in the adsorption of nickel ions with ASI-NC dose was due to the introduction of more binding sites for adsorption and the availability more surface area.

3.3 Effect of pH

The experience carried out at different pH show that there was a change in the percentage removal of nickel ions over the entire pH range shown in Fig. 3. This indicates the strong force of interaction between the nickel ions and ASI-NC that either H^+ or OH^- ions could influence the adsorption capacity. In other words, the adsorption of nickel ions on ASI-NC does involve ion exchange mechanism that have been an influence on the nickel ions adsorption while varying the pH This observation is in line with the type I and II isotherm and positive $\triangle H^0$ value obtained, which indicates irreversible adsorption probably due to polar interactions.

3.4 Effect of other ions

The effect of other ions like Ca^{2+} and Cl^- on the adsorption process studied at different concentrations. The ions added to 50mg/L of nickel ions solutions and the contents were agitated for 60 min at 30^0C. The results had shown in the Fig. 4 reveals that low concentration of Cl^- does not affect the percentage of adsorption of nickel ions on ASI-NC, because the interaction of Cl^- at available sites of adsorbent through competitive adsorption is not so effective. While the concentration of other ion Ca^{2+} increases, the interference of these ions at available surface sites of the sorbent through competitive adsorption increases that, decreases the percentage adsorption. The interference was more in the presence of Ca^{2+} compared with Cl^- ion. This is so because ions with smaller hydrated radii decrease the swelling pressure within the sorbent and increase the affinity of the sorbent for such ions.

3.5 Adsorption Isotherms

Adsorption isotherm describes the relation between the amount or concentration of adsorbate that accumulates on the adsorbent and the equilibrium concentration of the dissolved adsorbate. Equilibrium studies were carried out by agitating a series of beakers containing 50 mL of nickel solutions of initial concentration 20 mg/L with 0.025 g of activated nano carbon at 30 ^{0}C with a constant agitation. Agitation was provided for 1.0 h, which is more than sufficient time to reach equilibrium.

3.5.1 Freundlich adsorption isotherm

The Freundlich adsorption isotherm is based on the equilibrium sorption on heterogeneous surfaces. This isotherm is derived from the assumption that the

http://dx.doi.org/10.22161/ijeab/2.1.4 *ISSN: 2456-1878*

adsorption sites are distributed exponentially with respect to heat of adsorption. The adsorption isotherm is expressed by the following equation

$$q_e = K_F C_e^{1/nF} \quad \ldots\ldots\ldots\ldots (3)$$

Which, can be linearized as

$$\ln q_e = \ln K_F + \frac{1}{n_F} \ln C_e \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where, q_e is the amount of Nickel adsorbed at equilibrium (mg/g) and C_e is the concentration of nickel in the aqueous phase at equilibrium (ppm). K_F (L/g) and $1/n_F$ are the Freundlich constants related to adsorption capacity and sorption intensity, respectively.

The Freundlich constants K_F and $1/n_F$ were calculated from the slope and intercept of the $\ln q_e$ Vs $\ln C_e$ plot, and the model parameters are shown in Table 2. The magnitude of K_F showed that ASI-NC had a high capacity for nickel adsorption from the aqueous solutions studied. The Freundlich exponent, n_F, should have values in the range of 1 and 10 (i.e., $1/n_F < 1$) to be considered as favourable adsorption [5]. A $1/n_F$ value of less than 1 indicated that nickel is favorably adsorbed by ASI-NC. The Freundlich isotherm did not show a good fit to the experimental data as indicated by SSE and Chi-square statistics.

3.5.2 Langmuir adsorption isotherm

The Langmuir adsorption isotherm is based on the assumption that all sorption sites possess equal affinity to the adsorbate. The Langmuir isotherm [6] in a linear form can be represented as:

$$\frac{C_e}{q_e} = \frac{1}{q_m K_L} + \frac{C_e}{q_m} \quad \ldots\ldots\ldots\ldots (5)$$

Where q_e is the amount of nickel adsorbed at equilibrium (mg/g), C_e is the concentration of nickel in the aqueous phase at equilibrium (ppm), q_m is the maximum nickel uptake (mg/g), and K_L is the Langmuir constant related to adsorption capacity and the energy of adsorption (g/mg).

A linear plot of C_e/q_e Vs C_e was employed to determine the value of q_m and K_L the obtained data's were also presented in Table 2. The model predicted a maximum value that could not be reached in the experiments. The value of K_L decreased with an increase in the temperature. A high K_L value indicates a high adsorption affinity. Weber and Chakraborti expressed the Langmuir isotherm in term of dimensionless constant separation factor or equilibrium parameter (R_L) defined in the following equation:

$$R_L = \frac{1}{1 + K_L C_0} \quad \ldots\ldots\ldots\ldots\ldots (6)$$

Where, C_0 is the initial nickel concentration (ppm). Four scenarios can be distinguished:

The sorption isotherm is unfavorable when $R_L > 1$, the isotherm is linear when $R_L = 1$, the isotherm is favorable when $0 < R_L < 1$ and the isotherm is irreversible when $R_L = 0$. The values of dimensionless separation factor (R_L) for nickel removal were calculated at different concentrations and temperatures. As shown in Table 3, at all concentrations and temperatures tested the values of R_L for nickel adsorptions on the ASI-NC were less than 1 and greater than zero, indicating favorable adsorption.

The Langmuir isotherm showed a better fit to the adsorption data than the Freundlich isotherm. The fact that the Langmuir isotherm fits the experimental data well may be due to homogeneous distribution of active sites on the ASI-NC surface, since the Langmuir equation assumes that the adsorbent surface is energetically homogeneous.

3.5.3 Temkin adsorption isotherm:

The Temkin adsorption isotherm assumes that the heat of adsorption decreases linearly with the sorption coverage due to adsorbent-adsorbate interactions [7]. The Temkin isotherm equation is given as:

$$q_e = \frac{RT}{bT} \ln(K_T C_e) \ldots\ldots\ldots\ldots\ldots (7)$$

Which, can be represented in the following linear form

$$q_e = \frac{RT}{b} \ln K_T + \frac{RT}{b} \ln C_e \ldots\ldots\ldots\ldots\ldots (8)$$

Where, K_T (L/g) is the Temkin isotherm constant, b_T (J/mol) is a constant related to heat of sorption, R is the ideal gas constant (8.314 J/mol K), and T is absolute temperature (K). A plot of q_e versus $\ln C_e$ enables the determination of isotherm constants K_T and b_T from the slope and intercept, the model parameters are listed in Table 2. The Temkin isotherm appears to provide a good fit to the nickel adsorption data.

The adsorption energy in the Temkin model, b_T, is positive for Nickel adsorption from the aqueous solution, which indicates that the adsorption is endothermic. The experimental equilibrium curve is close to that predicted by Temkin model. Consequently, the adsorption isotherm of nickel on ASI-NC can be described reasonably well by the Temkin isotherm.

3.5.4 Hurkins-Jura adsorption isotherm

The Hurkins-Jura [8] adsorption isotherm can be expressed as

$$q_e = \sqrt{\frac{A_H}{B_H + \log C_e}} \ldots\ldots\ldots\ldots\ldots (9)$$

This can rearranged as follows:

$$\frac{1}{q_e^2} = \frac{B_H}{A_H} - \frac{1}{A_H} \log C_e \ldots\ldots\ldots\ldots\ldots (10)$$

Where, A_H (g^2/L) and B_H (mg^2/L) are two parameters characterizing the sorption equilibrium.

The isotherm equation accounts for multilayer adsorption and can be explained by the existence of a heterogeneous pore distribution. The Harkins–Jura isotherm parameters are obtained from the plots of of $1/q_e^2$ versus log C_e

http://dx.doi.org/10.22161/ijeab/2.1.4 *ISSN: 2456-1878*

enables the determination of model parameters A_H and B_H from the slope and intercept.

3.5.5 Halsay adsorption isotherm

The Halsay [9] adsorption isotherm can be given as

$$q_e = \exp\left(\frac{\ln K_{Ha} - \ln C_e}{n_{Ha}}\right) \quad \text{.....(11)}$$

A linear form of the isotherm can be expressed as follows

$$\ln q_e = \frac{\ln K_{Ha}}{n_{Ha}} - \frac{\ln C_e}{n_{Ha}} \quad \text{.....(12)}$$

Where, K_{Ha} (mg/L) and n_{Ha} are the Halsay isotherm constants.

A plot of $\ln q_e$ Vs $\ln C_e$, enables the determination of n_{Ha} and K_{Ha} from the slope and intercept. This equation is suitable for multilayer adsorption and the fitting of the experimental data to this equation attest to the heteroporous nature of adsorbent. The experimental data and the model predictions based on the non-linear form of the Halsay models. The model parameters are listed in Table 2. This result also shows that the adsorption of Nickel on ASI-NC was not based on significant multilayer adsorption. The Halsay model is also not suitable to describe the adsorption of nickel on ASI-NC, because this model also assumes a multilayer behavior for the adsorption of adsorbate onto adsorbent.

3.5.6 Redlich-Peterson adsorption isotherm

The Radlich-Peterson [10] adsorption isotherm contains three parameters and incorporates the features of Langmuir and Freundlich isotherms into a single equation. The general isotherm equation can be described as follows:

$$q_e = \frac{K_R C_e}{1 + a_R C_e^g} \quad \text{.....(13)}$$

The linear form of the isotherm can be expressed as follows:

$$\ln \frac{C_e}{q_e} = g \ln C_e - \ln K_R \quad \text{.....(14)}$$

Where, K_R (L/g) and a_R (L/mg) are the Radlich-Peterson isotherm constants and g is the exponent between 0 and 1. There are two limiting cases: Langmuir form for g = 1 and Henry's law for g = 0.

A plot of $\ln C_e/q_e$ versus $\ln C_e$ enables the determination of isotherm constants g and K_R from the slope and intercept. The values of K_R, presented in Table 2, indicate that the adsorption capacity of the ASI-NC decreased with an increase temperature. Furthermore, the value of g lies between 0 and 1, indicating favorable adsorption.

3.5.7 Dubinin-Radushkevich adsorption isotherm

The Dubinin-Radushkevich [11] adsorption isotherm is another isotherm equation. It is assumed that the characteristic of the sorption curve is related to the porosity of the adsorbent. The linear form of the isotherm can be expressed as follows:

$$\ln q_e = \ln Q_D - B_D \left[RT \ln\left(1 + \frac{1}{C_e}\right)\right]^2 \quad \text{.....(15)}$$

Where, Q_D is the maximum sorption capacity (mol/g), and B_D is the Dubinin-Radushkevich constant (mol^2/kJ^2). A plot of $\ln q_e$ Vs $R_T \ln(1+1/C_e)$ enables the determination of isotherm constants B_D and Q_D from the slope and intercept.

3.5.8 Jovanovic adsorption isotherm

The model of an adsorption surface considered by Jovanovic[16] is essentially the same as that considered by Langmuir. The Jovanovic model leads to the following relationship [12]:

$$q_e = q_{max}\left(1 - e^{K_J C_e}\right) \quad \text{.....(16)}$$

The linear form of the isotherm can be expressed as follows:

$$\ln q_e = \ln q_{max} - K_J C_e \quad \text{.....(17)}$$

Where, K_J (L/g) is a parameter. q_{max} (mg/g) is the maximum Nickel (II) uptake.

The q_{max} is obtained from a plot of ln q_e and C_e. Their related parameters are listed in Table 2. By comparing the values of the error functions, it was found the Langmuir and Temkin models are best to fit the Nickel adsorption on the ASI-C. Both models show a high degree of correlation. This is clearly confirming the good fit of Langmuir and Temkin models with the experimental data for removal of nickel from the solution.

3.5.9 The Brunauer-Emmett-Teller (BET) isotherm model

Brunauer-Emmett-Teller (BET) isotherm is a theoretical equation, most widely applied in the gas-solid equilibrium systems [13]. It was developed to derive multilayer adsorption systems with relative concentration ranges from 10 to 50 mg/L corresponding to a monolayer coverage lying between 10 and 30 mg/L. Its extinction model related to liquid-solid interface is exhibited as:

$$q_e = \frac{q_s C_{BET} C_e}{(C_s - C_e)[1 + (C_{BET} - 1)(C_e/C_s)]} \quad \text{.....(18)}$$

Where, C_{BET}, Cs, q_s and q_e are the BET adsorption isotherm (L/mg), adsorbate monolayer saturation concentration (mg/L), theoretical isotherm saturation capacity (mg/g) and equilibrium adsorption capacity (mg/g), respectively. As C_{BET} and C_{BET} (C_e/C_s) is much greater than 1,

In the linear form as used is represented as

$$\frac{C_e}{q(C_s - C_e)} = \frac{1}{q_s C_{BET}} + \left(\frac{C_{BET} - 1}{q_s C_{BET}}\right)\left(\frac{C_e}{C_s}\right) \quad \text{.....(19)}$$

Where, C_e is equilibrium Concentration (mg/l), C_s is adsorbate monolayer saturation concentration (mg/l) and C_{BET} is BET adsorption relating to the energy of surface interaction (l/mg).

http://dx.doi.org/10.22161/ijeab/2.1.4 *ISSN: 2456-1878*

3.6 Kinetic parameters

The rate and mechanism of the adsorption process can be elucidated based on kinetic studies. Nickel adsorption on solid surface may be explained by two distinct mechanisms: (1) An initial rapid binding of nickel molecules on the adsorbent surface; (2) relatively slow intra-particle diffusion. To analyze the adsorption kinetics of the nickel, the pseudo-first-order, the pseudo-second-order, and intra-particle diffusion models were applied [14]. Each of these models and their linear modes of them equations presented in below.

Kinetic Models and Their Linear Forms

Model	Nonlinear Form	Linear Form	Number of Equation
Pseudo-first-order	$dq_t/d_t = k_1(q_e-q_t)$	$\ln (q_e-q_t) = \ln q_e - k_1 t$	(20)
Pseudo-second-order	$dq_t/d_t = k_2(q_e-q_t)^2$	$t/q_t = 1/k^2q_e^2 + (1/q_e)t$	(21)

Where, q_e and q_t refer to the amount of nickel adsorbed (mg/g) at equilibrium and at any time, t (min), respectively and k_1 (1/min), k_2 (g/mg min) are the equilibrium rate constants of pseudo-first order and pseudo-second order models, respectively.

Pseudo-first order model is a simple kinetic model, which was proposed by Lagergren during 1898 and is used for estimation of the surface adsorption reaction rate. The values of ln (q_e - q_t) were linearly correlated with t. The plot of ln (q_e - q_t) Vs t should give a linear relationship from which the values of k_1 were determined from the slope of the plot. In many cases, the first-order equation of Lagergren does not fit well with the entire range of contact time and is generally applicable over the initial stage of the adsorption processes.

In the pseudo-second order model, the slope and intercept of the t/q_t Vs t plot were used to calculate the second-order rate constant, k_2. The values of equilibrium rate constant (k_2) are presented in Table 5. According to Table 5, the value of R^2 (0.999) related to the pseudo-second order model revealed that nickel adsorption followed this model.

Nevertheless, pseudo-first order and pseudo-second order kinetic models cannot identify the mechanism of diffusion of nickel into the adsorbent pores.

3.6.1 Simple Elovich Model:

The simple Elovich model [15] is expressed in the form,

$$q_t = \alpha + \beta \ln t \quad \text{.....(22)}$$

Where, q_t is the amount adsorbed at time t, α and β are the constants obtained from the experiment. A plot of q_t Vs ln 't' should give a linear relationship for the applicability of the simple Elovich kinetic. The Elovich kinetics of nickel on to ASI-NC for various initial concentrations (10, 20, 30, 40 and 50 mg/L) of volume 50 mL (each), adsorbent dose 0.025g, temperature 30 ^oC and pH 6.5.

3.6.2 The Elovich equation

The Elovich model equation is generally expressed as

$$dq_t / d_t = \alpha \exp(-\beta q_t) \quad \text{.....(23)}$$

Where; α is the initial adsorption rate (mg g^{-1} min^{-1}) and β is the desorption constant (g/mg) during any one experiment. To simplify the Elovich equation [16] assumed αβt>>t and by applying boundary conditions q_t = 0 at t= 0 and q_t = q_t at t = t Eq.(23) becomes:

$$q_t = 1/\beta \ln(\alpha\beta) + 1/\beta \ln t \quad \text{.....(24)}$$

If nickel ions adsorption fits with the Elovich model, a plot of q_t vs. ln(t) should yield a linear relationship with a slope of (1/β)and an intercept of (1/β)ln (αβ). The Elovich model parameters α, β, and correlation coefficient (γ) are summarized in table 5. The experimental data such as the initial adsorption rate (α) adsorption constant (β) and the correlation co-efficient (γ) calculated from this model indicates that the initial adsorption (α) increases with temperature similar to that of initial adsorption rate (h) in pseudo-second–order kinetics models. This may be due to increase the pore or active site on the ASI-NC adsorbent.

3.6.3 The Intraparticle diffusion model

The kinetic results were analyzed by the Intraparticle diffusion model [17, 20] to elucidate the diffusion mechanism. The model is expressed as:

$$q_t = K_{id}\, t^{1/2} + I \quad \text{.....(25)}$$

Where, 'I' is the intercept and K_{id} is the intra-particle diffusion rate constant. The intercept of the plot reflects the boundary layer effect. Larger the intercept, greater is the contribution of the surface sorption in the rate controlling step. The calculated diffusion coefficient K_{id} values are listed in Table 6. The K_{id} value was higher at the higher concentrations. Intraparticle diffusion is the sole rate-limiting step if the regression of q_t versus $t^{1/2}$ is linear and passes through the origin. In fact, the linear plots at each concentration did not pass through the origin. This deviation from the origin is due to the difference in the rate of mass transfer in the initial and final stages of the sorption. This indicated the existence of some boundary layer effect and further showed that Intraparticle diffusion was not the only rate-limiting step.

It is clear from the Table 5 that the pseudo- second-order kinetic model showed excellent linearity with high correlation coefficient (R^2>0.99) at all the studied concentrations in comparison to the other kinetic models.

http://dx.doi.org/10.22161/ijeab/2.1.4 *ISSN: 2456-1878*

In addition the calculated q_e values also agree with the experimental data in the case of pseudo-second-order kinetic model. It is also evident from Table 5 that the values of the rate constant k_2 decrease with increasing initial nickel concentrations. This is due to the lower competition for the surface active sites at lower concentration but at higher concentration the competition for the surface active sites will be high and consequently lower sorption rates are obtained.

3.7. Thermodynamic treatment of the adsorption process

Thermodynamic parameters associated with the adsorption, via standard free energy change (ΔG^0), standard enthalpy change (ΔH^0), and standard entropy change (ΔS^0) were calculated as follows. The free energy of adsorption process considering the adsorption equilibrium constant K_0 is given by the equation

$$\Delta G^0 = -RT \ln K_0 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (26)$$

Where, ΔG^0 is the free energy of adsorption (kJ/mol), T is the temperature in Kelvin and R is the universal gas constant(8.314 J mol/K).The adsorption distribution coefficient K_0 for the sorption reaction was determined from the slope of the plot of $\ln(q_e/C_e)$ against C_e at different temperature. The adsorption distribution coefficient may be expressed in terms of enthalpy change (ΔH^0) and entropy change (ΔS^0) as a function of temperature,

$$\ln K_0 = (\Delta H^0/RT)+(\Delta S^0/R) \quad \ldots\ldots\ldots\ldots (27)$$

Where, ΔH^0 is the standard heat change of sorption (kJ/mol) and ΔS^0 is standard entropy change (kJ/mol). The value of ΔH^0 and ΔS^0 can be obtained from the slope and intercept of plot of ln K_0 against 1/T. The value of thermodynamic parameter calculated from equation 26 and 10 are shown in table 4. The thermodynamic treatment of the sorption data indicates that ΔG^0 values were negative at all temperature. The results point out that physisorption is much more favorable for the adsorption of nickel ions. The positive values of ΔH^0 show the endothermic nature of adsorption and it governs the possibility of physical adsorption [18]. Because in the case of physical adsorption, while increasing the temperature of the system, the extent of nickel ions adsorption increases, this rules out the possibility of chemisorptions. The low ΔH^0 value depicts nickel ions is physisorbed onto adsorbent ASI-NC.

The negative ΔG^0 values table 4 were conform the spontaneous nature of adsorption nickel ions onto ASI-NC**.** The lesser values of ΔG^0 suggest that adsorption is physical adsorption process. The positive values of ΔS^0 in table 4, showed increased randomness of the solid solution interface during the adsorption of nickel ion onto ASI-NC.

In order to support that physical adsorption is the predominant mechanism, the values of activation energy (E_a) and sticking probability (S*) were calculated from the experimental data. They were calculated using modified Arrhenius type equation related to surface coverage (θ) as follows:

$$\theta = \left(1 - \frac{C_e}{C_i}\right) \quad \ldots\ldots\ldots\ldots (28)$$

$$S^* = (1-\theta)_e \frac{-E_a}{RT} \quad \ldots\ldots\ldots (29)$$

The sticking probability, S*, is a function of the adsorbate/adsorbent system under consideration but must satisfy the condition 0 < S*< 1 and is dependent on the temperature of the system. The values of Ea and S* can be calculated from slope and intercept of the plot of ln(1-θ) versus 1/T respectively and are listed in Table 4.

From Table 4 it is clear that the reaction is spontaneous in nature as ΔG^0 values are negative at all the temperature studied. Again positive ΔH^0 value confirms that the sorption is endothermic in nature. The positive value of ΔS^0 reflects the affinities of the adsorbents for the nickel. The result as shown in Table 4 indicate that the probability of the nickel to stick on surface of biomass is very high as S*<< 1, these values confirm that, the sorption process is physisorption.

3.8 Desorption studies

Desorption studies help to elucidate the nature of adsorption and recycling of the spent adsorbent and the nickel ions. If the adsorbed nickel ions can be desorbed using neutral pH water, then the attachment of the nickel ions of the adsorbent is by weak bonds. The effect of various reagents used for desorption studies. The results indicate that hydrochloric acid is a better reagent for desorption, because we could get more than 90% removal of adsorbed nickel ions. The reversibility of adsorbed nickel ions in mineral acid or base is in agreement with the pH dependent results obtained. The desorption of nickel ions by mineral acids and alkaline medium indicates that the nickel ions was adsorbed onto the ASI-NC through physisorption as well as by chemisorptions mechanisms [19].

IV. CONCLUSION

ASI-NC prepared from Syringodium Isoetifolium Leaves was found effective in removing nickel ion from aqueous solution. The adsorption is faster and the rate is mainly controlled by intra-particle diffusion. Using the sorption equation obtained from the Langmuir and Freundlich isotherms, it was found that ASI-NC is an effective one for the removal of nickel ion. The equilibrium data conformed well to the Langmuir and BET isotherm models. The temperature variation study showed that the

nickel ion adsorption is endothermic and spontaneous with increased randomness at the solid solution interface. Significant effect on adsorption was observed on varying the pH of the nickel ion solution. pH dependent results and desorption of metal ion in mineral acid suggest that the adsorption of nickel ions on ASI-NC involves chemisorptions as well as physisorption mechanism.

ACKNOWLEDGEMENT

The authors sincerely thank the University Grants Commission, New Delhi for providing the fund from Major Research Project.

REFERENCES

[1] Zolfaghari, G., Esmaili-Sari, A., Anbia, M., Younesi, H. and Ghasemian, M.B. "A zinc oxide-coated nanoporous carbon adsorbent for lead removal from water" Optimization, equilibrium modeling, and kinetics studies, (2013). *International Journal of Environmental Science and Technology*, 10 (2), 325-340.

[2] Memarian, R. and Ramamurthy, A.S., "Modeling of lead and cadmium uptake by plants in the presence of surfactants" (2013), *Environmental Monitoring and Assessment*, 185 (3), 2067-2071.

[3] Vijayaraghavan, K. and Joshi, U.M. Chicken Eggshells "Remove Pb(II) Ions from Synthetic Wastewater" (2013), *Environmental Engineering Science*, 30 (2), 67-73.

[4] Shukla, N.B. and Madras, G. "Kinetics of adsorption of methylene blue and rhodamine 6G on acrylic acid-based superabsorbents" (2012), *Journal of Applied Polymer Science*, 126 (2), 463-472.

[5] Usman, A.R.A., Sallam, A.S., Al-Omran, A., El-Naggar, A.H., Alenazi, K.K.H., Nadeem, M. and Al-Wabel, M.I. "Chemically Modified Biochar Produced from Conocarpus Wastes: An Efficient Sorbent for Fe(II) Removal from Acidic Aqueous Solutions" (2013), *Adsorption Science & Technology*, 31 (7), 625-640.

[6] Diaz, P.V., Medina, E.C., Nunez, F.U. and Fabila, M.G. "Reuse of Citrullus Lanatus Peel As Biosorbent to Remove Aluminum in Aqueous Phase" (2013), *Fresenius Environmental Bulletin*, 22 (5), 1432-1440.

[7] Bulgariu, L., Lupea, M., Bulgariu, D., Rusu, C. and Macoveanu, M. "Equilibrium Study of Pb(Ii) and Cd(Ii) Biosorption from Aqueous Solution on Marine Green Algae Biomass" (2013), *Environmental Engineering and Management Journal*, 12 (1), 183-190.

[8] Duran-Blanco, J.M., Lopez-Munoz, B.E. and Olguin, M.T. "Influence of pH on U(VI) Adsorption using a Thermally-Treated Mg-Al Hydrotalcite and a Natural Zeolite in a Batch System" (2013), *Separation Science and Technology*, 48 (5), 797-804.

[9] Vistuba, J.P., Nagel-Hassemer, M.E., Lapolli, F.R. and Recio, M.A.L. "Simultaneous adsorption of iron and manganese from aqueous solutions employing an adsorbent coal" (2013), *Environmental Technology*, 34 (2), 275-282.

[10] Frendlich H. "The dye adsorption is losungen (Adsorption in Solution)" (1906), *Z Phys. Chem*.,57, 385 – 470.

[11] Taghdiri, M. and Zamani, N. "Hexamine adsorption study on activated carbon from aqueous solutions for application in treatment of hexamine industrial wastewater" (2013), *International Journal of Environmental Science and Technology*, 10 (1), 19-26.

[12] Bulgariu, L., Hlihor, R.M., Bulgariu, D. and Gavrilescu, M. "Sorptive Removal of Cadmium(II) Ions from Aqueous Solution by Mustard Biomass" (2012), *Environmental Engineering and Management Journal*, 11 (11), 1969-1976.

[13] Hadi, M., Mckay, G., Samarghandi, M.R., Maleki, A. and Aminabad, M.S. "Prediction of optimum adsorption isotherm: comparison of chi-square and Log-likelihood statistics" (2012), *Desalination and Water Treatment*, 49 (1-3), 81-94.

[14] Langmuir I. "The adsorption of gases plane surfaces of glass, mica and platinum" (1918), *J. Am. Soc*., 579, 1361 – 1403.

[15] Hang, X.F., Xu, Y.M., Wang, L., Sun, Y.B., Lin, D.S., Sun, Y., Qin, X. and Wan, Q. "Sorption of Pb^{2+} on mercapto functionalized sepiolite" (2013), *Chemosphere*, 90 (2), 548-555.

[16] Zhou, Y.N., Wang, Z., Zhang, Q., Xi, X.J., Zhang, J. and Yang, W.T. "Equilibrium and thermodynamic studies on adsorption of BSA using PVDF microfiltration membrane" (2012), *Desalination*, 307, 61-67.

[17] Khan, A A, Singh, R P. "Adsorption thermodynamics of carbofuran on Sn (IV) arsenosilicate in H^+, Na^+ and Ca^{2+} forms" (1987), *Colloid & Surfaces* (24) 33 – 42.

[18] Ghosh, A. and Das Saha, P. "Optimization of Nickel adsorption by chemically modified fly ash using response surface methodology modeling" (2012), *Desalination and Water Treatment*, 49 (1-3), 218-226.

[19] Chien S H. and Clayton.W R. "Application of Elovich Equation to the kinetics of Phosphate release and sorption on soil" (1980), *Soil Sci. Sco. Am, J*., 44, 265 – 268.

[20] W. J. Weber and Morris J C. "Kinetics of adsorption on Carbon from solution" (1964), *J. Sanitary Engg. Div*. 90, 79.

http://dx.doi.org/10.22161/ijeab/2.1.4 *ISSN: 2456-1878*

Table: 1: Equilibrium Parameters for the adsorption of nickel (II) ion onto ASI-NC

M_0	Ce (mg / L)				Qe (mg / g)				Removed (%)			
	30 °C	40 °C	50 °C	60 °C	30 °C	40 °C	50 °C	60 °C	30 °C	40 °C	50 °C	60 °C
10	2.451	1.951	1.701	1.676	15.10	16.10	16.60	16.65	75.49	80.49	82.99	83.24
20	3.951	3.702	3.461	2.951	32.10	32.60	33.08	34.10	80.25	81.49	82.69	85.24
30	7.221	6.652	5.951	5.602	45.56	46.70	48.10	48.80	75.93	77.83	80.16	81.33
40	9.972	9.222	8.809	8.338	60.06	61.56	62.38	63.32	75.07	76.95	77.98	79.16
50	14.20	13.75	13.47	12.74	71.60	72.50	73.05	74.51	71.60	72.50	73.05	74.51

Table: 2: Langmuir and Freundlich Isotherm Parameter for the adsorption of nickel (II) ion onto ASI-NC

Model	Constant	Temperature (°C)			
		30	40	50	60
Freundlich	K_f (mg/g) $(L/mg)^{1/n}$	8.241	10.62	12.39	13.32
	n	1.176	1.301	1.384	1.391
Langmuir	Q_m (mg/g)	228.0	161.8	140.0	141.2
	b (L/mg)	0.034	0.062	0.085	0.093
Temkin	b_T (J/mol)	31.44	29.06	27.81	28.34
	K_T (L/mg)	0.851	0.948	1.003	1.023
Hurkins-Jura	A_H (g^2/L)	-204.8	-245.2	-273.4	-285.4
	B_H (mg^2/L)	-1.058	-1.037	-1.020	-0.997
Halsay	K_{Ha} (mg/L)	11.94	21.62	32.53	36.68
	n_{Ha}	1.176	1.301	1.384	1.391
Radlich-Peterson	g	0.150	0.231	0.277	0.281
	K_R (L/g)	0.121	0.094	0.081	0.075
Dubinin-Radushkevich	q_s (mg/g)	65.80	62.94	62.72	66.45
	$K_D \times 10^{-4}$ mol^2 kJ^{-2}	1.506	1.491	1.486	1.496
Jovanovic	K_J (L/g)	0.119	0.117	0.115	0.119
	q_{max} (mg/g)	16.07	17.74	19.21	19.88
BET	C_{BET} (L/mg)	6.265	3.754	4.897	6.194
	qs (mg/g)	0.160	0.266	0.204	0.161

Table: 3: Dimensionless Separation factor (R_L) for the adsorption of nickel (II) ion onto ASI-NC

(C_i)	Temperature °C			
	30°C	40°C	50°C	60°C
10	0.540	0.392	0.319	0.302
20	0.369	0.244	0.190	0.178
30	0.281	0.177	0.135	0.126
40	0.227	0.139	0.105	0.098
50	0.190	0.114	0.086	0.080

Table: 4: Thermodynamic Parameter for the adsorption of nickel (II) ion onto ASI-NC

C_0	ΔG°				ΔH°	ΔS°	E_a	S^*
	30° C	40° C	50° C	60° C				
10	-2834.3	-3688.6	-4255.7	-4437.9	13.57	54.62	1019.8	0.0032
20	-3531.3	-3857.2	-4200.3	-4855.6	9.484	42.75	7849.6	0.0089
30	-2894.0	-3267.6	-3750.5	-4073.8	9.313	40.28	7332.0	0.0131
40	-2777.1	-3136.4	-3395.3	-3694.3	6.347	30.18	4897.7	0.0355
50	-2329.3	-2522.7	-2678.4	-2970.0	3.944	20.66	2880.5	0.0909

http://dx.doi.org/10.22161/ijeab/2.1.4 *ISSN: 2456-1878*

Table: 5: The Kinetic Parameters for the adsorption of nickel (II) ion onto ASI-NC

C_0	Temp °C	Pseudo Second Order				Elovich Model			Intraparticle Diffusion		
		q_e	k_2	γ	h	α	β	γ	K_{id}	γ	C
10	30	15.31	0.016	0.978	3.716	2.979	3.562	9.971	1.801	9.877	1.866
	40	17.42	0.010	0.978	2.906	6.795	4.787	9.898	1.445	9.869	1.642
	50	17.38	0.016	0.984	4.946	3.016	7.172	9.900	8.986	9.896	1.755
	60	17.38	0.017	0.981	5.278	5.642	7.557	9.911	8.469	9.907	1.765
20	30	35.49	0.003	0.984	4.283	4.178	2.003	9.954	1.784	9.929	1.571
	40	35.74	0.004	0.979	4.845	6.173	2.097	9.909	1.66	9.896	1.605
	50	36.09	0.004	0.982	5.639	9.854	2.200	9.904	1.535	9.922	1.639
	60	36.58	0.005	0.979	6.536	2.285	2.427	9.921	1.339	9.939	1.684
30	30	48.59	0.004	0.979	8.731	5.699	1.997	9.958	1.210	9.880	1.656
	40	50.32	0.004	0.985	10.21	4.710	1.837	9.899	1.268	9.871	1.665
	50	52.34	0.003	0.984	9.347	2.043	1.576	9.922	1.454	9.863	1.645
	60	51.95	0.005	0.985	13.38	8.262	1.856	9.900	1.197	9.865	1.702
40	30	64.52	0.004	0.983	15.27	4.878	1.362	9.938	1.332	9.884	1.646
	40	66.02	0.003	0.978	15.00	4.938	1.336	9.915	1.331	9.892	1.654
	50	66.66	0.004	0.981	16.36	6.765	1.369	9.962	1.276	9.913	1.670
	60	67.62	0.004	0.985	16.85	7.741	1.370	9.918	1.254	9.924	1.681
50	30	76.55	0.002	0.979	12.63	4.483	1.162	9.920	1.340	9.911	1.602
	40	77.59	0.002	0.985	12.75	4.466	1.145	9.913	1.343	9.932	1.607
	50	77.76	0.002	0.979	13.77	7.295	1.212	9.920	1.250	9.880	1.628
	60	79.51	0.002	0.981	13.20	5.096	1.133	9.949	1.320	9.880	1.622

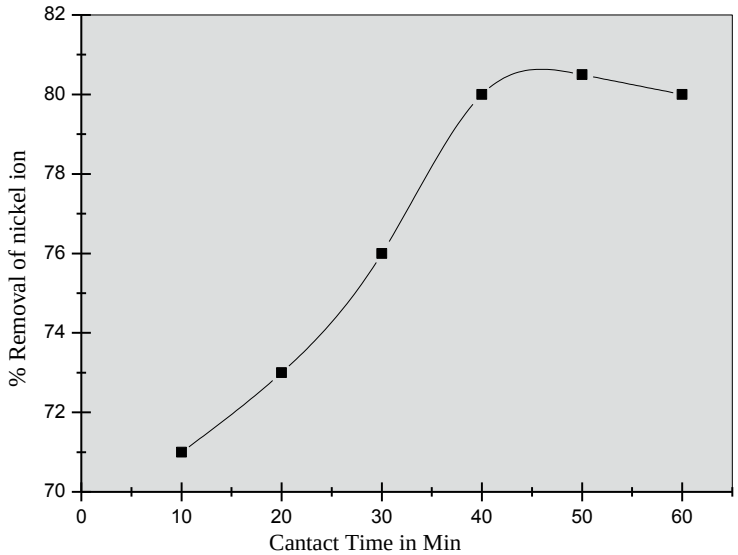
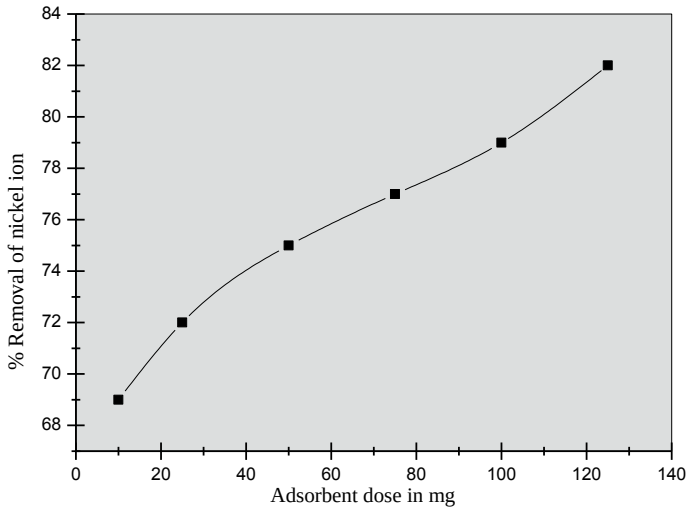
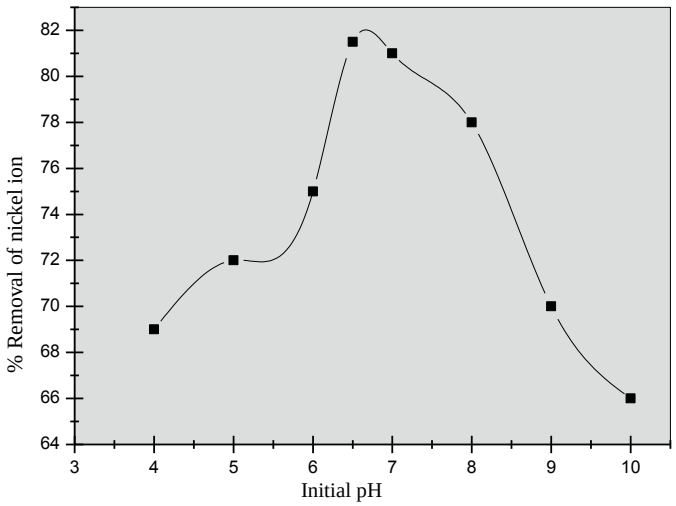


Fig:1- Effect of contact time on the removal of nickel ion
[Ni]=20 mg/L;Temprature 30°C;Adsorbent dose=25mg/50ml

http://dx.doi.org/10.22161/ijeab/2.1.4 *ISSN: 2456-1878*



Fig; 2- Effect of Adsorbent dose on the removal of nickel ion
[Ni]= 20mg/L; Contact time =60 min; Temprature 30^0C



Fig;3- Effect of initial pH on the removal of nickel ion
[Ni]= 20 mg/L;Temprature 30^oC; Adsorbent dose=25mg/50ml

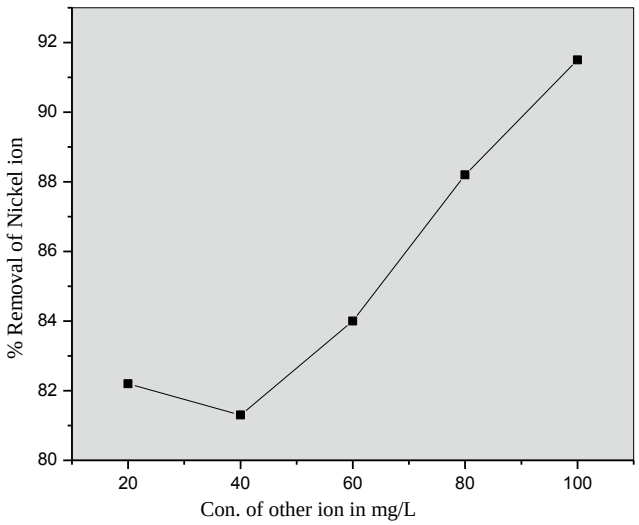


Fig.4-Effect of ionic strength on the adsorption of nickel ion
[Ni]= 20 mg/L;pH=6.5; Adsorbent dose=25mg/50 ml

http://dx.doi.org/10.22161/ijeab/2.1.7 *ISSN: 2456-1878*

Quantitative Analysis of some Germplasms of lablab Bean in Uttar Pradesh

Arvind Kumar

Department of Botany, Raja Balwant Singh College, Khandari, Agra, U.P., India

Abstract— *Lablab purpureus (L.) sweet is an ancient multipurpose legume that combines use as human food and forage in addition to serving as a cover crop for soil conservation. The crop is believed to be cultivated in south India as early as 1400 – 1500 BC. Although wide variability for agro-morphological traits exists in India, a more extensive germplasm collection and evaluation has not been reported so far. Hence the present study was undertaken with a set of 50 lablab accessions mainly collected from south India including nine accessions of exotic origin. All the 50 accessions were characterized for 29 qualitative and 10 quantitative traits. Further, there exists very high genetic differentiation between the exotic and the lines of Indian origin as also evident from biplot and scatter plot analysis. Although the exotic lines deviated for the Indian lines for majority of the traits, much of the useful variation for genetic improvement of vegetable traits existed among the Indian accessions while, the exotic lines possessed traits of forage importance.*

Keywords— Lablab purpureus, genetic structure, exotic accessions, genetic variability.

I. INTRODUCTION

Lablab bean or field bean or dilichos bean (*Lablab purpureus* L. sweet) is one of the ancient multipurpose crops widely distributed in India. Africa and South East Asia (Maass et al.2005). The crop is predominantly grown for grain, vegetable and fodder. Studies in *Lablab* have shown that the perennial types have considerable genetic and morphological diversity. For its use as pulse, white or cream coloured seeds are preferred over dark seed types. The most preferred types for vegetable are long pods, bold seeded with high pod fragrance. Cultivars with high biomass yield and drought tolerance are mostly suited for forage purposes. The crop has also been used as garden plant in USA for generations due to its beautiful dark green purple veined foliage with large spikes clustered with deep violet and white pea like blossoms. Lablab bean is better adapted to drought conditions as compared to common bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*). The Rongai cultivar was derived from Rongai distric of Kenya and released in Australia in 1962 (Wilson and Murtagh 1962) the crop is adaptable to diverse climate conditions such as arid, semi-arid, sub-tropical and humid conditions. In India, it is concentrated in the peninsular region and is cultivated in Karnataka, Tamil Nadu, Maharashtra and Andhra Pradesh.

Sufficient agro-morphological variation for yield and its attributes is found in Indian accessions but molecular diversity studies conducted with a set of 62 Indian accessions using AFLP and EST-SSR markers revealed narrow genetic diversity (Venkatesha *et al*.2007). Substantial variation for adro-morphological traits among lablab accessions was reported (Uddin and Newaz 1997, Mahadevu and Byre Gowda 2005, Islam 2008, Savitha and Ravikumar 2009, Girish and Byregowda 2009, Mohan et al.2009, Latha et al.2009, Upadhyay and Mehta 2010). Research information on this crop is not widwspread and scattered in a limited number of journal and reports. Despite its wide distribution in the Asian and African countries it is still considered a neglected crop with underused potential. For use as a legume, vegetable or forage crop, an understanding of available genetic diversity is the first step for future crop improvement. Information on genetic diversity and population structure is pre-requisite for planning breeding programs as it assists in choosing of divergent parents for crossing, providing more rational basis for expanding genes pool and for identifying genotypes that harbor valuable genes for its incorporation in breeding programs. Moreover, understanding the genetic structure in the populations facilitates appropriate genetic conservation strategies (Bhosale et al.2011). a better utilization and a fuller exploitation of collected materials required better knowledge of the variability existing among the collections. Therefore the present study was aimed at accessing patter of variation among the 50 Indian and exotic accessions for 10 quantitative and 29 qualitative traits, genetic structure among the accession and to identify trait relationship for important yield attributing traits.

II. MATERIALS AND METHODS

2.1. Genetic materials

A total of 50 lablab accessions were used in the present study. All the 50 germplasm accessions were raised in

http://dx.doi.org/10.22161/ijeab/2.1.7 *ISSN: 2456-1878*

augmented design at the R. B. S. College, Agriculture Dept. Bichpuri, Agra, Uttar Pradesh, India. Each accession was sown as single rows of 3 M length with inter row spacing of 60 cm and 15 cm between plants. All recommended package as per the requirement of the crop was adopted. Observations were recorded on 29 qualitative characters (table 1) and 10 quantitative characters (days to 50% flowering, plant height (cm), pods per plant, green pod yield per plant (g), dry seed yield per plant (g), days to maturity, seeds per pod, 100 seed weight (fresh and dry), shall recovery percent (dry) based on the descriptors provided by the International Plant Genetic Resources Institute.

III. RESULTS AND DISCUSSION

3.1 Frequency distribution for qualitative traits

3.1.1 Seedling and plant characteristics

The frequency distribution for 29 qualitative traits is given in table 1. The emerging cotyledon colour was observed green in 40 (13.08%) accessions and white in 10 (1.92%) accessions. The hypocotyl colour was observed to be white in all the 50 accessions. Majority of the accessions 38 (13.22%) were found to be non-pigmented, whereas, 8 (0.438%) extensively pigmented. In 3 (1.33%) accessions, the stem pigmentation was found to be almost solid and in 1 (0.12%) accessions, it was localized nodes. The growth habit was observed to be intermediate in 44 (14.60%) accessions while 6 (0.40%) were found to be determinate. With regard to branch orientation, 27 (11.33%) accessions possessed branches tending to be perpendicular to main stem while, 4 (1.33%) were found to have short and erect lateral branches.

3.1.2 Leaf characteristics

The vein colour of fully developed primary leaves was observed to be green in 48 (13.50%) of the individuals while, 2 (1.50%) recorded purple colour. The leaf anthocyanin was absent in all the 50 accessions studied. Leaves of almost half the number of accessions 22 (7.23%) were pale green while, 28 (7.77%) were observed to be green. High variation was observed for leaf hairiness. The number of accessions belonging to glabrous group were 20 (5.58%) while, 26 (8.39%) individuals possessed low pubescence and 4 accessions (1.03%) were moderately pubescent. A total of 45 (14.06%) accessions possessed round leaf shape, 2 (0.12%) ovate and 3 (0.82%) ovate-lanceolate. Leaf persistence was observed when 90% pods were ripened. A total of 45 (12.61%) accessions were classified under most leaves remaining category.

3.1.3 Flower characteristics

Wide variability was observed for flower bud colour. The flower bud colour recorded white in 2 (1.00%) accessions, cream in 39 (11.00%) accessions, light yellow in 1 (0.52%) accessions, pink in 6 (1.48%) accessions and purple in 2 (1.00%) accessions. The standard petal colour, wing petal colour and keel petal colour were white in 40 (13.06%) accessions, pink in 7 (0.94%) and purple in 3 (1.00%) accessions respectively.

3.1.4 Pod characteristics

The pod curvature was observed straight in 20 accessions, slightly curved in 20 accessions respectively. High variability was observed for pod pubescence, one of the most important character which attributes to resistance for insect pests. Maximum numbers of accessions (42) were found to be moderately pubescent, 1 were found to be pubescent and 7 accessions were found to be glabrous. Pod beak was recorded short in 7 accessions, medium in 4, long in 38 and thick in 1 accessions. Pod fragrance is one of the characters influencing consumer acceptability. Pod fragrance was estimated to be absent in 3 individuals, low in 7, medium in 30 and 10 accessions with high fragrance. With respect to constriction on the pods, most of the accessions (45) were slightly constricted while, 4 accessions constricted and 6 accessions have no constriction. The distribution of pod colour was 0.28% as white, 1.06% in cream, 12.26% in green, 0.56% in green with purple stature and 0.28% purple. Erect type of pod attachment was observed in 19 accessions, 30 with intermediate and 1 accession with pendent type. Pod attachment at maturity was found to be erect in 10 accessions, 35 with intermediate and 5 with pendant type.

3.1.5 Seed characteristics

Almost all the individuals (48) recorded green seed colour on fresh seeds, while fresh seeds of 1 and 1 accessions recorded cream and purple colour respectively. Oval seed shape (46) was found to be dominant over the round seed shape (4). Seed colour at maturity was observed to be green in maximum of accessions whereas, only 9 and 1 accessions recorded cream and white colour respectively. The dry seed colour was found to be highly variable as compare to the fresh seed colour. A total of individuals had 20 cream colour, 5 purple, 19 brown, 4 black and 1 green in colour. The seed shape of the dry seeds was classified as oval in a total of 25 accessions, round in 20 accessions; only one accession was classified under flat category. No variability was recorded for seed texture at maturity and all the accessions (50) were found to be moderately ridged.

IV. CONCLUSIONS

50 lablab accessions sown as single rows of 3 M length with inter row spacing of 60 cm and 15 cm between plants were observed for 10 quantitative characters (days to 50% flowering, plant height (cm), pods per plant, green pod yield per plant (g), dry seed yield per plant (g), days to maturity, seeds per pod, 100 seed weight (fresh and

dry), dhall recovery percent (dry) based on the descriptors provided by the International Plant Genetic Resources Institute.They were found to be different and are promising candidates for breeding.

REFERENCES

[1] Bhosale SU, Stich B, Rattunde HFW, Weltzien E, Haussmann BIG, Hash CT, Melchinger AE and Parzies HK (2011). Population structure in sorghum accessions from West Africa differing in race and maturity class. Genetic **139**:453-463.

[2] Das LDV (1990). Performance of forage accessions of Dolichos lablab (L.) Madras Agricultural Journal **77**:555-556.

[3] Earl DA (2011). Structural harvester v0.6.1. http://taylor0.biology.ucla.edu/structureHarvester/Accessed 5 Dec 2012.

[4] Elangaimannan R, Anbuselvam Y and Karthikeyan P (2008). Genetic diversityin blackgram [Vigna mungo 9L.0 hepper]. Legume Res **31**:57-59.

[5] Evanno G. Reagnaut S and Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a stimulation study. Molecular Ecology **14**:2611-2620.

[6] Falush D, Stephens M and Pritchard JK (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes **7**:574-578.

[7] Fuller DQ and Harvey EL (2006). The archaeobotany of Indian pulses: Identification, processing and evidence for cultivation. Environ Archaeol **11**:220-246.

[8] Fuller DQ, Korisettar R, Venkatasubbaiah PC and MK Jhones (2004). Early plant domestications in southern India: some preliminary archaeobotanical results. Vegetation Hist Archaeobot **13**:115-129.

[9] Girish G and Byre Gowda M (2009). Inheritance of qualitative characters in Dolichos bean Lablab purpureus L. Sweet. Environ Ecol **27**:571-580.

[10] Hartl DL and Clark AG (1997). Principles of population genetics. 3rd Edition. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, USA

[11] Islam MT (2008). Morpho-agronomic diversity of hyacinth bean (*Lablab purpureus* (L.) Sweet) accessions from Bangladesh. Plant Genetics Resourses Newsletter **156**:72-77.

[12] Kimani EN, Wachira FN and Kinyua MG (2012). Molecular diversity of Kenyan lablab bean(*Lablab purpureus* (L.) Sweet) accessions using amplified fragment length polymorphism marker. American J Plant Sci **3**:313-321.

[13] Lablab bean website (http://www/lablab.org)

[14] Latha M, Mani S, Lissy MB, Abraham Z, Asokan NR, Hanuman L and Mishra SK (2009). Collection and characterization of field bean (*Lablab purpureus* (L.) Sweet var. *purpureus*) Indian J Plant Genet Resou **22**:93-97.

[15] Liu CJ (1996). Genetic diversity and relationships among *Lablab purpureus* genotypes evaluated using RAPD as markers. Euphytica **90**:115-119.

[16] Maass BL, Jamnadass RH, Hanson J and pengelly BC (2005).determining sources of diversity in cultivated and wild *Lablab purpureus* related to provenance of germplasm by using amplified fragment length polymorphism. *Lablab purpureus* (L.) **52**:683-695.

[17] Maass BL, Knox MR, Venkatesha SC, Angessa TT, Ramme S and Pengelly BC (2010) *Lablab purpureus* – A crop lost for Africa? Tropical Plant Biology **3**:123-135.

[18] Mahadevu P and Byre Gowda M (2005). Genetic improvement of Dolichos bean (*Lablab purpureus* (L.) Sweet) through use of exotic and indigeneous germplasm. Indian J Plant Genetic Resources **18**:47-48.

[19] Mohan N, Aghora TS and Devraju (2009). Evalution of dolichos (*Lablab purpureus* L.) germplasm for pod yield and pod related traits. J Hort Sci **4**:50-53.

[20] Nadarajan N and Gunasekaran M (2005). Basic principles In: Quantitative genetics and biomaterical techniques in plant breeding. Kalyani Publishers, New Delhi.

[21] Prakash P, Venkatesha SC, Ashok TH, Gowda TKS, Byre Gowda M (2009). Genetic diversity in field bean as revealed with AFLP markers. J Food Legumes 22:18-22

[22] Pengelly BC and BL Maass (2001). *Lablab purpureus* (L.) Sweet- diversity, potential use and determination of a core collection of this multi-purpose tropical legume. Genetic Resources Crop Evol **48**:26-272.

[23] Pritchard JK, Stephens M and Donnelly P (2000). Inferences of population structure using multilocus genotype data. Genetics **155**:945-959.

[24] Savitha BN and Ravikumar RL (2009). Comparative analysis of phenotypic and molecular diversity in selected pendal and non-pendal genotypes of field bean [*Lablab purpureus* (L.) Sweet]. Indian J Plant Genetic Resources **69**:232-236.

[25] STRUCTURE software pakage (http://pritch.bsd.uchicago.edu/structure.html)

[26] STRUCTURE Harvester online program (http://taylor0.biology.ucla.edu/structureHarvester)

[27] Uddin MS and Newaz MA (1997). Genetic parameters and the association among flower and pod

characteristics of hyacinth bean. Legume Res **20**:82-86.

[28] Upadhyay D and Mehta N (2010). Biometrical studies in Dolichos Bean (Dolichos lablab L.) for Chhattisgarh plains. Research J Agric sci **1**:441-447

[29] Ushakumari R and chandrasekharan P (1992). Genetic variability and correlation studies in fodder lablab (*Lablab purpureus* (L.) Indian J Genet plant Breed **52**:169-173.

[30] Venkatesha SC, byre Gowda M, Mahadevu P, Mohan Rao, Kim DJ, Ellis N and Knox MR (2007). Genetic diversity within *Lablab purpureus* and the application of gene specific marker from a range of legume species. Plant Genetic resour **5**:154-171.

[31] Whitbread AM, Pengelly BC (eds) (2004). Tropical legumes for sustainable farming systems in southern Africa and Australia. ACIAR Proc.no.115, Canberra, Australia, Pp 180.

[32] Wilson G P, Murtagh GJ (1962) Lablab - New forage crop for the north coast. New south Eales agricultural Gazette **73**:460-462.

[33] Wright S (1978) Evolution and the genetics of populations vol 4. Variability within and among natural populations, University of Chicago Press, Chicago, Pp 580.

Table.1: Characterization of lablab accessions using descriptors provided by the international plant Genetic resources Institute.

S.No	Character	State of Expression	Score	No. of accessions	Frequency
1	Emerging cotyledon colour	White	1	10	1.92
		Green	2	40	13.08
		purple	3	0	0.00
2	Hypocotyl colour	White	1	50	15.00
		green	2		0.00
3	Stem pigmentation	No pigmentation	0	38	13.2
		Localized to nodes	3	1	0.12
		Extensive	5	8	0.438
		Almost solid	7	3	1.33
4	Vein colour (fully developed primary leaves, on inner face)	Green	1	48	13.50
		Purple	2	2	1.50
5	Leaf anthrocyanin	Absent	0	50	15.00
		present	2		0.00
6	Leaf colour	Pale green	1	22	7.23
		Green	3	28	7.77
7	Leaf hairiness	Glabrous	0	20	5.58
		Low pubescent	3	26	8.39
		Moderately pubescent	5	4	1.03
		Highly pubescent	7	0	0.0
8	Leaf shape	Round	1	45	14.06
		Ovate	3	2	0.12
		Ovate-lanceolate	5	3	0.82
		Lanceolate	7		0.0
		Linear- lanceolate	9		0.0
9	Leaf persistence (when 90% pods are ripe)	Few leaves remaining	3	45	12.61
		Intermediate	5		0.00
		Most leaves remaining	7	5	2.39
10	Growth habit	Determinate		6	0.40
		Semi-determinate	2		0.00
		Indeterminate	3	44	14.60
		Others (specify)	4		0.00

http://dx.doi.org/10.22161/ijeab/2.1.7 *ISSN: 2456-1878*

11	Branch orientation	Short and erect lateral branches	3	4	1.33
		Branches tending to be perpendicular to main stem, medium in length	5	27	11.33
		First lateral branches long and spreading over ground	7	19	2.34
12	Flower bud colour	White	1	2	1.00
		Cream	2	39	11.00
		Light yellow	3	1	0.52
		Pink	4	6	1.48
		purple	5	2	1.00
13	Standard petal colour	White	1	40	13.06
		Cream	2		0.00
		Light yellow	3		0.00
		Pink	4	7	0.94
		purple	5	3	1.00
14	Wing petal colour	White	1	40	13.06
		Cream	2		0.00
		Light yellow	3		0.00
		Pink	4	7	1.00
		purple	5	3	0.94
15	Keel petal colour	White	1	40	13.06
		Cream	2		0.00
		Light yellow	3		0.00
		Pink	4	7	1.00
		purple	5	3	0.94
16	Pod curvature	Straight	0	20	5.10
		Slightly curved	3	20	4.00
		Curved	5	10	5.90
17	Pod pubescence	Glabrous	0	7	1.00
		Moderately pubescent	3	42	13.39
		Pubescent	5	1	0.61
18	Pod beak	Short beak	1	7	3.00
		Medium beak	2	41	0.66
		Long beak	3	38	11.22
		Thick beak	4	1	0.12
19	Pod fragrance	Absent	0	3	0.38
		Low	1	7	3.56
		Medium	2	30	10.06
		high	3	10	1.00
20	Pod constriction	No constriction	0	1	0.78
		Slightly constricted	3	45	13.22
		constricted	5	4	1.00
21	Pod colour	White	1	1	0.28
		Cream	2	3	1.06
		Green	3	43	12.26
		Green with purple suture	4	1	0.56

http://dx.doi.org/10.22161/ijeab/2.1.7 *ISSN: 2456-1878*

		Purple	5	1	0.28
		Dark purple	6	1	0.56
		Red	7		0.00
22	Pod attachment	Erect	1	19	6.78
		Intermediate	2	30	7.94
		Pendent	3	1	0.28
23	Pod attachment at maturity	Erect	1		3.11
		Intermediate	2	35	10.28
		Pendent	3		1.61
24	Seed colour (fresh)	Green	1	48	14.16
		Cream	2	1	0.56
		Purple	3	1	0.28
		Brown	4		0.00
		Black	5		0.00
25	Seed shape (fresh)	Round	1	4	3.06
		Oval	2	46	11.94
		Flat	3		0.00
		Others (specify)	4		0.00
26	Seed colour at maturity	White	1	1	0.56
		Green	2	40	10.28
		Cream	3	9	4.72
		Purple			0.00
		Brown			0.00
		Black			0.00
27	Seed colour (dry)	Green	1	1	0.83
		Cream	2	20	4.61
		Purple	3	5	1.17
		Brown	4	19	6.28
		Black	5	4	2.11
28	Seed shape (dry weight)	Round	1	20	6.78
		Oval	2	25	8.22
		Flat	3	1	0.28
		Others (specify)			0.00
29	Seed texture at maturity	Smooth	3		0.00
		Moderately ridged	5	50	15.00
		Markedly ridged	7		0.00

http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*

Fertility of agricultural soils in the area of Jorf Lasfar (El Jadida-Morocco)

K. Moustarhfer[1], N. Saber[1], H. Mohcine[1], C. Marrakchi[2]

[1] Laboratory of geology of old chain, Department of Geology, Faculty of Sciences Ben M'sik, University Hassan II of Casablanca, Morocco.

[2] Laboratory of applied geology, geomatics and environment, Department of Geology, Faculty of Sciences Ben M'sik , University Hassan II of Casablanca, Morocco.

Abstract— *The Jorf Lasfar area has known for some decades a remarkable change in lands use. In fact, the installation of the port of Jorf Lasfar, the phosphate complex and an industrial area allowed the invasion of agricultural soils and influenced their fertility. The present work aims to assess the soil fertility through some physico-chemical parameters such as (pH, OM, $CaCO_3$, Total Nitrogen, phosphorus and potassium); the soil samples were carried out according two horizons [0-2.5cm] and [2.5-10cm] in fifty-three points during two agricultural years 2014 and 2015. The main results indicate that the pH ranges from 7.32 to 8.7; the organic matter content is often higher than 3%. Nutritive elements (Total Nitrogen, phosphorus and potassium) record values that respectively vary from 0.001 to 0.31%, 100.15 to 261.62ppm and 102.36 to 672.17ppm. The high rate of phosphorus is probably due to the excessive use of fertilizers or to the nearby phosphate processing plant.*

Keywords— Soils fertility, pH, Organic Matter, Nutrients elements, Jorf Lasfar.

I. INTRODUCTION

Under a Mediterranean climate, Morocco experiences climatic variations which generally translated by inter and intra annual irregular rainfall. Morocco is now the cereals importer while in the beginning of the last century was exporter.

Doukkala region by the quality of its agricultural lands was a suitable area for the production of cereals in a non-irrigated environment but over time we notice a decline in the productivity despite the efforts at widespread mechanization, the use of selected seeds and the recommendations of fertilization [1,2,3] In the area of Jorf Lasfar (coastal Doukkala), lands which are destined for cereal crop experience nowadays a remarkable urban and industrial invasion which influenced in a negative way the cereal yields.

In fact, with climatic constraints added to bad agricultural practices in which the soil fertility is affected by intensive work that caused by the destabilization of the cycles of principle nutrients (nitrogen, phosphorus and potassium). These essential elements of the development of the plant that are present in the soil [4] allow attributing a fertilising power. However, these mineral elements present in insufficient quantities will influence the optimal crop growth, where the necessity for a rationa and reasoned use of agricultural inputs in these elements. The fertilizing potential mainly depends on soil properties and their interactions [5]. [6] In fact, according to these authors, the maintain of the fertilizing potential of a soil is determined by a combination of several parameters, knowing, its structure and texture, the presence of the OM and the dynamics and the availability of minerals from which the main objective of this work is the evaluation of the fertility of agricultural soils of Jorf Lasfar area through the fertilizer nutrients pH, OM, Total Nitrogen, Phosphorus and Potassium.

II. MATERIALS AND METHODS

2.1. Description of the study area:

The study area is part of the Doukkala plain that belongs to the Western Meseta. It is located about 24 km from the Southwest of El Jadida city and about 110 km from Casablanca city. It extends over a length of 22 Km and a width of 15 km, an area of 300 Km^2. It is bordered to the Northeast by Moulay Abdallah, to the South-West by Sidi Abed and to the North and to the West by the Atlantic Ocean (Fig. 1). The area is characterized by an important demography (850000 habitants, census of 2004). The economic activities are mainly industrials (phosphates, thermal power, metallurgy...) and secondarily agricultural (market gardening, cereal ...).

The climate of the region is semi-arid Mediterranean, characterized by a fresh and humid winter and a hot and dry summer. The annual average temperature is 17.7°C, with a maximum of 21°C and a minimum of 14°C. The annual average rainfall is about 389 mm, the rainiest months are November, December and January, from 68 to 75 mm and the less rainy months are June, July, August and September (Meteorology station of El Jadida). The dominant winds are often oriented South-West to

http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*

Northeast. The Jorf Lasfar site is part of the Moroccan Western Meseta (specifically coastal Meseta) characterized by tabular regim of Mesozoic and Cenozoic deposits that overcome angular unconformity the formations of Paleozoic basement affected by the Hercynian orogeny [7].

The study area is characterized by Cenomanian age deposits consist calcareous sandstone rich in shells prints, with the alternation of argillaceous limestone and marl benches. The whole is topped by conglomerates that passelocally to yellow or red sands of Plio-quaternary. The Pliocene and the Quaternary are associated and make large superimposed dunes.

2.1. Sampling of soils and analysis techniques:

A sampling of 53 samples was carried out during two crop years. They have been realized by using an auger on two horizons [0-2.5cm] and [2.5-10cm].These sampling concerned plots destined for non-irrigated cereal crops (Fig. 1). After a drying in open air for at least 5 days, grinding by using an agate mortar and then sieving through a sieve of 2 mm, we have carried out the following analysis:

The determination of the texture was carried out by the Robinson pipette method at the Agronomy and Veterinary Institute of Rabat. The dosage of nutrients elements TN, P_2O_5 and K_2O were performed at the National Institute of Agricultural Research of Settat (soil fertility and plant nutrition laboratory) and at the Analysis Center at the Department of Geology (Faculty of Sciences Ben M'sik). They focus on the analysis of organic matter by [8], which consists of a cold oxidation of the organic fraction of carbon by potassium dichromate; the pH analysis by the glass electrode in a solution ½ soil / water by the method of [9]; the measurements of calcium carbonates were determined by volumetry according to Bernard's method described by [10]; the electric conductivity was measured by the method of [11]; the phophorea analysis was carried out by the method of [12] wherein the extraction is carried out by sodium hydrogen carbonate to pH = 8.5. This method is based on the formation and reduction of a complex by ortho phosphoric acid and molybdic acid (sky blue coloration); the potassium and the total nitrogen were measured respectively by the method of [13] and [14].

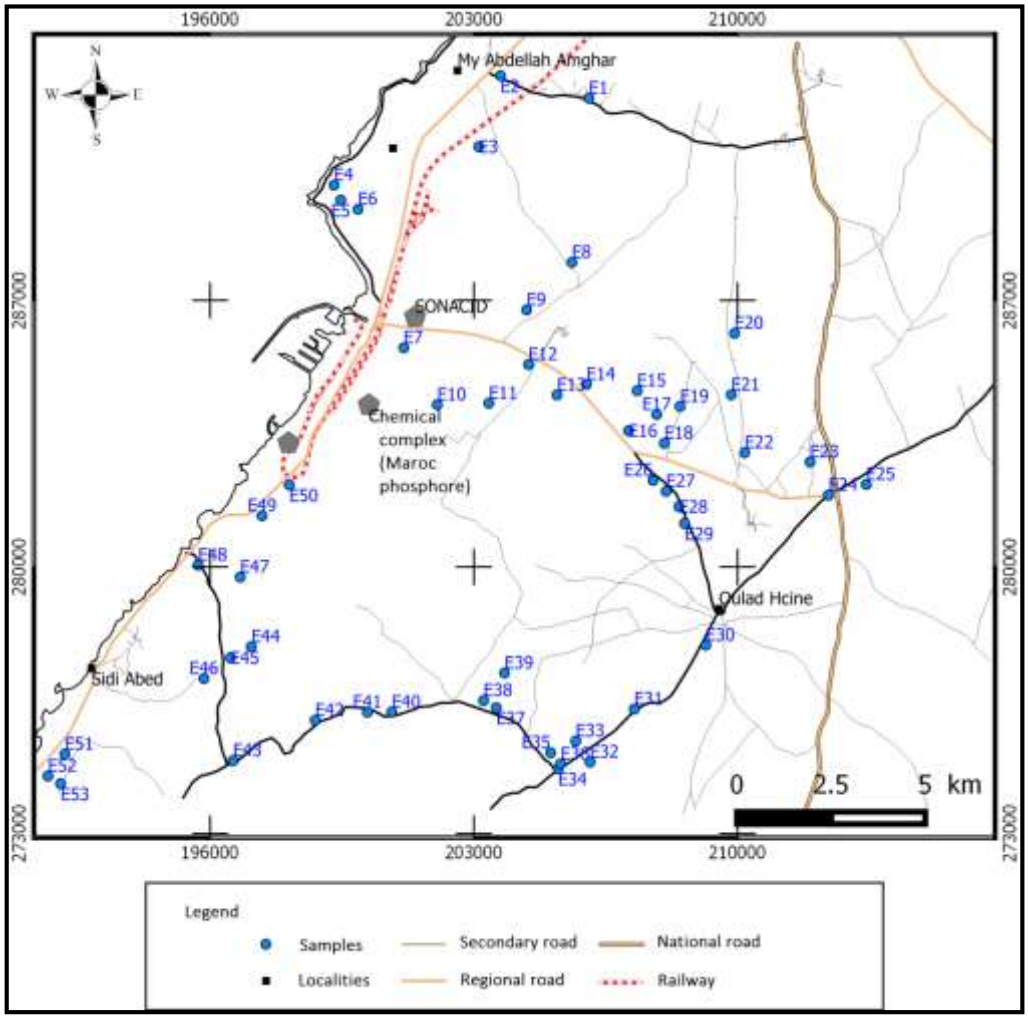


Fig. 1: Location of the sampling points

http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*

2.3. Treatment and data analysis:

The statistical analysis was used in order to detect the significant correlations between the different parameters with XLSTAT 2015 software. The spatial variations of the physicochemical parameters of soils are determined based on the geographic information system (GIS).

II. RESULTS AND DISCUSSION

3.1. Physico-chemical characterization:

The textural analysis of the soils studied revealed that the majority of the analyzed agricultural soils are classified among the limono-sandy-clay soils to sandy-clay with a percentage of clay that hardly exceeds 35% and a percentage of sand to approximately 51% (Fig. 2).

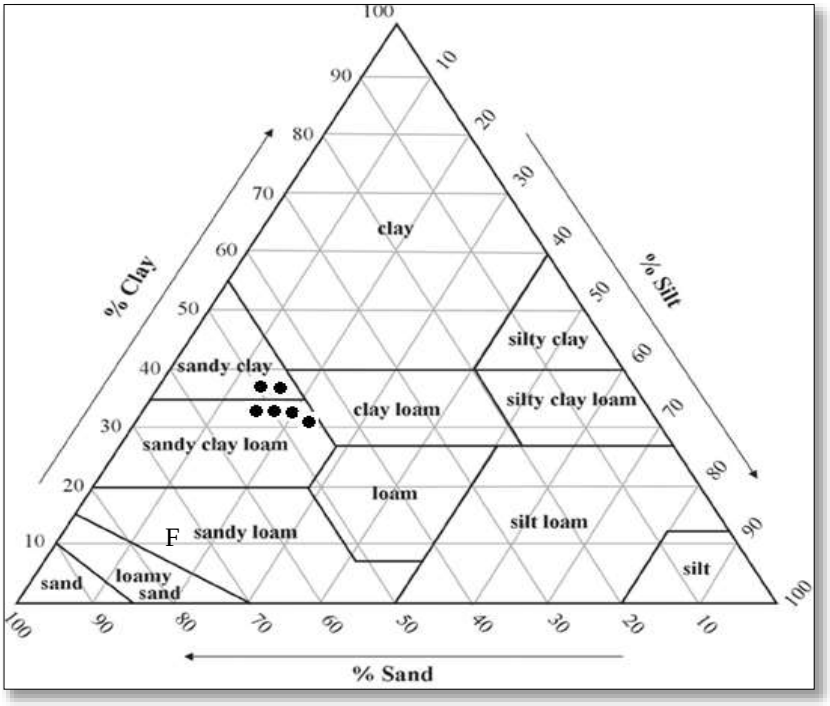


Fig. 2: Particle size classification of soil samples

The tables 1 and 2 present the descriptive statistics of the results of physico-chemical parameters of two horizons [0-2.5cm] and [2.5-10cm].

The pH values show that the majority of soils are moderately alkaline to basic tendency. They vary from 7.32 to 8.52 with a mode around 8.12 for the horizon of the surface and they range from 7.48 to 8.70 with an average of 8.24 for the horizon of depth. The rate of the organic matter is significantly higher in the horizon [2.5-10cm] even in the surface horizon. The contents recorded at this last range from 0.46 to 5.87%, unlike those registered in depth ranging from 0.77 to 6.49%. The $CaCO_3$ values don't show any significant differences between these two horizons, according to the distribution of limestone rate of the international standard NF ISO 10693 [15], the soils of this region are few to moderately limestone.

The electrical conductivity of the soils sampled do not reveal any significant differences between the two horizons, it varies respectively from 0.1 to 0.74 mS/cm, (average = 0.24) and from 0.05 to 1.36 mS/cm (average = 0.21) in surface than depth. These values are lower than the agricultural standards cited by [16].

The phosphorus concentrations are variable and range between 104.31 and 261.62ppm for the horizon [0-2.5cm] and between 100.15 and 199.99ppm for the horizon [2.5-10cm]. Following the standards [17], the agricultural soils of the site studied present high phosphorus levels as compared to normal soils and they are higher in surface than depth. The potassium contents are higher in surface (60.1 to 672.17ppm) as those in deep (58.9 to 461.15ppm), according to the classification of [17], the analyzed soils manifest high levels to very high in potassium.

The analyzes revealed very low nitrogen contents, which are of the order of 0.08% in surface and 0.06% in depth. Noting that there was no significant difference between the two horizons [0-2.5cm] and [2.5-10cm].

http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*

Table.1: Descriptive statistics of physic-chemical parameters to the horizon surface [0-2.5cm]

	pH	TOM (%)	$CaCO_3$* (%)	EC* (mS/cm)	P_2O_5 (ppm)	K_2O (ppm)	TN* (%)
Maximum	8,52	5,87	32,11	0,74	261,62	672,17	0,31
Minimum	7,32	0,46	0,19	0,1	104,31	60,1	0,001
Mean	8,12	2,96	5,24	0,24	177,33	285,61	0,087
Td. deviation	0,21	1,39	7,47	0,13	29,11	145,57	0,077

Table.2: Descriptive statistics of physic-chemical parameters to the horizon of depth [2.5-10cm]

* TOM = Total Organic Matter; EC = Electrical Conductivities; TN = Total Nitrogen.

	pH	TOM (%)	$CaCO_3$* (%)	EC* (mS/cm)	P_2O_5 (ppm)	K_2O (ppm)	TN* (%)
Maximum	8,7	6,49	33,94	1,36	199,99	461,15	0,23
Minimum	7,48	0,77	0,16	0,05	100,15	58,9	0,002
Mean	8,24	3,04	5,19	0,21	152,21	189,94	0,06
Td. deviation	0,26	1,41	7,26	0,2	24,64	100,99	0,045

3.2. Spatial variation of physico-chemical parameters:

The spatial distribution maps of different parameters of the two horizons [0-2.5cm] and [2.5-10cm] are reported in the figures (3 to 9).

- **pH:**

According to the pH distribution maps (Fig. 3a and 3b), the pH of the soils studied shows significant spatial variation. It is generally a homogeneous alkaline trend throughout the study area, and those for the two horizons [0-2.5cm] and [2.5-10cm].

The Alkaline trend of these soils may be associated with the chemical composition of the original material (limestone) that plays a role in the valuation of nutrients elements in the rhizosphere. It can also be due to the close relationship between annual rainfall and pH: more the rain is important more the soil is acidic. Remembering that the study area is characterized by a semi-arid climate with low rainfall which would explain in part the pH values found. High pH values were already reported in soils with high content of carbonates [18, 19, 20, and 21].

- **Organic Matter:**

The figure 4 (a and b) shows the spatial distribution of the organic matter in the study area. From this figure, the North zone and the South-West zone are characterized by high contents of organic matter exceeding 3%. However, the Northeast zone and Southeast manifest low contents of OM do not exceed 3%. These results show that the studied soils are fairly rich in organic matter and they are comparable with those found by [22] in the Jorf Lasfar region where the organic matter ranges from 1.9 to 6.54%. These high rates can be related to the role which the type of cereal rotation acts as [23] proposed, where they indicate that the rotation (wheat-wheat) allows a high rate in OM.

- **Calcium carbonates:**

From the figure 5 (a and b), the samples showing the highest contents of calcium carbonates are those located in the North-West and in the South-West of Jorf Lasfar. In fact, the spatial distribution of these concentrations presents a remarkable variability, registered rates at Sebt Ouald Douib are of the order of 1.19 and 0.91% in surface and depth respectively, while those recorded in North-East of Sidi Abed are of the order of 28.8 and 29.08%, as well as those revealed in the North-West of Moulay Abdellah attain 32.11 and 33.94%.

This variability is mainly due to the proximity of the mother rock and sampled horizon where the lithological nature of the rock (limestone Cenomanian) is responsible for high contents of $CaCO_3$ found. The relative similarity between the horizons [0-2.5cm] and [2.5-10cm] is due, especially to the precipitation of carbonates and to their insolubility in the soil [24]. This can be eventually added to tillage and the crops grown that make soils homogeneous.

- **Electrical Conductivities**

From the figure 6 (a and b), the distribution of the electrical conductivity do not show any remarkable spatial variation across the study site. The East zone and the Southeast zone show the lowest values compared from North to South-West. These results allow to suggest that the soils of the region are not affected by the problem of salinization despite the significant variation of the overall mineralization of groundwater that are not widely used in the irrigation of these soils [22].

- **Phosphorus (P_2O_5):**

The spatial distribution of phosphorus (P_2O_5) of the studied the site presents significant variations in the recorded concentrations (Fig. 7a and 7b). The agricultural

http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*

soils of the study area show high contents of P_2O_5 and they are higher in surface than in depth.

The highest concentrations are located in South-West of the industrial area where the P_2O_5 values could reach 261.62ppm and 199.99ppm respectively at surface and at depth. However, the phosphorus contents increase while going away from the phosphate chemical complex, toward South-West (toward Sidi Abed) and decrease toward the North and Northeast. This seems to indicate a probable influence whether to the clearances dust coming from the plant of Morocco Phosphorus III and IV. Knowing that according to climatological studies winds mainly flow from the phosphate industries to Sidi Al Abed. Whether to the massive amount of phosphorus present in the soil that will be probably linked to the richness of mother rock [25] or the excessive use of phosphate fertilizers in these agricultural fields .We can conclude that the most affected sectors match those located in South-West of the phosphate industry complex.

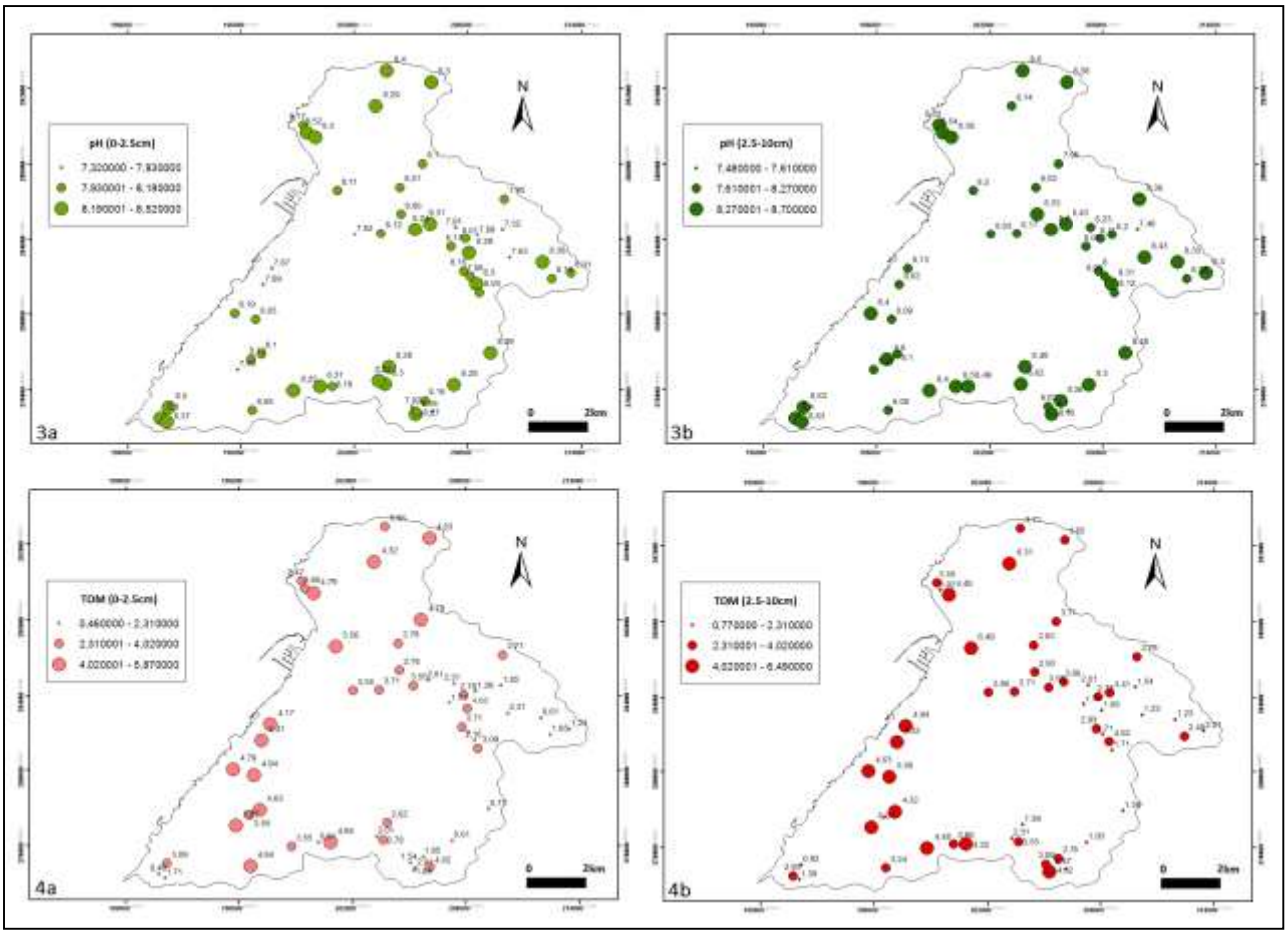
- **Potassium (K_2O):**

The figure 8 (a and b) show the spatial distribution of the potassium contents of the two horizons. The potassium concentrations are higher in the superficial samples than in depth samples. The distribution of these concentrations shows some variability throughout the study area. The potassium values are higher in the Northeast and South-West parts. These concentrations reflect the richness of these soils in this element (> 100ppm).

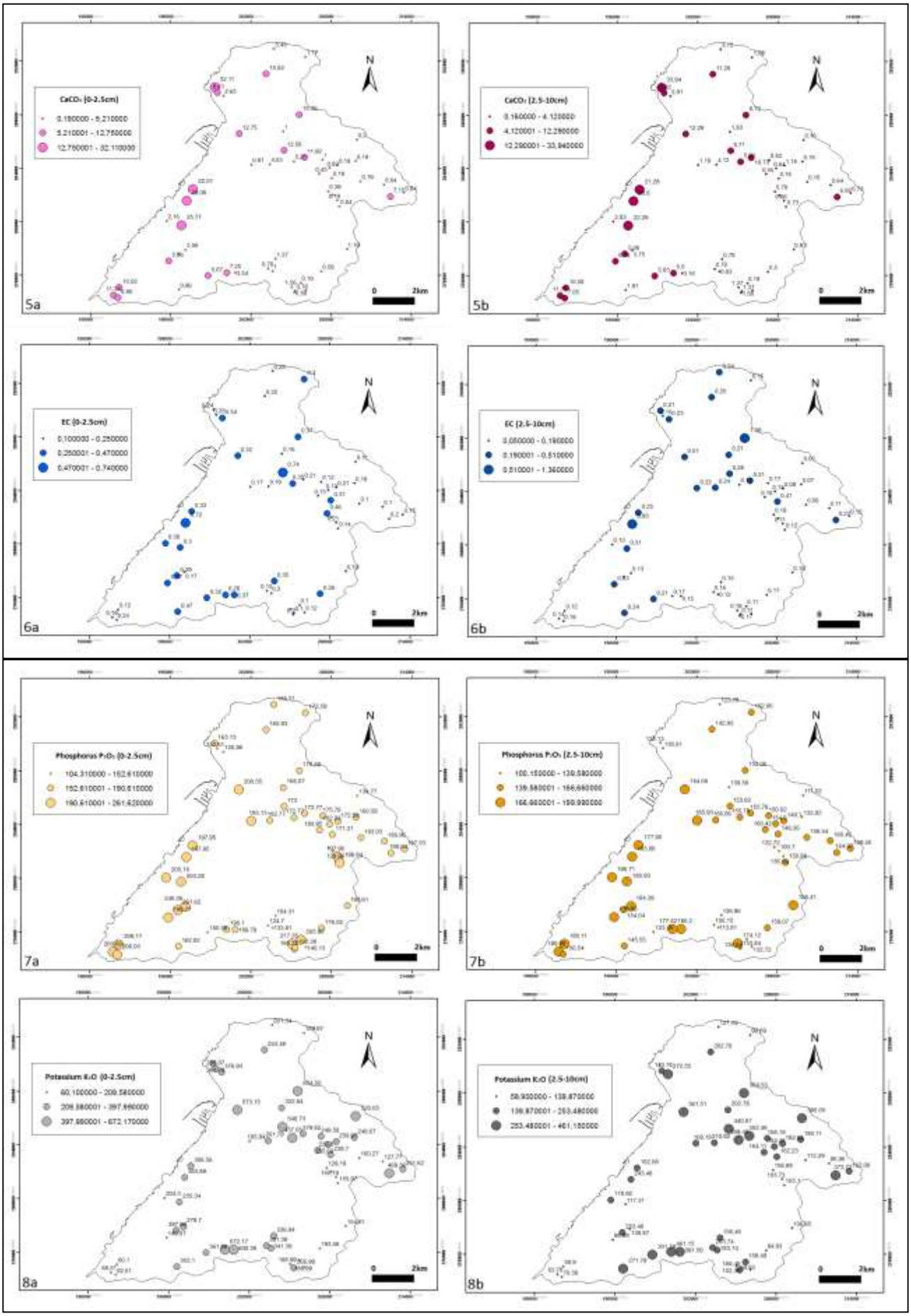
Moreover, these values are comparable to those obtained by [22]. Therefore, we can consider the potash status of cultivated land steady over the past 10 years. The richness of these potassium soils may be could be related to the use of potash fertilizers. In fact, the crops grown receive lots of fertilizers, including potash fertilizers.

- **Total nitrogen:**

The spatial distribution of total nitrogen of the region shows that there are no significant differences in the values recorded throughout the area (Fig. 9a and 9b). At the two horizons [0-2.5 cm] and [2.5-10 cm] the total nitrogen values vary respectively from 0.005 to 0.31 and from 0.002 to 0.23%, which shows that there is no significant variation between the two horizons. According to [26], the decrease in the quantity of N mineralizable can probably be linked to an increase in pH which promotes the mineralization of the organic matter and this by stimulation of the biological activity.



http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*



http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*

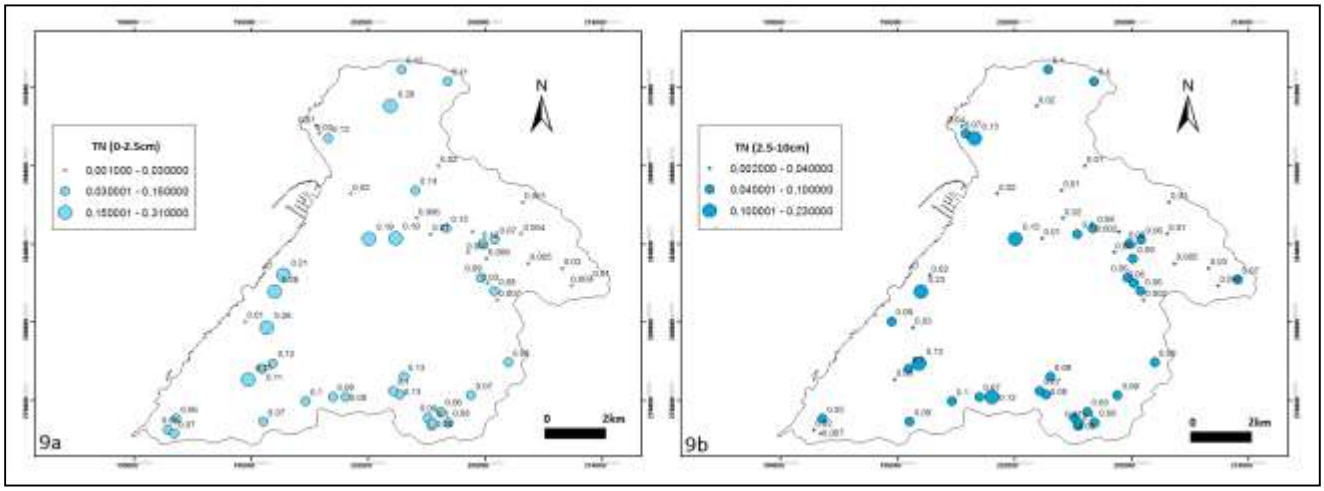


Fig. 3 to 9: Spatial distribution maps of different parameters of the two horizons [0-2.5cm] and [2.5-10cm]

3.3. Statistical analyzes of physico-chemical parameters:

- **Principal Component Analysis (PCA)**

To identify the relationships that may exist between soil parameters, seven variables were used for principal component analysis (PCA). Soil parameters refer to the results of physicochemical analyzes (pH, total organic matter, calcium carbonate, electrical conductivity, total nitrogen, potassium and phosphorus) of the two horizons [0-2.5cm] and [2.5-10cm] of soils. They are used in the analysis as active or explanatory variables.

a. Correlation between parameters and soils of the superficial horizon [0-2.5cm]

The information provided by the factorial axes is shown in Table 3. These results range from 2.24 to 0.38%, whether from 32.06 to 5.49%. The F1 axis alone accounts almost the information whether 32.06% of the total inertia. The other axes (F2, F3, F4, F5, F6 and F7) provide respectively 18.42%, 14.74%, 13.21%, 9.01%, 7.04% and 5.49%. The first four factorial axes explain about 78.45% of the total variability of the different active variables.

Table.3: Participation rate of each factorial axis in the preparation of the projection plans. Fn represents the factorial axes of the ACP

Fn	F1	F2	F3	F4	F5	F6	F7
Eigenvalue	2,245	1,290	1,032	0,925	0,631	0,493	0,384
% Variability	32,066	18,423	14,748	13,216	9,015	7,043	5,490
% Cumulative	32,066	50,489	65,237	78,452	87,467	94,510	100,000

The axis 1 alone accounts for 32.06% of the total variation of the dispersion matrix of individuals and axis 2 accounts for 18.42%. The first two axes (F1 and F2) define the principal plan; they bring about 50.49% of the information. On this plan that the important of the analysis has been established (Fig. 10). The axis 1 is characterized by OM, $CaCO_3$ and EC. The axis 2 is characterized by pH, K_2O and P_2O_5. This axis can be considered as the axis of soil fertility.

http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*

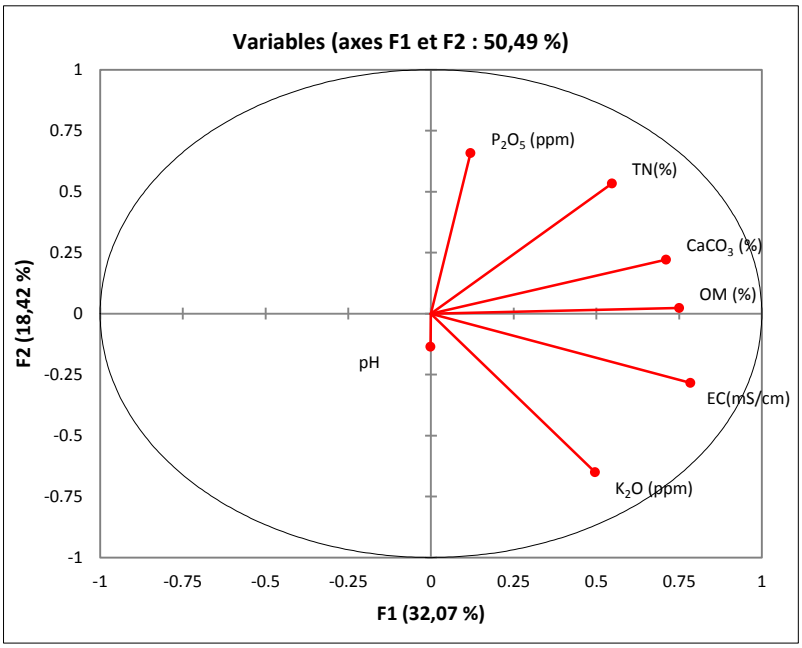


Fig. 10: Principal component analysis between superficial soil parameters on the principal plan (plan 1 - 2) of Jorf Lasfar

b. Correlation between parameters and soils of the depth horizon [**2.5-10cm**]

The figure 11 shows the results of the PCA of seven variables. The first two axes present respectively 29.29 and 16.89% of the information, whether a total of 46.18% of the total variability. The correlation matrix of the variables analyzed represents the correlation circle of the variables on the principal plan (plan 1 - 2). The first four factorial axes account for 74.82% of the total variability of the different active variables. The factorial axis F1 allows distinguishing five parameters $CaCO_3$, OM, K_2O, P_2O_5 and EC. The factorial axis F2 allows distinguishing two parameters: pH and TN.

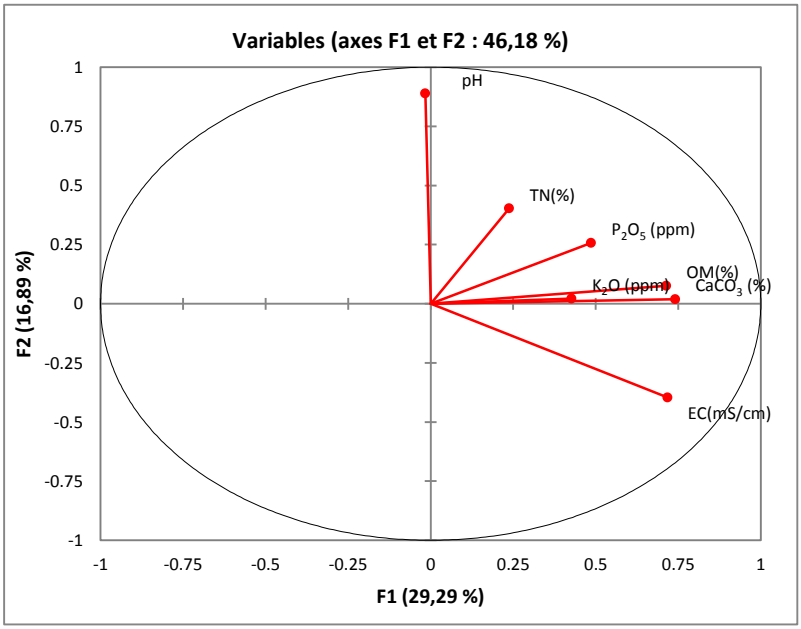


Fig. 11: Principal component analysis between depth soil parameters on the principal plan (plan 1 - 2) of Jorf Lasfar

http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*

IV. CONCLUSION

The objective of this work was the evaluation the fertility of agricultural soils in the Jorf Lasfar area through fertilizer elements: pH, OM, Total Nitrogen, phosphorus and potassium.

The results obtained from the analysis of the various samples taken from the different points of the study area allowed demonstrating:

The soils with limono-sandy-clayey to sandy-clayey texture with a moderately alkaline pH. The majority of the soils of the region are characterized by high levels of organic matter overcome largely 3%. The electrical conductivity remains below the agricultural standards which range of 4mS/cm, the $CaCO_3$ rates of these soils allowed to classify them among the soils that are moderately rich in limestones. The evaluation of the phosphorus and potassium soil contents shows that they have a richness of these elements in the whole study area studied.

REFERENCES

[1] P. Jouve, " Analyse des modèles de conduite des céréales et voies d'amélioration des rendements", Hommes, Terres, et Eau, Vol 9, No: 35, pp. 79-85, 1979.

[2] P. Jouve, F. Papy, " Les systèmes de culture dans les zones semi-arides du Maroc occidental ", RGM, NO 7, pp. 3-30, 1983.

[3] M. Ait Hamza, " Les céréales dans le Maroc du Centre-Ouest, Méditerranée", pp. 27-32, 1988.

[4] L. P. Delville, " Gérer la fertilité des terres dans les pays du sahel", pp. 397, 1996.

[5] D. Soltner, " Les bases de la production végétale. Tome I : sol 18 è édition ", Collection Science et Technique Agricole, pp. 467, 1990.

[6] V. Genot, G. Colinet, L. Bock, "La fertilité des sols agricoles et forestiers en région wallonne", Rapport analytique 2006 sur l'État de l'Environnement Wallon. Gembloux, Belgique : Laboratoire de Géopédologie, Unité Sol-Écologie-Territoire, Faculté universitaire des Sciences agronomiques, 2007.

[7] M. Fadili, " Etude hydrogéologique et géophysique de l'extension de l'intrusion marine dans le Sahel de l'Oualidia (Maroc) : analyse statistique, hydrochimie et prospection électrique", Thèse de Doctorat Es-Science, Univ. Chouaib Doukkali, pp. 15, 2014.

[8] A. Walkley, I. A. Black," An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents", Soil Sci. 63, pp. 251-263, 1934.

[9] E. O. Mc lead, "lime requirements. In : Page, A.L. et al. (Eds.), Methods of Soil Analysis, Part 2", second ed., Agronomy, vol. 9 Soil Society of America, Madison, WI, pp. 199-244, 1982.

[10] H. Chamley, " Guide des techniques du laboratoire de Géologie Marine de Luminy", pp. 198, 1966.

[11] J.D. Rhoades, D.L. Corwin, " Monitoring soil salinity", J. Soil and Water Cons., 39(3), pp. 173-175, 1984.

[12] S. R. Olsen, " Estimation of available phophorous in soils by extraction with sodium bicarbonate", Cir.U.S. Dep.Agr. 939, pp.1-19, 1954.

[13] E. Lakanen, R. Ervio, " A comparison of eight extractions for the détermination of plant available micronutrients in soils", ActrAgr.Fenn.123, pp. 223-232, 1971.

[14] Seal analytical, ''Total Kjeldahl Nitrogen in Acid Digests'', Method No. G-188-97, Rev. 6, 2008.

[15] D. Baize, "Guide des analyses courantes en pédologie : choix, expression, présentation et interprétation", INRA Editions, Paris, 1988.

[16] DIAEA /DRHA /SEEN, "Direction de l'irrigation et de l'aménagement de l'espace Agricole, Service des Expérimentations, des Essais et de la Normalisation –Rabat", 2008.

[17] A. Delaunois, Y. Ferrie, M. Bouche, C. Colin and C. Rionde, " Guide pour la description et l'évaluation de la fertilité des sols ", 2008.

[18] D. Wenming, G. Zhijun, D. Jinzhou, Z. Liying, and T. Zuyi, "Sorption characteristics of zinc (II) by calcareous soil", radiotracer study.Applied Radiation and Isotopes, vol. 54, pp. 371-375, 2001.

[19] R. Moral, A. Cortés, I. Gomez, J. Mataix-Beneyto, " Assessing changes in Cd phytoavailability to tomato in amended calcareous soils", Bioresource Technology, vol. 85, n° 1, pp. 63-68, 2002.

[20] M. Jalali, Z. V. Khanlari, "Effect of aging process on the fractionation of heavy metals in some calcareous soils of Iran", Geoderma, vol. 143, pp. 26-40, 2008.

[21] G. Sayyad, M. Afyuni, S. F. Mousavi, K. C. Abbaspour, B. K. Richards, R. Schulin, "Transport of Cd, Cu, Pb and Zn in a calcareous soil under wheat and safflower cultivation", a column study. Geoderma, vol. 154, pp. 311-320, 2010.

[22] P. El Hassnaoui, " Impacts environnementaux de la zone industrielle (province d'El Jadida) : Approche pluridisciplinaire et épidémiologie", Thèse de doctorat, Univ. Chouaib Doukkala, 2011.

[23] R. Mrabet, N. Saber, A. El-Brahli, S. Lahlou, F. Bessam, " Total, Particulate Organic Matter and Structural Stabihty of a Calcixeroll soi1 under different wheat rotations and tillage systems in a

http://dx.doi.org/10.22161/ijeab/2.1.8 *ISSN: 2456-1878*

semiarid area of Morocco", Soi1 & Tillage Res. 57, pp: 225-235, 2001.

[24] A. Ruellan, "Individualisation et Accumulation du Calcaire dans les sols et les dépôts quaternaires du MAROC", Cah. O.R.S.T.O.M., sér. Pédol., vol. V, n°4, 1967.

[25] P. M. V. Compos, "Contribution à l'étude des transporteurs de phosphate de la famille PHT1 chez le Peuplier (Populustrichocarpa Torr. & Gray) et le champignon ectomycorhizien Laccariabicolor (Maire) P. D. Orton", Thèse de doctorat. Biologie Végétale et Forestière. Nancy (France), pp. 141, 2008.

[26] R. L. Halstead, J. M. Lapansee, K. Irvason," Mineralization of soil organic phosphorus with particular reference to the effect of lime", Can. J. Soil Sci. 43, pp. 97-106, 1963.

http://dx.doi.org/10.22161/ijeab/2.1.9 *ISSN: 2456-1878*

Isolation and Pathogenicity Evaluation of Postharvest Fungal of Some Fruits in Cameroon

Yaouba Aoudou*, Mpounze Essoua Gaelle Phalone

Phytopathology Laboratory, Department of Plant Protection, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 222 Dschang, Cameroon

***Abstract*—** *The present work was designed to study the biodiversity of fungal post-harvest decay of banana, mango and safou fruits sold in local markets in the Dschang locality, Western Region of Cameroon. A total of 90 infected fruit samples were collected from different local markets, small pieces of mouldy part were inoculated on prepared plates of Potato dextrose agar (PDA), after 7 days of incubation, pure isolated fungi were identified according to the recommended references. The pathogenicity of the some most prevalent fungi isolated was evaluated on uninfected fruits. Results obtained showed some variations in isolation frequency of the fungi from each fruit. Aspergillus, Colletotrichum, Fasarium and Veticillium were the most common genera that colonized banana, mango and safou fruits with different incidences. Cercospora capsici was present on safou (50%), C. mangiferae on mango (50.9%) and C. musae on banana (51.7%). Colletotrichum gloesosporioides appeared on banana (8.62%), mango (15%) and safou (22.92%); Colletotrichum musae on banana (22%). Cercospora spp caused injuries with lesions diameters that vary depending on the type of fruit and fungal species. Proper measures should be adopted to protect fruits from fungal decay.*

***Keywords*—Fruits, Post-harvest fungi, Isolation, Pathogenicity, Western Region, Cameroon.**

I. INTRODUCTION

Fruits play an important role in human nutrition by contributing the necessary growth factors such as vitamins and essential minerals in human daily diet maintaining a good and normal health. It has been recognized that fruits are commercially and nutritionally important food product. Rot diseases caused by fungal pathogens provoke severe losses of agricultural and horticultural crops every year (Salman, 2005; Parveen *et al*., 2016). Fruits have wide distribution in nature. Tropical fruit production knows more and more increased with fresh bananas which was ranked 1st, with more than 145 million tons produced in 2011 globally (FAO, 2011). Cameroon's production of sweet banana in 2010 was 1 333 851 tons. The mango and the safou, despite their low production, also feature prominently after the banana. The relatively short shelf-life period provoked by pathogens is one of the most important limiting factors that impact the economic value of fruits. Approximately 20-25% of the harvested fruits are deteriorated by pathogens during post-harvest handling even in advanced countries (Droby, 2006). The postharvest losses are often more harsh in developing countries due to lack of storage and transportation facilities. Fruit infections by fungi may appear during the growth period, harvesting, handling, transportation and post-harvest stockpile and marketing conditions, or after procuring by the consumer. Fruits incorporate high levels of nutrients element and sugars and their low pH values make them exceptionally desirable to fungal decay (Singh *et al*., 2007). Fungi are considered as an essential post-harvest losses agent of different fruits, based on cultivar, season and production area amid other factors (Valiuskaite *et al*., 2006; Ewekeye *et al*., 2016). Fungi are the most crucial and common pathogens and the main cause of crop diseases. It Infect a wide range of fruits and vegetables during storage and transportation (Sommer, 1985).

The importance of post-harvest diseases is now recognized by fruits producers since serious losses occurred during the transit. In Cameroon, banana and mango are almost produced all over the country (MINADER, 2012). Fruits play an important role in socioeconomic. Surveys conducted by Hartill and Everett (2002), Everett *et al.* (2007) showed that anthracnose, stem rot, galls, fruit spot and fruit rot were the most important fungal diseases. The incidence of these diseases can be up to 90% in areas with high relative humidity (COLEACP, 2008). Little information is available on the fungi associated with some fruits in Cameroon. This study was aimed at isolating and identifying the fungi associated with post-harvest decay of bananas, mangoes and safou from different localities in the Dschang market, Cameroun.

II. MATERIALS AND METHODS

Collection of samples :

Ninety samples of infected and uninfected fruits were randomly collected from some markets in the city of Dschang in May 2016. Thirty samples of each the fruits

http://dx.doi.org/10.22161/ijeab/2.1.9 *ISSN: 2456-1878*

banana, mangos and Safou were collected. Samples were separately kept in clean plastic bags, transferred to the Phytopathology laboratory of the University of Dschang and stored in a refrigerator for mycological analysis.

Isolation and identification of fungi :

The direct plating technique described by Pitt and Hocking (1985) was employed. The fruit samples were surface sterilized for 3 minutes with 1% NaOCl and rinsed in four successive changes of sterile distilled water. Four small pieces from the margin of lesion of each sample were directly inoculated on prepared plates of Potato dextrose agar which contain (g/L): peeled potato100.0g, glucose 20.0g, agar 15.0g, water 1000.0 ml. The medium was supplemented with chloramphenicol (250 mg per liter) as a bacteriostatic agent (Smith and Dawson, 1944). The plates were inoculated at 28 ± 1 °C for 5 to 7 days. Three replicates were prepared for each sample. The resulting fungi were isolated, purified and identified according to their macro and micro characteristics.

Identification of fungal genera and species :

The pure isolated fungi were identified following the most documented keys in fungal identification (Raper and Fennell, 1965; Barnett and Hunter, 1972; Pitt, 1985, Moubasher, 1993; Alexopoulos and Mims, 1996; Klich, 2002; Agrios, 2005).

Pathogenicity test :

The pathogenicity test was done on apparently healthy mature fruits. The method of inoculation by wound of fruits was used Rivera-Vargas *et al*. (2006). The inoculated fruits were kept in laboratory conditions (22 ±2° C) for seven days. Data collected on the lesions developed by the fungus. For this test, the 3 species of *Cercospora* genus isolated from fruits were used, namely *C. capsici, C. mangiferae* and *C. musae*.

Statistical analysis :

Frequency occurrence of isolation of each fungus and diameters of lesions developed on fruits were calculated. Data obtained was analyzed statistically using SPSS (Version 17).

III. RESULTS AND DISCUSSION

The biodiversity of fungal species listed on Table 1 could be regarded as common post-harvest decay agents of various studied fruits. Through this investigation at 28 ± 2°C nine fungal species attributed to six genera were isolated. *Aspergillus, Cercospora, Colletotrichum, Fasarium* and Veticillium were the most common genera that colonized banana, mango and safou fruits with different incidences (Fig. 1). In which *Aspergillus* was represented by *A. niger*, *Cercospora* (3 species), *Fusarium* and *Verticillium* by one spece. *Cercospora* contained 3 species namely *C. capsici, C. mangiferae* and *C. musae. Fusarium* and *Verticillium genera* were represented by one specie for each namely *F. oxysporum* and *Verticillium albo-atrum. Cercospora* was by far the most common genus affecting the different kinds of fruits. It appeared on 50 % each of banana, mango and safou fruits (Fig. 1). *Aspergillus, Colletotrichum, Fusarium* and *Verticellium* were the second most common genus affecting these fruits. A. niger was found on banana (8.62%), and mango (15%) and safou (12%). *Colletotrichum gloesosporioides* appeared with variable incidences on banana (8.62%), mango (15%) and safou (22.92%). Other species showed higher affinity towards certain fruits such as *Rhizoctonia solani* on mango and safou fruits.

Pathogenicity of fungal species:

Table 2 shows the diameters of lesions caused by the 3 species of *Cercospora* genus on the fruits of banana, mango and safou. Different species of *Cercospora* caused injuries with lesions diameters that vary depending on the type of fruit and fungal species. It should be noted that all the types of fruits used presented lesions.

Developed lesions varied from 22 mm to 36 mm on banana fruit, from 19 mm to 45 on mango fruit and from 16 mm to 24 mm on safou fruit. *C. mangiferae* and *C. capsici* caused injury significantly greater than that caused by *C. musae* on bananas. On mango fruit, *C. mangiferae* and *C. musae* caused injuries to 45 and 36.5 mm respectively while that caused by *C. capsici* was 19 mm.

It should be noted that damage caused by fungi on the safou fruits were weak compared to those caused on the banana and mango. However the safou fruits showed more likely *C. capsici* with a lesion of 24 mm. *C. mangiferae* confirmed its pathogenicity to the mango fruits. Figure 2 presents some of the lesions caused by species of genus *Cercospora* on the fruits.

This investigation embraces an extensive survey of the fungi associated with post-harvest rot of fruits in samples collected from markets in Dschang. The tested samples comprised of banana, mango and safou fruits. In this respect, Akinmusire (2011) and Chukwuka *et al.* (2010) mentioned that fruits can be affected by a wide range of microorganisms such as fungi which have a serious threat to production of fruits. Spoilage attributed to any change in the condition of food makes it less palatable, or even toxic; these alterations may be accompanied by changes in taste, smell, appearance.

During the first part of this investigation, it was possible to isolate 9 species belonging to 6 fungal genera from the samples of fruits. Some of these fungi are reported by several authors to be commonly implicated in the postharvest deterioration of many fruits and vegetables in the Tropics (Hartil and Evertt, 2002; Everett *et al.*, 2005; Regnier *et al.*, 2010; Onyeani *et al*., 2012; Didy *et al*., (2013); Amadi *et al.*, 2014 ; Djeugap *et al*., 2015)

http://dx.doi.org/10.22161/ijeab/2.1.9 *ISSN: 2456-1878*

During this study, *Colletotrichum musae* was also isolated and it is known as the causative agent of banana rot. Scott, (2001) found that the two primary post-harvest rots of banana fruits in Hawai'i were crown rot and anthracnose caused by the fungus *C. musae*. Raut and Ranade (2004) and Ranasinghe *et al.* (2005) reported that, banana suffer from serious post-harvest losses caused by fungal infections, especially *C. musae*.

Colletotrichum spp have been reported to affect fruits, causing disease on immature and growing fruits in the field conditions, and damage fruits during transportation and storage (Wharton *et al.*, 2004).

Species such as *Cercospora musae* or *Mycophaerella musae*, *Cercospora mangiferae* and *Cercospora capsici* were the most frequent compared with other fungal species isolated. These three species are generally reported to cause significant damage to fruits.

A. niger, *R.solani*, *F. oxysporum* and *F. solani* were relatively less important on these in respect to their low isolation frequencies. These fungi however have been reported as pathogenic in some fruits including mango, apple, banana and grape in other part in the tropics (Kortsen *et al*., 1994; Bashar *et al.*, 2012). Several reports showed the implication of *A. niger* in spoilage of many fruits and vegetables (Bali *et al*., 2008; Tafinta *et al.*, 2013). The origin of fruit contamination by fungi is difficult to determine. Generally, contamination of agricultural product is a function of many factors including infestation in the field prior to harvest, handling during harvesting and methods of packaging and transportation of the product to the market (Amadi *et al*., 2014).

IV. CONCLUSION

Several fungal species belonging to 6 fungal genera could be regarded as the most common causes of post-harvest deterioration of banana, mango and safou fruits in the Dschang markets, Western region of Cameroon. Results suggested the need of developing appropriate management strategy to control post-harvest diseases caused by *Cercospora* spp, especially since their pathogenicity has been proven on these fruits.

ACKNOWLEDGEMENTS

The authors express their gratitude to all the members of the Phytopathology Laboratory of the Faculty of Agronomy and Agricultural Sciences for their kind collaboration.

Fig.1: Lesions caused by Cercospora spp on banana and safou fruits

REFERENCES

[1] Agrios, G.N., 2005. Plant pathology. 5th Ed. Elsevier Academic Press, California, USA, 922p.

[2] Akinmusire, O.O., 2011. Fungal Species Associated with the Spoilage of Some Edible Fruits in Maiduguri Northern Eastern Nigeria. *Advances in Environmental Biology*, 2011; 5(1): 157-161.

[3] Alexopoulos, C.J., C.W. Mims and Blackwell, M., 1996. Introductory Mycology. 4th Ed. Wiley, New York.

[4] Amadi, J.E., P. Nwaokike, G.S. Olahan and Garuba, T., 2014. Isolation and identification of fungi involved in the post-harvest spoilage of guava (*Psidium guajava*) in Awka Metropolis. *Inter J Eng Appl Sci*, 4: 7-12.

[5] Bali, R.V., Bindu, M.G., Chenga, R.V., Reddy, K., 2008. Post-harvest fungal spoilage in sweet orange (*Citrus sinensis*) and acid lime (*Citrus aurentifolia* Swingla) at different stages of marketing. *Agric. Sci. Digest*., 28:265-267.

[6] Barnett, H.L. and Hunter, B.B., 1972. Illustrated genera of imperfect fungi. 3rd Edition. Burgess Publishing Company, 200 p.

[7] Bashar, M.A., S. Shamsi and Hossain, M., 2012. Fungi associated with rotten fruits in Dhaka Metropolis. Bangladesh *J Bot*, 41: 115-117.

[8] Chukwuka, K.S., Okonko, I.O., Adekunle, A.A., 2010. Microbial ecology of organisms causing pawpaw (*Carica Papaya L.*) Fruit decay in Oyo State, Nigeria. *American-Eurasian J. Toxicol. Sci*., 2 (1): 43-50.

[9] COLEACP (Comité de Liaison Europe-Afrique/Caraïbes/Pacifique). 2008. Guide de bonnes pratiques phytosanitaires pour l'avocat (*Persea americana*) issu de l'agriculture biologique en pays ACP.

[10] Didy O., Odimba A., Legreve B. and Dhed'A, D., 2013. Caractérisation des populations de *Mycosphaerella jiensis* et épidémiologie de la cercosporiose noire du bananier dans la région de Kisangani. Thèse de Doctorat Université Catholique de Louvain, French.72p.

[11] Djeugap, F.J., G.N. Tsompbeng, K.E. Keuete, A. Yaouba and Serferbe, S., 2015. Isolation and Identification of Fungi Associated with Avocado fruits from Local markets of the West Region of Cameroon. *Inter J Agri Biosci*, 4(2): 64-68. www.ijagbio.com

[12] Droby, S., 2006. Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Horticul*., 709: 45–51.

[13] Ewekeye, T.S., Oke, O.A. and Esan O.O., 2016. Studies on post harvest rot of apple (*Malus domestica* Borkh). *Indian Journal of Plant Sciences* ISSN: 2319–3824. Vol.5 (1) January-March, pp.36-41

[14] Everett, K.R., L.M. Boyd, H.A. Pak and Cutting, J.G.M., 2007. Calcium, fungicide sprays and canopy density influence postharvest rots of avocado. *Australasian Plant Pathology* 36: 223-230.

[15] Everett, K.R., S.G. Owen and Cutting, J.G.M., 2005. Testing efficacy of fungicides against postharvest pathogens of avocado (*Persea Americana var* Hass). *New Zealand Plant Protection* 58: 89-95.

[16] FAOSTAT., 2011. Statistiques des productions, d'exportations et d'importations fruitières du Cameroun.

[17] Hartill, W.F.T. and Everett, K.R., 2002. Inoculum sources and infection pathways of pathogens causing stem-end rot of Hass avocado (*Persea americana*). New Zealand *J Crop Hortic Sci*, 30: 249-260.

[18] Klich, M.A., 2002. Identifiction of common *Aspergillus* species. United state Department of Agriculture. Agriculutre Research Service, Southern Regional research center New Oluisiana USA, 116.

[19] Korsten, L., G.M. Sanders and Grosse-Weischede, E., 1994. Isolation and pathogenicity of avocado post-harvest pathogens from Westfalia and other avocado producing areas. South African Avocado Growers' Association Year book 17: 46-48.

[20] MINADER, 2012. Ministère de l'Agriculture et du Développement Rurale. Annuaire Statistique du Cameroun 2010.

[21] Moubasher, A.H., 1993. Soil fungi in Qater and Other Arab Countries. *The Scientific and Appl. Res. Center* Univ.of Qater. 670.

[22] Onyeani, C.A., S. Osunlaja, O.O. Oworu and Olufemi, S., 2012. First report of fruit anthracnose in mango caused by *Colletotrichum gloeoesporioides* in Southwestern Nigeria. *Inter J Sci Technol Res*, 1: 30-34.

[23] Parveen, S., Wani, A.H., Bhat, M.Y., Koka, J.A., Wani, F.A., 2016. Management of postharvest fungal rot of peach (Prunus persica) caused by Rhizopus stolonifer in Kashmir Valley, *India. Plant Pathology & Quarantine* 6(1): 19–29. doi 10.5943/ppq/6/1/4.

[24] Pitt, J.I., Hocking, A.D., 1985. Fungi and food spoilage. Sydney: *Acad. Press*, 1- 413.

[25] Ranasinghe, L., Jayawardena, B., Abeywickrama, K., 2005. An integrated strategy to control post-harvest decay of Embul banana by combining essential oils with modified atmosphere packaging. *Int. J. Food Sci. Technol*., 40: 97–103.

[26] Raper, K.B., Fennell, D.J., 1965. The genus *Aspergillus*, Williams and Wikins, Baltimore USA.

[27] Raut, S.P., Ranade, S., 2004. Diseases of banana and their management. In: Naqvi, S.A.M.H. (Ed.), *Diseases of Fruits and Vegetables*, Kluwer Academic Publishers, Netherlands, 2: 37–52.

[28] Regnier, T.S. Combrinck, Y. Du Plooy and Botha, B., 2010. Evaluation of *Lippia scaberrima* essential oil and some pure terpenoid constituents as postharvest mycobiocides for avocado fruits. *Postharvest Biolog Technol*, 57: 176-182.

[29] Rivera-Vargas, L.I., Y. Lugo-Noel, R.J. McGovern, T. Seijo and Davis, M.J., 2006. Occurrence and distribution of *Colletotrichum* spp. on mango (*Mangifera indica* L.) in puerto rico and Florida, USA. *Plant Pathol. J*., 5: 191-198.

[30] Salman, M.A.M., 2005 – Biological Control of *Rhizopus* Soft Rot on Apple, Pear and Peach by Trichoderma harzianum. Doctoral Thesis, National University, India.

[31] Scott, P.M., 2001. Analysis of agricultural commodities and foods for *Alternaria* mycotoxins. *J. Am. Oil Chem. Soc*., 6: 1809–17.

[32] Singh, R. P., Dhania, G., Sharma, A. and Jaiwal, P. K., 2007. Biotechnological approaches to improve phytoremediation efficiency for environment contaminants. In: Environmental bioremediation technologies, Singh, S. N.;Tripahti, R. D. (Eds) Springer, 223-258

[33] Smith, N.R., Dawson, V.T., 1944. The bacteriostatic action of rose bengal in media used for plate counts of soil fungi. *Soil Sci.*,58: 467- 471.

[34] Sommer, N.F., 1985. Strategies for control of post-harvest disease of selected commodities. In: Post-harvest Technology of Horticultural Crops. University of California Press,. 83-98.

[35] Tafinta I.Y., K. Shehu, H. Abdulganiyyu, A.M. Rabe and Usman, A., 2013. Isolation and

Identification of Fungi Associated with the Spoilage of Sweet Orange (Citrus Sinensis) Fruits In Sokoto State. *Nigerian Journal of Basic and Applied Science*, 21(3): 193-196

[36] Valiuskaite, A., Kvikliene, N., Kviklys, D. and Lanauskas, J., 2006. Post-harvest fruit rot incidence depending on apple maturity. *Agronomy Research* 4(Special Issue) 427-431

[37] Wharton, S.J., Basu, S.P., Ashe, H.L. 2004. Smad affinity can direct distinct readouts of the embryonic extracellular Dpp gradient in Drosophila. *Curr. Biol.* 14(17): 1550-1558.

Table.1: Different types of fungal species isolated from deteriorated fruit samples during this investigation.

Genus	**Species**	**Banana**	**Mango**	**Safou**
Aspergillus	*A. niger*	+	+	+
Cercospora	*C. capsici*	-	-	+
	C. mangiferae	-	+	-
	C. musae	+	-	-
Colletotrichum	*C. gloesosporioides*	+	+	+
	C. musae	+	-	-
Fusarium	*F. oxysporum*	+	+	+
Rhizoctonia	*R. solani*	-	+	+
Verticellium	*V. albo-atrum*	+	+	+
No. of species		6	6	6

+ = Present; - = absent

Table.2: Diameters (mm) of the lesions developed on fruits by Cercospora genus

	Diameters (mm) of the lesions			
Fruit	*C. musae*	*C. mangiferae*	*C. capsici*	Witness
Banana	22.12 ± 4.82^{b}	36.00 ± 5.84^{a}	31.75 ± 2.33^{a}	0
Mango	36.50 ± 7.58^{a}	45.00 ± 11.22^{a}	19.88 ± 3.42^{b}	0
Safou	16.12 ± 6.70^{b}	16.37 ± 4.46^{b}	24.31 ± 5.50^{b}	0

Values followed by the same aphabetical letter in the same column are not significantly different according to Duncan test.

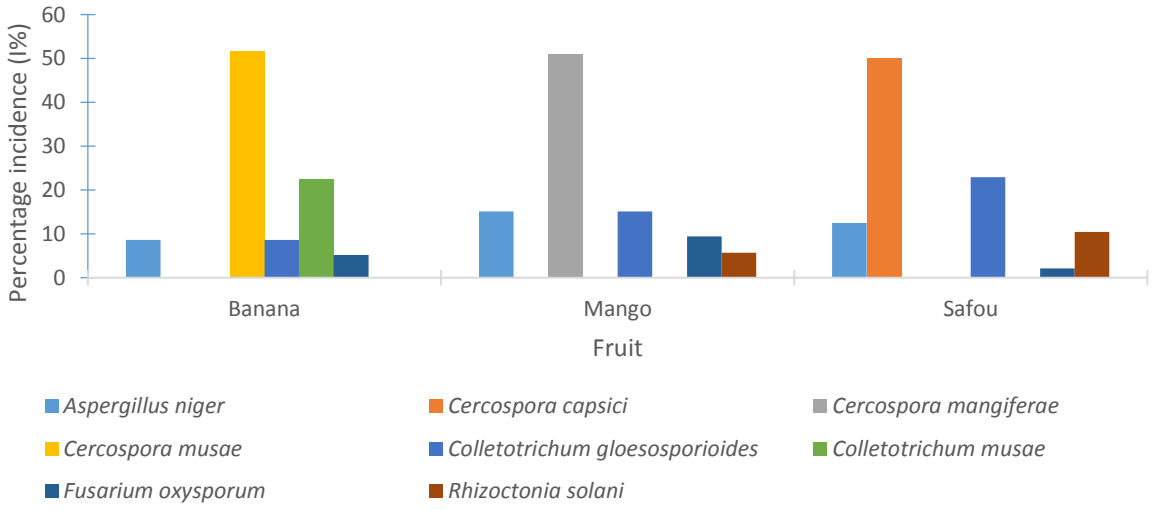


Fig.2 : Prevalence of the most common fungal species isolated from different fruits

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

Determination of Thermal Bioclimatic Conditions for Touristsin west and North West of Iran using PET

Ali. Hanafi[1], Hasan. Atashgahi[2]

[1]AssistantProfessor of Climatology, Faculty of Basic sciences, University of Imam Ali, Tehran, Iran
[2]MSc of Geographic Information System, Faculty of Basic sciences, University of Imam Ali, Tehran, Iran

***Abstract*—** *Tourism has become an important sector that has an impact on development of country economy. The main benefits of tourism are income creation and generation of jobs. For many regions and countries it is the most important source of welfare.The purpose of this study is to determine the most suitable months for human thermal comfort and tourism activities in west and North Westof Iran by using Physiologically Equivalent Temperature(PET).The data, which covering the period 1985–2010, from a dense network of 32 meteorological stations in west and north west f of Iran was used to compute the PET. Mean air temperature, relative humidity, vapor pressure, wind speed, and cloud cover data were obtained from theIran Meteorological Organization (IRIMO).Ray Man model was used to calculate the PET. Based on the calculations of PET in the region, it is shown that the monthsMarch and Decemberin the west parts of Kermanshah, the monthsApril, May and October in Kermanshah and Kurdistan and the June and September in the northern parts of the region such as Azerbaijan, and Hamadan are laying in the comfortable class that representing the most suitable months for tourism and tourist activities.*

***Keywords*— Tourism, physiologically equivalent temperature (PET), climatic comfort, Ray Man, west and North West of Iran.**

I. INTRODUCTION

Nowadays tourism industry is developing into one of the most important and most lucrative industries worldwide. In 2012, international tourist arrivals exceeded one billion for the first time in history, contributing 9 % to global GDP, 1.3 US$trillion in exports (United Nations World Tourism Organization 2013, Rutty and Scott 2014).Tourism is heavily depended on weather and climate in such a way that fair weather and climate knowledge of destination play a key role in tourism industry and they can be attracting or off putting factors for tourists (Fallahi et al, 2012).Usually, geographical location, topography, landscape, vegetation and fauna are factors that influence decisions regarding areas to be visited. Weather and climate are two additional factors (Matzarakis, 2006; Farajzadeh and Matzarakis, 2009). Climate and tourism are closely related and the relationship between these two is indicated whereby "Climate Comfort Index"(Ataei et al, 2013).The climatic indices, which are primary used for tourism climate assessments and thermal comfort studies, present a certain number of important points. From the point of view of human-biometeorology they do not include the effects of short and long wave radiation fluxes which are generally not available in climate records. The required, for the human energy balance, short and long wave radiation fluxes are calculated using synoptic and climatological and astronomical data (VDI 1998; Matzarakis et al 2000; Matzarakis 2007).

Commonly used indices that measure the effect of the thermal environment on humans are PMV (Predicted Mean Vote) (Fanger 1972), PET (*Physiological Equivalent Temperature)* (Höppe, 1999, Matzarakis et al, 1999), and SET* (standard effectivetemperature) (Gagge et al. 1986). The advantage of these thermal indices is that they require the same meteorological input parameters i.e. air temperature, air humidity, wind speed, short and long wave radiation fluxes (Matzarakis 2007). The *physiologically equivalent temperature* (PET) is a thermal index derived from the human energy balance. It is well suited to the evaluation of the thermal component of different climates. PET is preferable to other thermal indexes such as the predicted mean vote because of its units (°C), which make results more comprehensible to urban or regional planners. PET results can be presented graphically or as bioclimatic maps(Matzarakis et al., 1999).In this regard Rayman which is an improved model calculates the average radiant temperature and thermal indexes in simple and complex environments based on the data from weather stations and climatic elements such as temperature, humidity and wind

speed (Matzarakis 2009). This model can be used to evaluate urban bioclimate and thermal indexes including (PET), (SET) and (PMV).

Iran, among the world's eighth leading countries considering its cultural and historical sites, and based on the diversity of landscape and continental ecotourism attractions, ranked in the top five countries of the world (Esmaili and Fallah Ghalhari, 2014).However, little research has been done to establish environmental and continental information for tourists that can notethefollowingresearch:

Matzarakis and Farajzadeh (2009), in a paper entitled "Quantification of climate for tourism in the northwest of Iran " concluded that all the stations in northwest Iran have a month with (TCI) over 80 which can be considered an ideal index comfort. Esmaili and Fallah Ghalhari (2014) havestudiedBioclimatic Conditions of Mashhad for Tourists activities and concluded themonths of April, May and October have thebestbioclimate condition in Mashhad.Ataei and Hashemi Nasab (2012), in a research Evaluated Human Bioclimate of Semnan Province by using (PET) and (PMV) and showed thatinthe months ofOctober and November comfortclimateprevailinginthe city.

Ranjbar et al (2009), in a paper studied the relation between climatic conditions and annual tourism trend in the city of Marvdasht. Ataei et al (2013) determineda suitable calendar for tourism in Ahwaz usingPET and Concluded that Ahwaz has comfortable and suitable conditions during the cold seasons of the year and is the best destination for tourists in the winter.

Because oftheavailability of largetourist attractionsinWestand Northwest of Iran, the purpose of this study was to examine and analyze the thermal bioclimate (physiologically equivalenttemperature) and determine the most suitable months for tourist activities in the west and north west of Iran.

II. STUDY AREA

The study area is located between 29° 58′ to 39° 49′ latitude and 44° 03′ to 50° 39′ longitude and includes the west and north west of Iran. This region has a diverse climate. In the northwest, winters are cold with heavy snowfall and subzero temperatures during December and January.

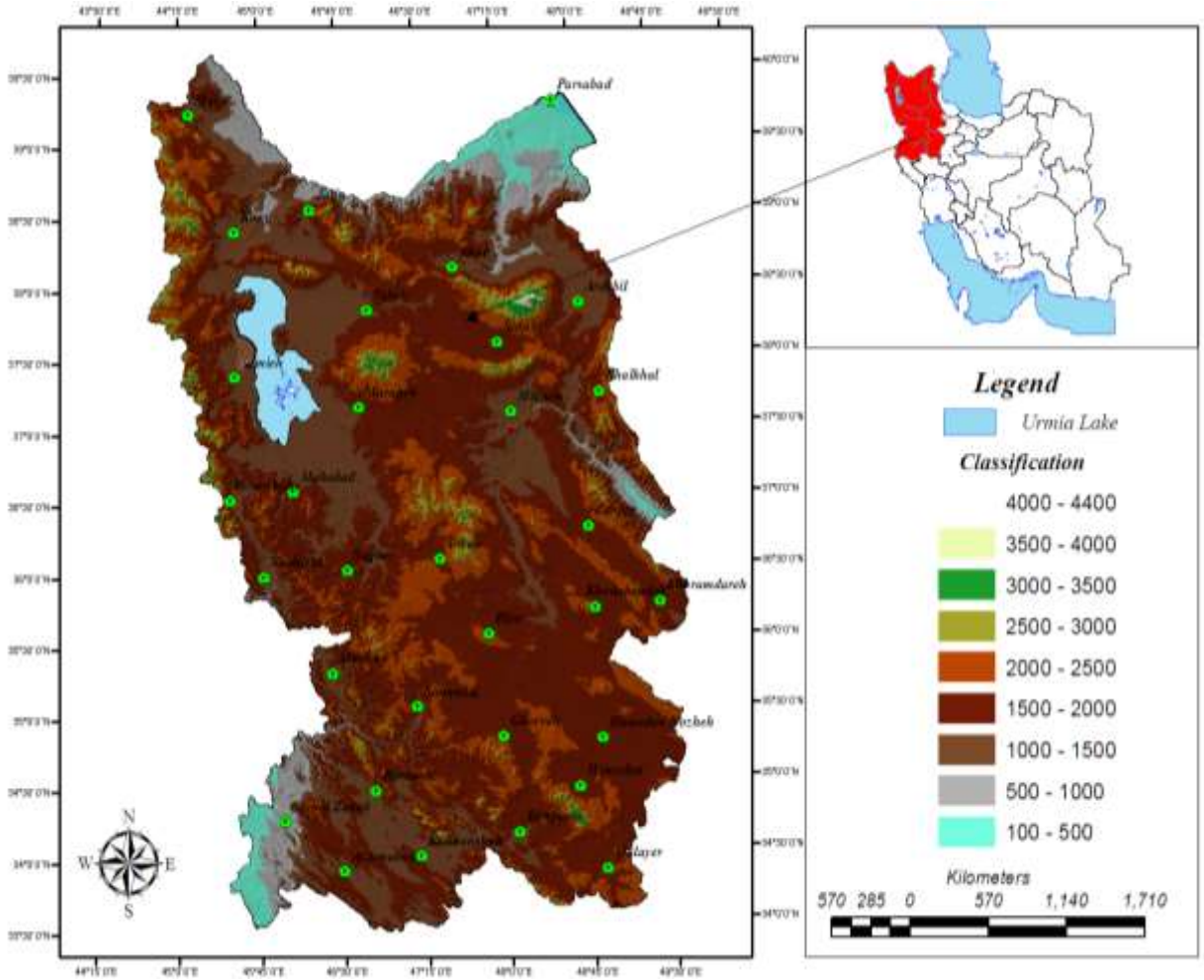


Fig.1: Topographic map, weather stations and geographical location of the study area

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

Spring and fall are rather mild, while summers are dry and hot. In the south, winters are mild and the summers are hot. In general, the west of Iran has plentiful cultural, historical and environmental attractions (ecotourism) consideringas one of the most potential spots for tourist capacity.More than 100 tourism landmarks, plentiful cultural, historical and natural attractions (ecotourism and geotourism) and variety of climates can be found in the region and are mostly visited by the domestic and foreign tourists.In this region, the following provinces are located: WestAzerbaijan, East Azerbaijan, Zanjan, Kurdistan, Ardabil, Kermanshah and Hamadan (Figure 1). Uremia lake and Sahulan cave palace in West Azerbaijan, Arasbaran forests and Kandovan village in East Azerbaijan, Qezel Ozan River and Soltanieh Dome in Zanjan, Sabalan Mountainsand Sar Ein Mineral Springs in Ardabil, Alisadr cave in Hamadan, zarivar lake in Kurdistan, Bistun vault in Kermanshah are also interesting locations for visitors.

III. DATA AND METHODS

In the present study to assess Physiological Equivalent Temperature, the climatic data of 32 synoptic stations over a 25- year time period (1985-2010) were obtained in a quality controlled format from the I. R.of *Iran* MeteorologicalOrganization (IRIMO). The meteorological elements dry temperature in Celsius, relative airhumidity in percent, wind speed in meter per second, vapor pressure in hPa, the cloud amount in Octa are necessary for calculating PET.The mean radiant temperature can be calculated by combining the theoretical maximum global radiation and the mean cloud cover within the radiation and bioclimate model Ray Man (Matzarakis et al, 2000). The obtained data were fed into Excel program. Next, considering compatibility with (PET) method they were fed into Ray Man and finally the outputs were analyzed. The Humanmeteorological conditions are analyzed by means of 25-day mean values of thermal sensation measured at 7am, 2pm and 9pm during the year.

The thermal sensation is defined by means of physiologically equivalent temperature PET as the physiologically significant assessment of the thermal environment derived from the human energy balance (Höppe, 1999, Matzarakis et al., 1999, Matzarakis 2007). Since the 1960s, heat balance models of the human body have become more andmore accepted in assessing thermal comfort. More universally applicable models take into account all basic thermoregulatory processes, like the constriction or dilation of peripheral blood vessels and the physiological sweat rate (Höppe 1993, 1999).The Munich energy balance model for individuals" (MEMI) (Höppe 1993) is such a thermo physiological heat balance model. It is the basis for calculating the physiologically equivalent temperature (PET).PET is defined to be equivalent to the air temperature that is required to reproduce in a standardized indoor setting and for a standardized person the core and skintemperatures that are observed under the conditions being assessed (VDI 1998; Höppe 1999, Matzarakis 2007). The standardized person is characterized by a work metabolism of 80 W of light activity, in addition to basic metabolism; andby 0.9 clo of heat resistance as a result of clothing.

The calculation of PET includes the following steps:

– Calculation of the thermal conditions of the body with MEMI for a given combination of meteorological parameters.

– Insertion of the calculated values for mean skin temperature and core temperature into the model MEMI and solving the energy balance equation system for the air temperature Ta (with v = 0.1 m/s, VP = 12 hPa and Tmrt = Ta).

Finally the resulting air temperature is equivalent to PET (Matzarakis 2007).PET allows the evaluation of thermal conditions in a physiologically significant manner, too. With respect to this, Matzarakis and Mayer (1996) transferred ranges of PMV for thermal perception and grade of physiological stress on human beings (Fanger 1972) into corresponding PET ranges (Table 1).

Table.1: Ranges of the physiologically equivalent temperature (PET) for different grades of thermal perception by human beings and physiological stress on human beings (Matzarakis and Mayer 1996, Matzarakis 2007)

PET	Thermal Perception	Grade of Physiological Stress
4 °C	very cold	extreme cold stress
8 °C	Cold	strong cold stress
13 °C	Cool	moderate cold stress
18 °C	slightly cool	slight cold stress
23 °C	Comfortable	no thermal stress
29 °C	slightly warm	slight heat stress
35 °C	Warm	moderate heat stress

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

41 °C	Hot	strong heat stress
	very hot	extreme heat stress

They are valid only for the assumed values of internal heat production and thermal resistance of the clothing.This model can be used to evaluate urban bioclimatic and thermal indexes including (PET), Standard Effective Temperature (SET), Predicted mean Vote (PMV). The model has been developed in Germany according to international guidelines between atmosphere and short-and-long-waive fluxes. Ray man model is a run model for evaluation of bio meteorological weather quality, urban and regional planning at micro and macro level (Matzarakis, 2009).

IV. RESULTS AND DISCUSSION

Choosing a travel time and destination in terms of climate comfort can positively affect the quality of tourism. Enjoying a fair weather while travelling will increase the tendency to stay longer and coming back to the same spot. In contrast an unfavorable climatic condition enhances a negative experience. According to the PET index (table 1), the numerical value of 18-23 range shows a comfort condition without the warm or cold tensions.In this classification, the numerical values of 23- 29 and 13-18 indicate a slightly warm and cool stresses that can wear light or heavy clothes to bring the comfort condition.

In order toobtainan overview ofPET changesin theregion, four stations of Kermanshah, Sanandaj, ,Tabriz and Ardebil were selected from different geographic regions andthe biometeorological conditions are analyzed by means of ten-day mean values of thermal sensation during the year. By means of the probability of occurrence of different thermal sensations that enable more detail information about bioclimate.The relative frequencies for PET values divided into10 classes in order to evaluate the thermal stress for the Period from 1985 until 2010.

The relative frequencies for PET in Kermanshah station is shown in Figure 2. In Kermanshah station, thermal comfort occurs from March to June and September to November, with highest Probability (>30 %) in the months May and October and minimum frequency in July to August (about 0 %). Cold stress (<4 °C) can be observed from November to March with highest Probability (>50 %) in the months January and February. Days with strong heat stress, can be observed from June to October with maximum frequencies (Morethan45%)in the months July andAugust.

Figure 3 shows the relative frequencies for PET in Sanandaj station.In this station, thermal comfort occurs throughout the yearexceptJuly and August, with maximum frequencies (>30 %) from 10st of May to 10th of June and 10th of September to 20st of Octoberand minimum frequency in December to March (about0%). Cold stress (<4 °C) can be observed from November to April with highest Probability (>80 %) in the months January and February.Days with strong heat stress, can be observed from 1st of June to 20th of September with maximum frequencies (Morethan30%) in the months July and August.

As can be seen in Figure4, in Tabriz station, thermal comfort occurs from April to October, with maximum frequencies (>30 %) from 20st of May to 20th of June and 1th of September to 10st of October and minimum frequencyfromNovembertoMarch (about 0 %).Cold stress (<4 °C) can be observed from October to May with maximum frequencies (>90 %) in the months December, January and February. Days with strong heat stress, can be observed from 20st of June to 10th of September with maximum frequencies (Morethan8%) from 20 st of July to 10th of August.

The relative frequencies for PET in Ardabil station is shown in Figure 5. Ardabilis consideredas oneof the coldestcities in Iran, inthis station, thermal comfort occurs from April to October, with maximum frequencies (>30 %) from 10st of June to 10th of Septemberand minimum frequency in November to April (about0 %). Cold stress (<4 °C) can be observed from September to June with maximum frequencies (>95 %) in the months December, January and February. Days with strong heat stressinArdabilrarelyhappens and can be observed from July to September with frequencies lessthan2%.

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

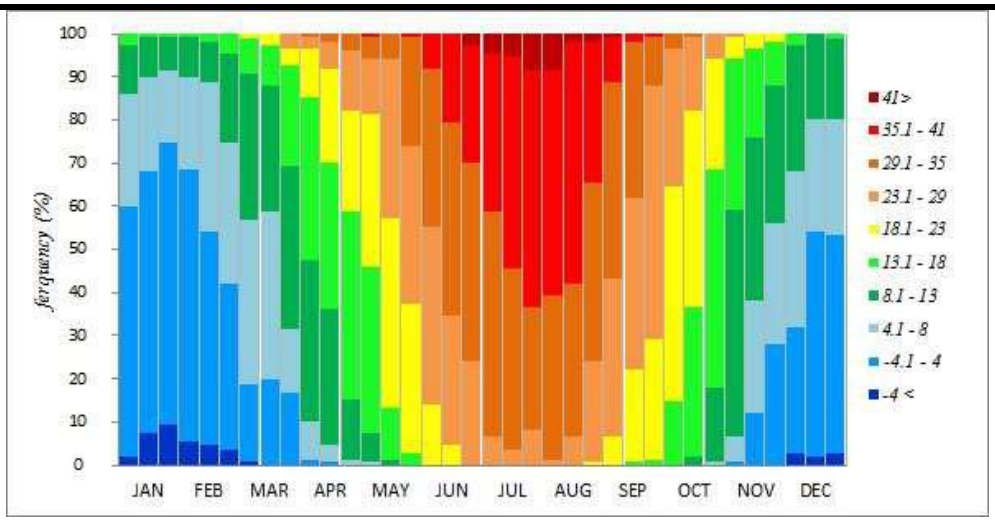


Fig.2: Probability of occurrence of different PET classes for the meteorological station Kermanshah for the period 1985–2010.

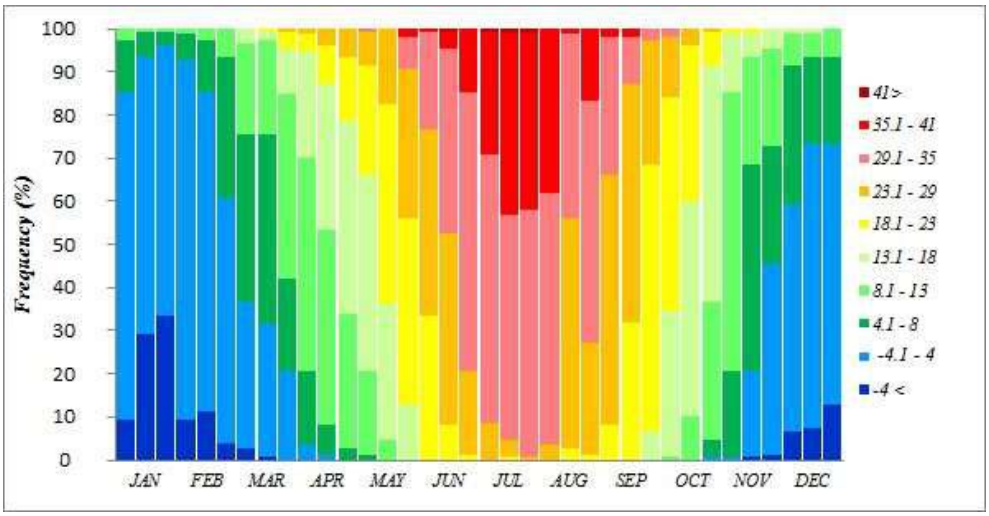


Fig.3: Probability of occurrence of different PET classes for the meteorological station Sanandaj for the period 1985–2010.

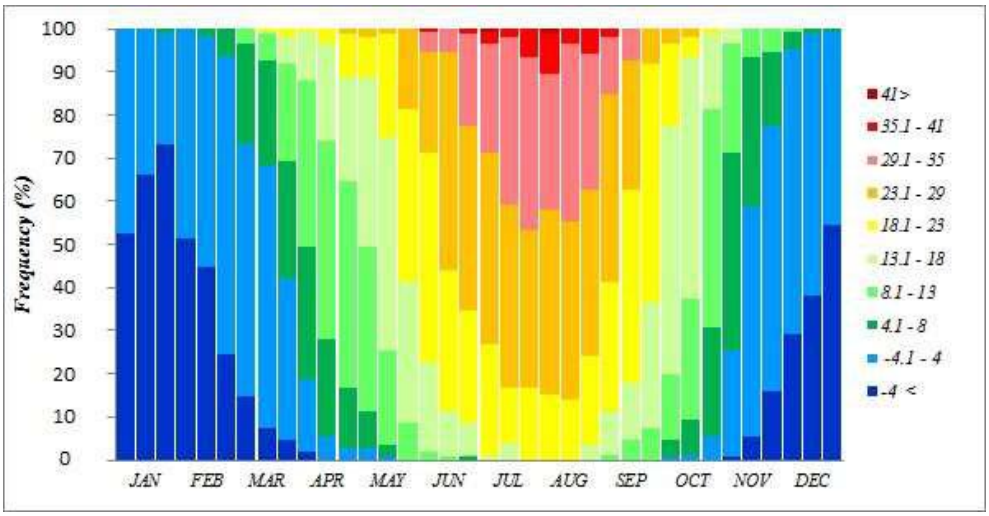


Fig.4: Probability of occurrence of different PET classes for the meteorological station Tabriz for the period 1985–2010.

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

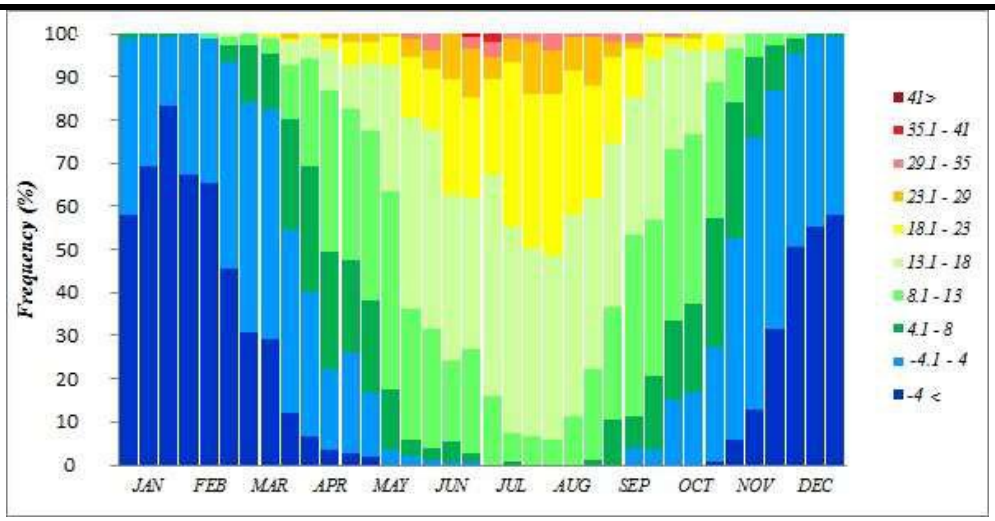


Fig.5: Probability of occurrence of different PET classes for the meteorological station Ardebil for the period 1985–2010.

Figure 6 shows the mean daily changes in the PET for Ardebil, Tabriz, Kermanshah and Sanandaj stations in the period 1985–2010. The thermal comfort zone (the numerical value of 13 to 29 degrees) is marked on the chart.

In Kermanshah and Sanandaj stations, there is a cold physiological stress with different intensities from 1st of January to 30th of April and 20th of October to 31st of December. From 1st of May to 15th of June and 10th of September to 20th of October the physiological stress is zero and there is a climate comfort condition. There is a warm physiological stress with different intensities from 15th of June to 10th of September.

Based on PET of Tabriz station, there is a cold physiological stress with different intensities from 1st of January to 10th of May and 15th of October to 31st of December. From 10th of May to 15th of July and 15th of August to 15th of October the physiological stress is zero and there is a climate comfort condition. There is a warm physiological stress with slight intense from 15th of July to 15th of August.

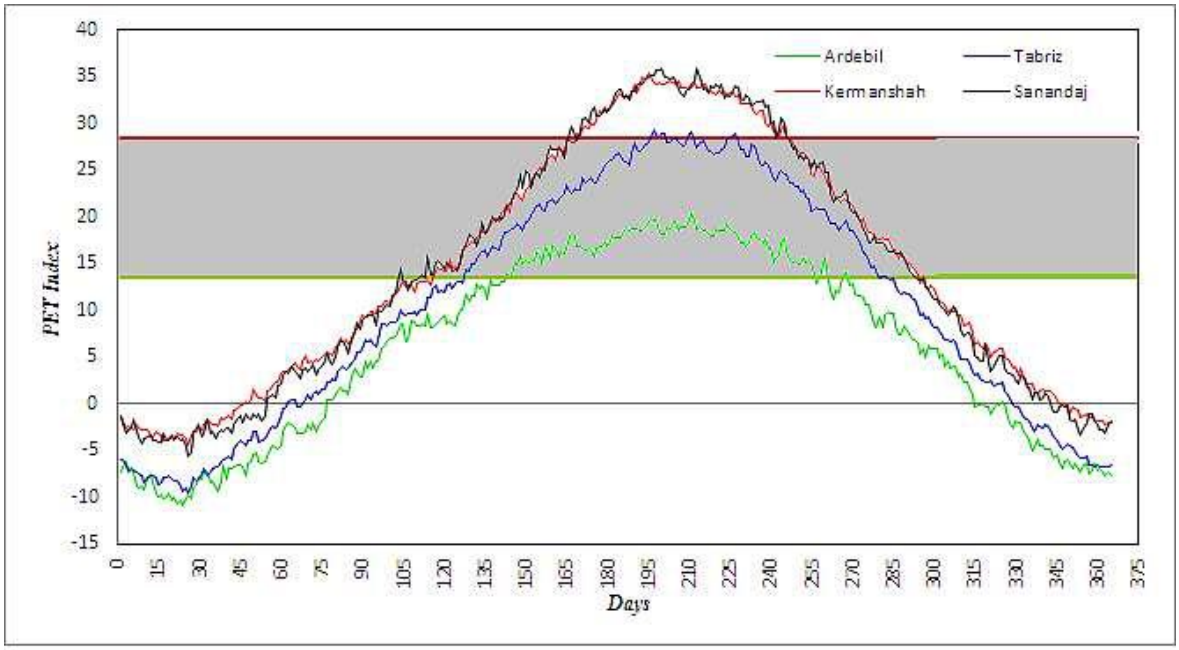


Fig.6: PET variations and comfort range at selected stations for 1985–2010

In Ardebil station, there is a cold physiological stress with different intensities from 1st of January to 15th of May and 20st of September to 31st of December. From 15st of May to 20th of September, the physiological stress is zero and there is a climate comfort condition. There isn't warm physiological stress in Ardebil station throughout the year.

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

In order to study the temporal and spatial changes in climate comfort, the monthly maps predicted and evaluated.Figure 7 shows the PET conditions for January. In this month,there isan extreme cold stress dominates in the provinces of Azerbaijan[1], Kurdistan andHamadan, whilethere is a strong cold stress in the provinces of Kermanshah.The PET condition for February isthe same asJanuary. There is an extreme cold stress in the northern parts of the study area such as Azerbaijan, Kurdistan and Hamadan, whilea moderate cold stress is dominant in west parts of Kermanshahsuch as Sarpole Zahab station (Fig. 8).In March, the amount of cold stress compared to the months of January and February have been reduced.There is an extreme and strong cold stress in the northern parts of the region such as Azerbaijan, Kurdistan and Hamadan and moderate cold stress in west parts of Kermanshah (Fig. 9).In April, the western parts ofKermanshah provincessuch as Sarpole Zahab are associated with comfortable perception,while there is a slight cold stress in Jolfa, Khoy, Parsabad, Miyaneh and Aslamabad stations and moderate to strong cold stress in other areas (Fig. 10).

In the month of May by increasing the air temperature, the Coldstressis reducedinthe study area, sothere is slight heat stress in the west parts of Kermanshah provinces andslightcold stress to comfort conditions in most parts of Azerbaijan, Kurdistan, Hamadan and Kermanshah (Fig. 11).The PET condition forJuneisindicateda strong to moderate heat stress in the western parts of Kermanshah provinces and Parsabad station, a slight heat stress in most parts of study area and climatic comfort conditions in theStationsof Ahar, Sarab, Ardebil, Khalkhal, Khodabandeh, Khorramdareh and Bijar (Fig. 12).

July is the hottest month in the study area. The PET condition forJulyisindicateda strong to moderate heat stress in the western parts of Kermanshah province, a slight tomoderate heat stress in most parts of Azerbaijan, Kurdistan, Hamadan and Kermanshah provinces and climatic comfort conditions in theStationsof Ahar, Sarab, Ardebil and Khalkhal (Fig. 13).Physiological equivalent temperature index in August is also similar to July, so that the PET condition shows a strong heat stress in the western parts of Kermanshah province such as Sarpole Zahap station, aslight to moderate heat stress in most parts of Azerbaijan, Kurdistan, Hamadan and Kermanshah provinces and climatic comfort conditions in theStationsof Sarab, Ardebil and Khalkhal(Fig. 14).In September, the intensity of the heat stress compared with the months of July and August have been reduced. There is a moderate heat stress in the western parts of Kermanshah province, slight heat stressin theStationsof Kermanshah, Aslamabad, Sanandaj, Khoy, Jolfa, Miyaneh and Parsabad, climatic comfort conditionsin most parts of region and slight cold stress in theStationsofSarab, Ardebil, Khalkhal and Khodabandeh (Fig. 15).In October,the heat stress reduced and cold stress has started in the northern parts of study area.The PET condition for October isindicatedclimatic comfort conditionsin the western parts of Kermanshah province, a slight to moderate cold stressin most parts of region and strong cold stress in theStationsofSarab, Ardebil and Khalkhal (Fig. 16).The physiological equivalent temperature condition in November shows a moderate cold stress in the western parts of Kermanshah province and strong to extreme cold stress in Otherareas (Fig. 17).In December, the severity of cold stress has increased. The PET condition in December shows a strong cold stress in the western parts of Kermanshah province and extreme cold stress in otherareas (Fig. 18).

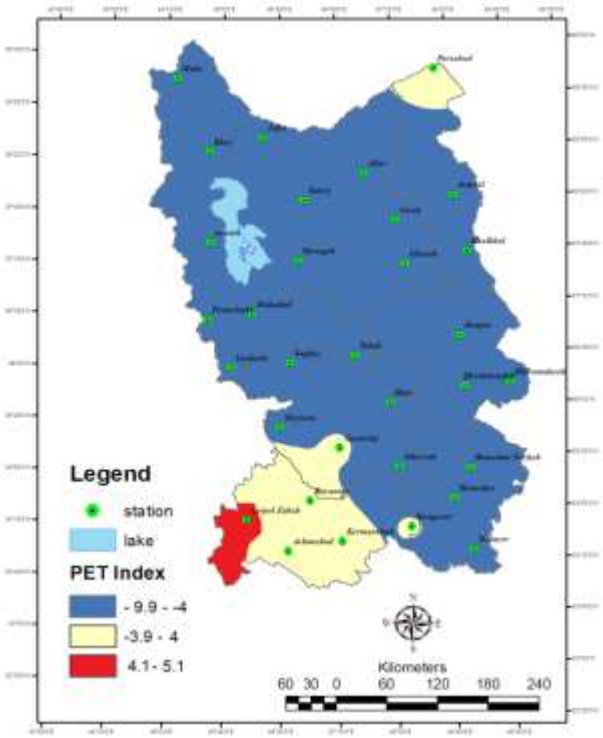


Fig.7: Geographical distribution of PET in January

[1] . Inthispaper,Azerbaijanincludesthe provinces ofEast Azerbaijan, West Azerbaijan, Ardabil and Zanjan

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

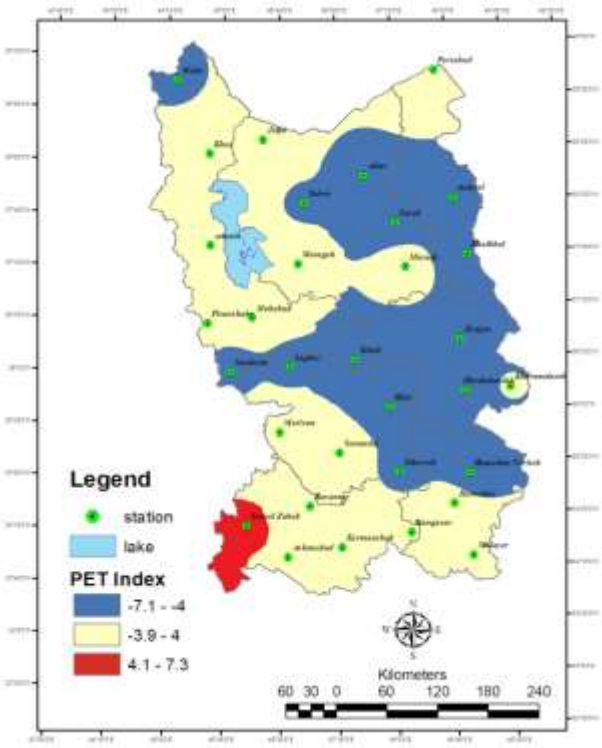


Fig.8: Geographical distribution of PET in February

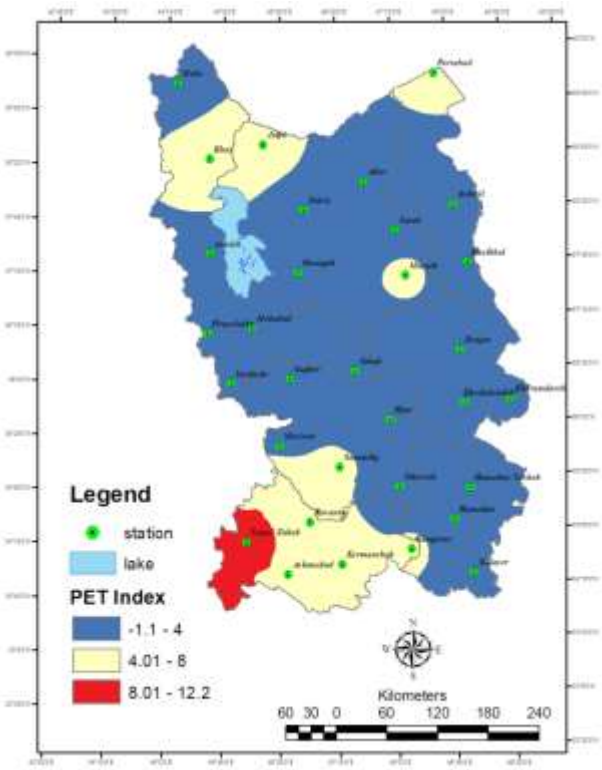


Fig.9: Geographical distribution of PET in March

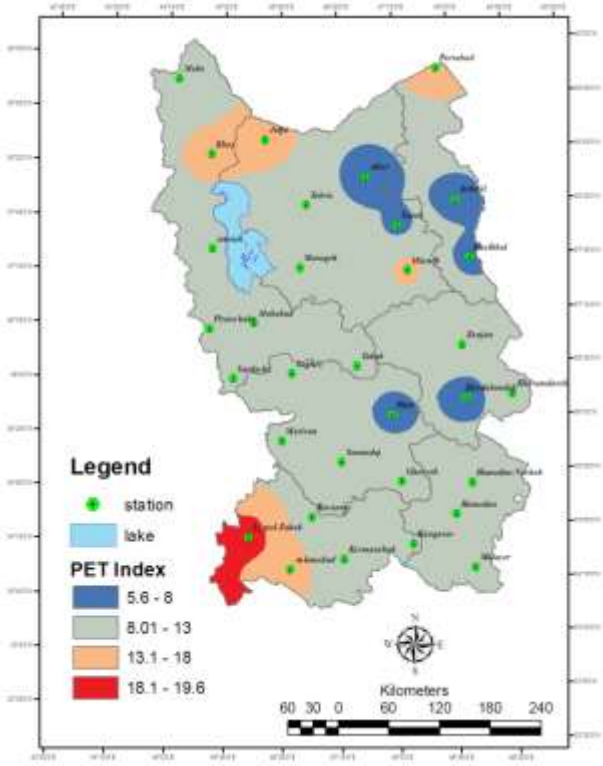


Fig.10: Geographical distribution of PET in April

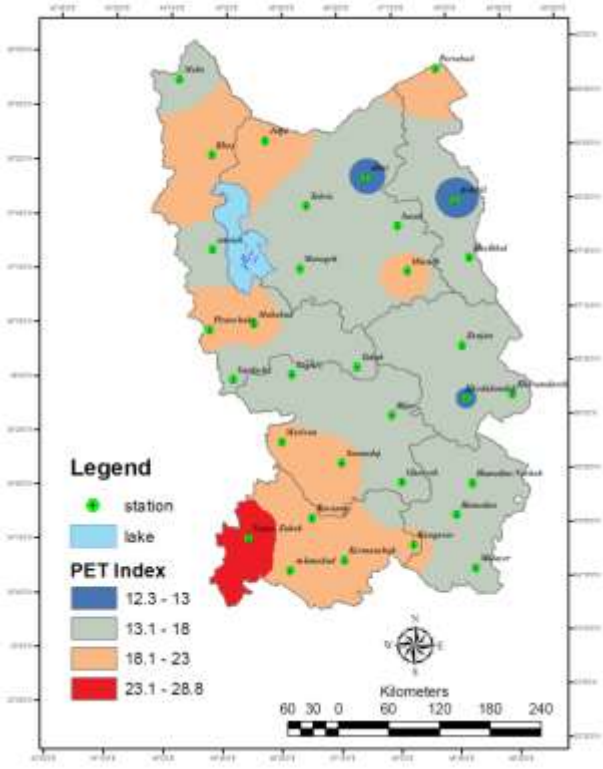


Fig.11: Geographical distribution of PET in May

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

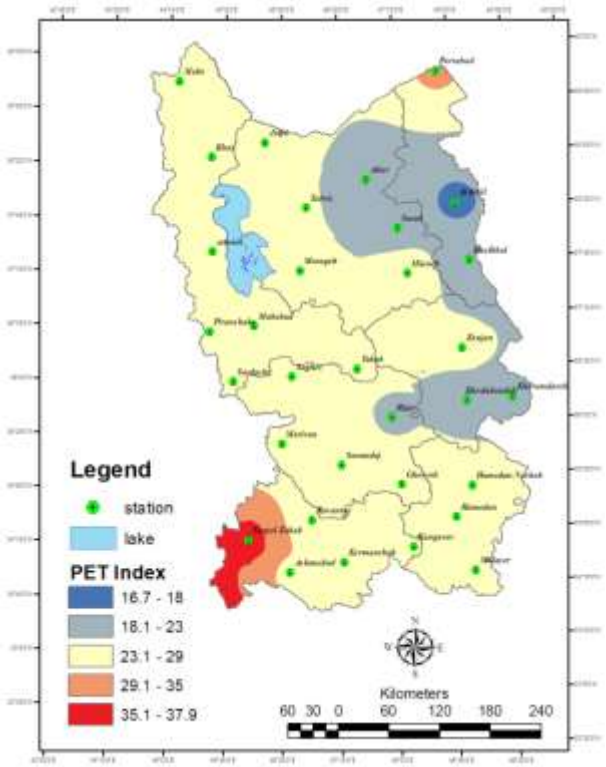


Fig.12: Geographical distribution of PET in June

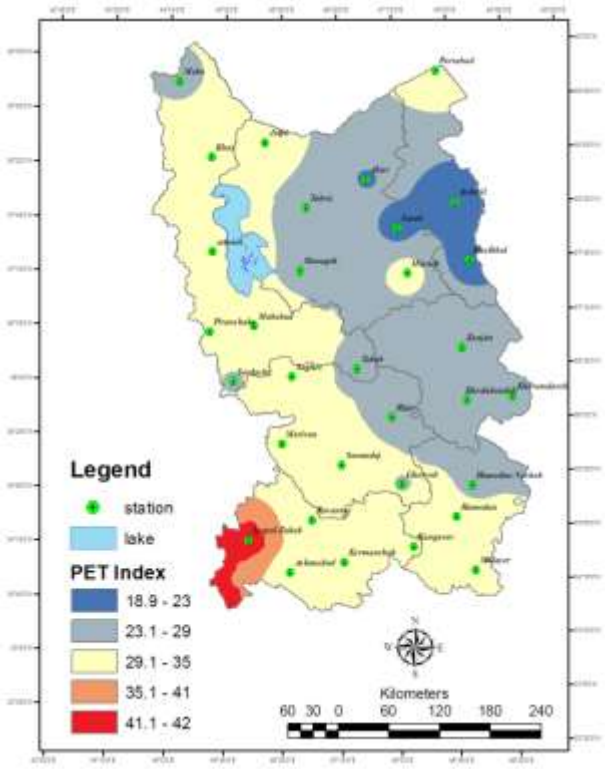


Fig.13: Geographical distribution of PET in July

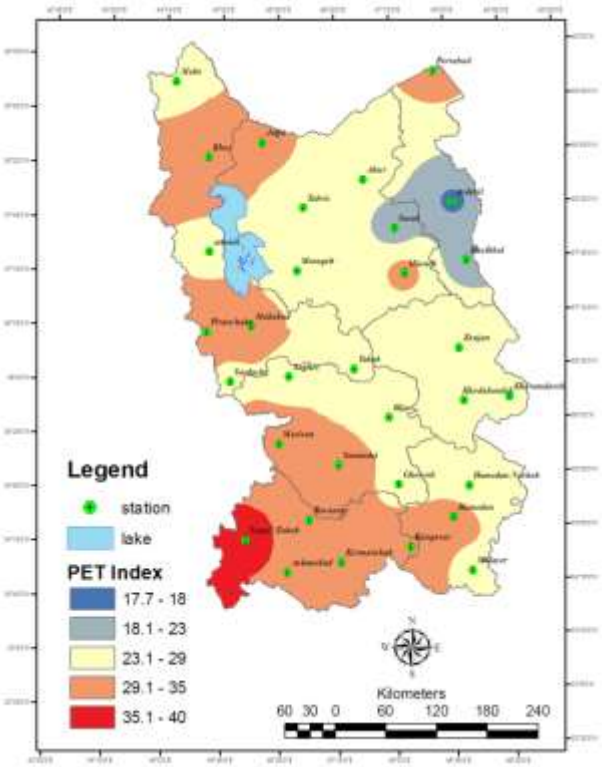


Fig.14: Geographical distribution of PET in August

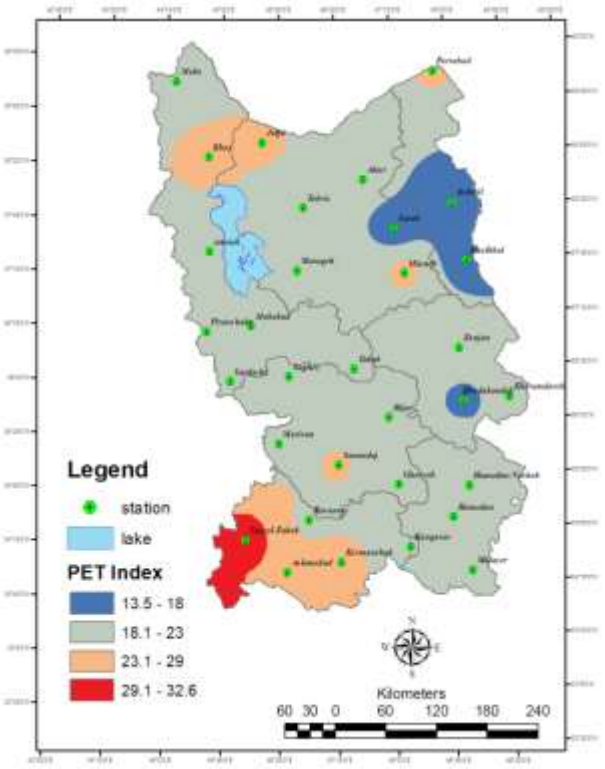


Fig.15: Geographical distribution of PET in September

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

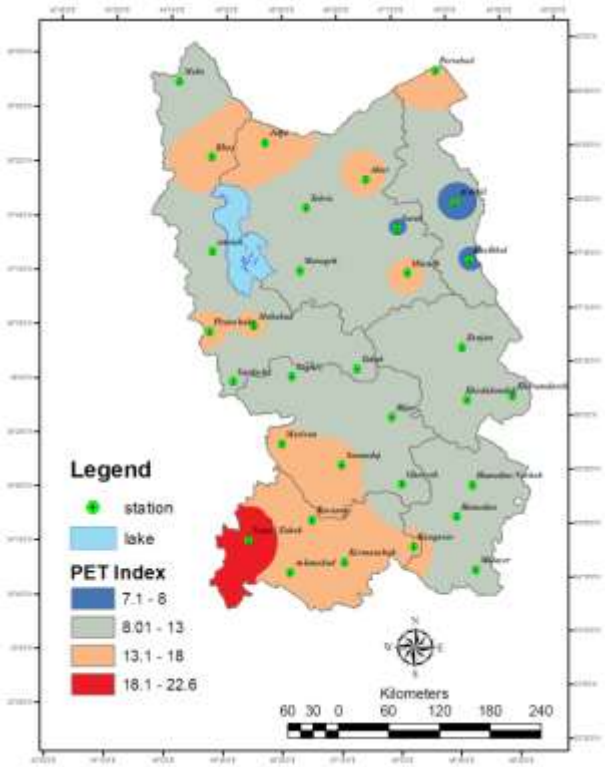


Fig.16: Geographical distribution of PET in October

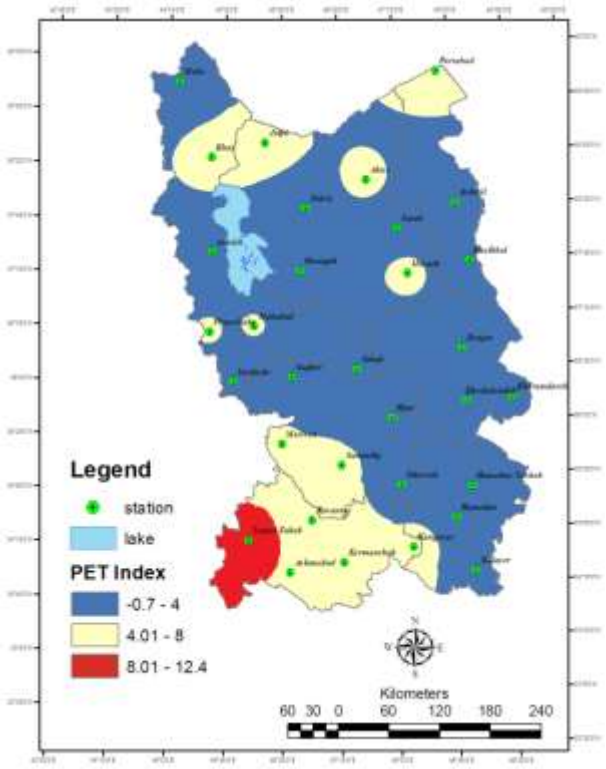


Fig.17: Geographical distribution of PET in November

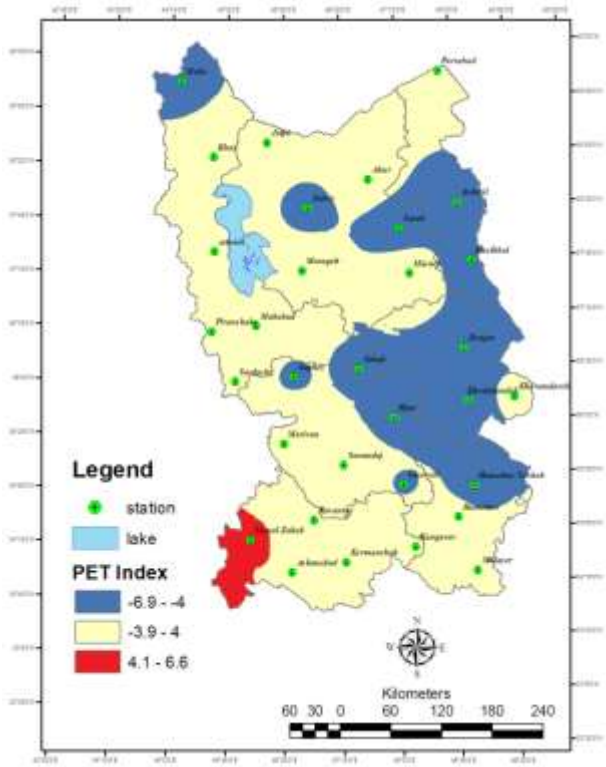


Fig.18: Geographical distribution of PET in December

V. CONCLUSIONS

The extent ofIranindifferentlatitudesandclimaticdiversity,providinggoodconditionsfortourist activities(in terms of bioclimate and climate comfort) in all seasons.In this paper, the climatic tourism potential and thermal comfort conditions during the period (1985–2010) were analyzed forwest and north westof Iran. In the study area, the most suitableareasfortourist activitiesin the summerareAzerbaijan and especially Ardebilprovince.Inspring andautumn, the bestthermal comfort conditions in the province of Kermanshahand Kurdistan and in winterthe bestthermal comfort conditions in the western parts ofKermanshahis provided.The findings of this study can be valuable for tourism in the region.The analysis of climate and bioclimate, especially if presented in a clear and simple way to beunderstandable for everyone, provides a basis for the promotion of tourism destinations (Zaninović and Matzarakis 2007). The information can be used by tourist managers in advertising, by tourists who want to decide when to take their holidays and by physicians to warn their patients for the periods that are unsuitable for health therapy. For example, the people who have difficulty tolerating thesummer heat, such as the elderlies, should choose the best period of bio meteorological conditions to take their vacations, which prevail in the southern parts of region such as Kermanshah in March and December, in the middle sectors such as

Kurdistanin April, May and October and in the northern parts such as Azerbaijan and Hamadan in June and September. For sportsmen who prefer an active anddynamic vacation, pleasant or even cool conditions would be more convenient than summer heat, when the body has to usesthe energy for defense from heat.

REFERENCES

[1] Ataei H, HasheminasabS. 2012. Regionalization and Evaluation of Seasonal Human Bioclimate of Semnan Province. Journal of Basic and Applied Scientific Research, vol 2(5), pp4740-4750.
[2] Ataei H, Hasheminasab S. 2013.Determination of suitable calendar for Tourism in Ahwazutilizing Physiological Equivalent Temperature (PET). Journal of Life Science. Vol 2 (7) June 2013: 104 – 109.
[3] FallahiA. 2012. Kurdistan Tourism Climate Index zoning using TCI. Proceedings of the Fourth Global Conference geography student.
[4] Esmaili R, Fallah Ghalhari G. 2014. An Assessment of Bioclimatic Conditions for Tourists - A Case Study of Mashhad, Iran. Atmospheric and Climate Sciences, 2014, 4, 137-146.
[5] Farajzadeh H, Matzarakis A. 2009. Climate Potential for Tourism in Northwest of Iran. Meteorological Applications 16, 545-555.
[6] Fanger P. O. 1972. Thermal comfort. New York: McGraw Hill.
[7] Gagge A. P, Fobelets A. P,Berglund L. G. 1986. A standard predictive index of human response of the thermal environment. ASHRAE Transactions 92, 709-731.
[8] Höppe P. 1999. The physiological equivalent temperature —a universal index for the biometeorological assessment of the thermal environment. Int. J. Biometeorol. 43, 71-75.
[9] Matzarakis A,Rutz F,Mayer H. 2007. Modeling Radiation flaxes in simple and complex environments – Application of the Ray man model, Int. J.Biometeoral. (51,323-334).
[10] Matzarakis A. 2007. Assessment Method for Climate and Tourism Based on Daily Data Development in Tourism Climatology. 52-58.
[11] Matzarakis A. 2009. The Seventh Internationalconference on urban climate. 29June – 3July 2009 Yokhama. Japan.
[12] Matzarakis A,Mayer H. 1996. Another kind of environmental stress: Thermal stress. WHO News 18, 7–10.
[13] Matzarakis A,Mayer H. 1997. Heat stress in Greece. Int. J. Biometeorol. 41, 34-39.
[14] Matzarakis A,Mayer H, Iziomon M. G. 1999. Applications of a universal thermal index: Physiological equivalent temperature. Int. J. Biometeorol. 43, 76-84.
[15] Matzarakis A, Rutz F, Mayer H. 2000. Estimation and calculation of the mean radiant temperature within urban structures. In: Biometeorology and Urban Climatology at the Turn of the Millennium (ed. by R.J. de Dear, J.D. Kalma, T.R. Oke and A. Auliciems): Selected Papers from the Conference ICB-ICUC'99, Sydney, WCASP-50, WMO/TD No. 1026, 273-278.
[16] Matzarakis A. 2006. Weather and climate related information for tourism. Tourism and Hospitality Planning and Development 3: 99–115.
[17] Ranjbar F, MuqbilM, Arslany M. 2010. Climate with anannual reviewof the tourist city MARVDASHT. Journal of Geography. Third year. No. 7, pp. 90-79.
[18] Rutty M, Scott D. 2014. Bioclimatic comfort and the thermal perceptions and preferences of beach tourists, Int. J. Biometeorol. 58, 1-9.
[19] Spagnolo J, R. de Dear .2003. A field study of thermal comfort in outdoor and semi-outdoor environments in subtropical Sydney Australia. Building and Environment, 38: 721-738.
[20] UNWTO .2008. WordTourism Barometer. Volume6, Number2. Madrid: United Nations Word Tourism Organization Word Tourism Organization. Madrid, Spain.
[21] United Nations World Tourism Organization (UNWTO) UNWTO tourism highlights.2013. Edition.UNWTO:http://dtxtq4w60xqpw.cloudfront.net/sites/all/files/pdf/unwto_highlights13_en_lr.pdf. Accessed 30 Sep 2013.
[22] VDI. 1998. Guideline VDI3787. Part I: Environmental meteorology. Methods for the human biometeorological evaluation of climate and air quality for the urban and regional planning at regional level. Berlin: Beuth. Düsseldorf: Verein Deutscher Ingenieure, 52 pp.
[23] World Meteorological Organization.1999. Climate and human health. World Climate News, 14, 3-5.
[24] Word Tourism Organization. 2006. Tourism 2020 Vision. WTO Publication Unit. Word Tourism Organization. Madrid. Spain.
[25] Zaninović K,Matzarakis A. 2009. The bioclimatological leaflet as a means conveying climatological information to tourists and the tourism industry, Int J Biometeorol (2009) 53:369–374.

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

Determination of Erosivity of Enugu State

G.I. Okolotu, S.I. Oluka, P.C. Eze.

Department of Agricultural and Bioresource Engineering, Enugu State University of Science and Technology, Enugu, Nigeria.

Abstract— *To combat erosion, there is need for adequate examination of soils and factors of erosion. The study of Erosivity is vital for effective soil conservational planning and agricultural activities in Enugu State of Nigeria of West Africa, and other parts of the world. In this study, rainfall amount and duration were obtained and used in the determination of rainfall intensities in Enugu regions. These rainfall records were used in calculating the three erosivity indices namely; the maximum 30-minute intensity, kinetic energy greater than 25, and peak storm intensity. With the aid of the above data, erosivity of Enugu was uncovered. This was achieved by taking the average of the calculated years. This Erosivity value can be used in modeling a general soil loss equation for all soil types in Enugu. This general soil loss equation can be used to predict erosion in Enugu region. Also, from the force impact of rain capable of causing erosion, erosive rain were separated from non erosive rain using the method based upon the concept that there is a threshold value of intensity at which rain starts to be erosive. With this insight, one can also predict future erosive rain and non erosive rain using the weather forecast data. With the knowledge of these predictions, erosion prevention technique may be applied in areas of possible future occurrence rather than remediating soil after erosion hazard.*

Keywords— Erosivity, Soil Erosion, Rainfall, Soil Loss, Storm Intensity.

I. INTRODUCTION

Erosivity refers to the intrinsic capacity of rainfall to cause erosion. Water erosion would not occur if all rain were non - erosive, therefore erosivity is fond of the physical properties of rainfall. Rainfall indices represent the climatic influence of rainfall on water related soil erosion (Yu, 1998). The basic erosivity factors include; Amount: This is the quantity of rain that falls in any given rainfall event. The higher the amount the more erosive it becomes. Intensity: This refers to the amount of rain received during a unit time. High intensity rain are usually received during short duration, thus low intensity are of long duration. High intensity storm have large drop sizes thus will cause more erosivity. Rain-drop size: Raindrops ranges from 0.0039 inches (0.1cm) to 0.35 inches (1.0cm) in mean diameter above which they tend to break up. Smaller drops are called cloud droplets and their shape is spherical. As raindrop increases in size, its shape becomes more oblate with its larges cross section facing the on-coming air flow. Large raindrops are increasingly flattened on the bottom like hamburger buns and very large ones are shaped like parachutes. The large size is explained by condensation on large smoke particles or by collision between drops of liquid water. Drop size distribution: Some rain are made up of drops of all sizes, the proportion of large and small drops (size distribution) and how it varies in different rain affects erosivity. Much bigger rain drop sizes from thunder storm have high intensities, and are highly erosive as they strike the soil with combine force. Terminal velocity: Falling rain drop reach a maximum (terminal) velocity when the force of gravitational acceleration is equated by resistance to the drop falling through the air. The terminal velocity is a function of the drop size and it increases up to $9ms^{-1}$ from the largest drop. Mathematically, terminal velocity is calculated as;

$$V_t = \frac{2gr^2(e-\sigma)}{9N},$$

where, V_t = Terminal velocity; g = Acceleration due to gravity; r = Radius of the raindrop, e = Density of water, σ = Density of air ($1kgm^{-3}$), N = Viscosity of air (Assume 1×10^{-3}). Another factor in Erosovity determinant is the Kinetic Energy of Rainfall. This is the force impact of rain and it is a major factor in splash erosion. It is mathematically calculated as;$KE = 210.3 + 89Log_{10}I$,

where, KE = Kinetic energy (metric ton ha^{-1}); I = Rainfall intensity ($mmhr^{-1}$).

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

II. THE STUDY AREA

The study area Enugu State of Nigeria in West Africa is bounded by several other states; in the North by Benue and Kogi states, in the South by Abia and Imo states, while in the West and East by Anambra and Ebonyi State respectively. Minerals mined in Enugu state includes; coal, iron ore, fine clay, silica sand, lime stone, and marble (Wikipedia, 2014). The climate is tropical hinterland in nature and is comparatively congenial, characterized by high temperature, high humidity and substantial rainfall which is entirely seasoned, most of it falling between May and October. Rainfalls in the tropical regions are more erosive than those in the temperate regions due to the presence of strong winds and high temperature. Annual distributions of rainfall also influence the erosivity of rain.

III. MATERIALS

The major materials used in this work are the rainguage, the automated scientific weather station, and rainfall data. Rainfall data include rainfall amounts and duration. These rainfall records were obtained using the rain gauge and the automated scientific weather station which is equipped with a standard set of sensors that record; air temperature, relative humidity, rainfall amount, duration, speed and direction, soil temperature and moisture, etc., automatically every five minutes interval.

IV. METHODOLOGY

Rainfall amount, A (mm) was obtained by daily reading of the rainfall while rainfall duration, T (hr) was obtained by timing each rainstorm using the scientific automated weather station. These rainfall records were carried out in the four geographical zones of the study area; Enugu North (Nsukka), Enugu South (Awgu), Enugu West (Udi), and Enugu East (Enugu capital city). Erosivity indices were calculated as below;

i. The Peak storm intensity index (AIM):

This erosivity index is the product of the amount of rain per storm and the peak intensity of storm. This index was developed by Lal (1976) and is generally referred to as AIM index.

The peak storm intensity index is stated as thus;

$$AIM = A(mm)x\ IM(mmhr^{-1}) \quad - \quad - \quad \textbf{(i)}$$

Where, A=Total Amount of rainfall; IM= Maximum rainfall intensity (peak intensity) within the period.

Procedures: Identify the total rainfall amount, A (total in month) from the daily rainfall data (Appendix 4) and multiply it with the maximum rainfall intensity that occurred in that month. That is; A (mm) x IM ($mmhr^{-1}$). Multiply the result by (10^{-2}) to convert the unit mm^2hr^{-1} to cm^2hr^{-1}. Example: In January 2014, Total amount, A = 58.00mm, and maximum Intensity, I occurred on 27th day with 229.17$mmhr^{-1}$(From appendix 2.1.1). Thus;

Jan. 2014 =58.00mm X 229.17$mmhr^{-1}$ =13291.86mm^2hr^{-1} x 10^{-2} =132.9186cm^2hr^{-1}; or Jan. 2014 = 58.00mm x 229.17$mmhr^{-1}$, = 5.8cm x 22.917$cmhr^{-1}$, = 132.9186cm^2hr^{-1}

ii. The Kinetic Energy of Rainfall (KE):

Kinetic energy of rainfall is the force impact of rainfall, its role in soil detachment has long been recognized (Ellison, 1944), It is mathematically defined using the energy intensity relationship equation developed by Wischemeier (1969) and it stated as thus;

$$KE = 210.3 + 89log_{10}I \quad - \quad - \quad \textbf{(ii)}$$

Where; KE = Kinetic energy (ton metric hr^{-1}); I = Rainfall intensity ($mmhr^{-1}$)

iii. The Maximum 30 Minutes Intensity Index (EI_{30}):

This is a product of kinetic energy of storm and the 30-minutes intensity (greatest intensity during any 30 minute period).EI_{30} Index was computed from rainfall data by locating the greatest amount of rain that fell in any 30 minutes intensity, and multiplying the value with Kinetic energy. Wischemeier (1969) found this index to be most significantly suitable for erosion.

$$EI_{30} = EXI_{30} \quad - \quad - \quad - \quad \textbf{(iii)}$$

Where; E = kinetic energy of rainfall; I_{30} = Maximum 30minutes intensity

iv. The Kinetic Energy Greater Than 25 Index (KE > 25)

This is the force impact of rain that is capable of causing erosion. Thus, this index is used to separate erosive rain from non erosive rain (KE > 25 index is used to identify

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

erosive rain). Hudson (1971) developed KE > 25 index after he found accumulated Kinetic energy of storms with intensity greater than 25mmhr^{-1} with soil loss. This method was based upon the concept that there is a threshold value of intensity at which it starts to be erosive. This value is 25mmhr^{-1}. Mathematically;

$$KE > 25 = 210.3 + 89log_{10}(I > 25) \quad \text{(iv)}$$

Where; I> 25 = Intensities above 25mmhr^{-1}; I = Rainfall intensity (mmhr^{-1}).

V. RESULTS AND DISCUSSION

The following results were generated using the procedures discussed above. The procedure was generated for other months and years and was tabulated as thus;

Table.1: Peak Storm Intensity Index (AIM) for year 2014.

Month	TOTAL RAINFALL AMOUNT, A (mm)	MAXIMUM RAINFALL INTENSITY, IM (mmhr^{-1})	PEAK STORM INTENSITY INDEX, AIM (cm^{2}hr^{-1}) = A x IM
Jan	58.00	229.17	132.9186
Feb	8.00	72.73	5.8184
March	78.50	145.16	113.9506
April	156.00	165.00	257.4000
May	169.50	139.29	236.0966
June	371.50	218.18	810.5387
July	214.50	84.62	181.5100
Aug	211.00	184.21	388.6831
Sept	381.00	187.50	714.375
Oct	59.50	115.38	68.6511
Nov	60.50	222.73	134.7517
Dec	0.00	0.00	0.0000
Total	1768.0	1763.97	3044.6938

Note: 2014 Total rainfall Amount (A) and maximum Intensity (IM) were taken from NIMET, NWFS, Enugu State Min. Of Agriculture, while AIM index is multiplication of both A and IM.

Table.2: Peak Storm Intensity Index (AIM) for year 2013.

Month	TOTAL RAINFALL AMOUNT, A (mm)	MAXIMUM RAINFALL INTENSITY, IM (mmhr^{-1})	PEAK STORM INTENSITY INDEX, AIM (cm^{2}hr^{-1}) = A x IM
Jan	47	75.81	35.6307
Feb	0.00	0.00	0.0000
March	28.00	121.43	34.0004
April	139.00	125.00	173.7500
May	279.00	115.38	321.9102
June	236.50	72.22	170.8003
July	235.50	185.71	437.3471
Aug	168.00	140.54	236.1072
Sept	417.10	183.33	764.6694
Oct	182.60	118.64	2042.2637
Nov	59.00	91.89	54.2151
Dec	2.00	40.00	0.8000
Total	1793.7	1269.95	4271.4941

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

Note: 2013 Total rainfall Amount (A) and maximum Intensity (IM) were taken from NIMET, NWFS, Enugu State Min. of Agriculture, while AIM index is multiplication of both A and IM.

Table.3: Peak Storm Intensity Index (AIM) for year 2012

Month	TOTAL RAINFALL AMOUNT, A (mm)	MAXIMUM RAINFALL INTENSITY, IM ($mmhr^{-1}$)	PEAK STORM INTENSITY INDEX, AIM (cm^2hr^{-1}) = A x IM
Jan	4.00	44.44	1.7776
Feb	3.20	41.67	1.3334
March	2.80	26.00	0.7280
April	139.50	136.36	190.2222
May	298.00	157.14	468.2772
June	269.90	195.45	527.5196
July	343.50	175.00	601.1250
Aug	161.00	133.33	214.6613
Sept	223.00	142.31	317.3513
Oct	265.00	172.22	456.3830
Nov	49.00	125.00	61.2500
Dec	0.00	0.00	0.0000
Total	1758.9	1348.92	2840.6286

Note: 2012 Total rainfall Amount (A) and maximum Intensity (IM) were taken from NIMET, NWFS, Enugu State Min. of Agriculture, while AIM index is multiplication of both A and IM.

Table.4: Peak Storm Intensity Index (AIM) for year 2011.

Month	TOTAL RAINFALL AMOUNT, A (mm)	MAXIMUM RAINFALL INTENSITY, IM ($mmhr^{-1}$)	PEAK STORM INTENSITY INDEX, AIM (cm^2hr^{-1}) = A x IM
Jan	0.00	0.00	0.0000
Feb	112.00	52.24	58.5088
March	96.60	68.00	65.6880
April	223.40	71.96	160.7586
May	454.80	164.55	748.3734
June	637.60	162.00	1032.9120
July	482.40	174.00	839.3760
Aug	281.60	153.85	433.2416
Sept	581.60	161.54	939.5166
Oct	175.50	111.11	194.9981
Nov	0.00	0.00	0.0000
Dec	0.00	0.00	0.0000
Total	3045.5	1119.25	4473.3731

Note: 2011 Total rainfall Amount (A) and maximum Intensity (IM) were taken from NIMET, NWFS, Enugu State Min. of Agriculture, while AIM index is multiplication of both A and IM.

Table.5: Peak Storm Intensity Index (AIM) for year 2010.

Month	TOTAL RAINFALL AMOUNT, A (mm)	MAXIMUM RAINFALL INTENSITY, IM ($mmhr^{-1}$)	PEAK STORM INTENSITY INDEX, AIM (cm^2hr^{-1}) = A x IM
Jan	16.60	70.91	11.7711
Feb	16.00	136.00	21.7600
March	14.40	160.00	23.0400
April	448.60	174.29	781.8649

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

May	354.40	166.67	590.6785
June	658.00	142.86	940.0188
July	808.20	157.14	1270.0055
Aug	734.40	197.27	1448.7509
Sept	852.60	152.00	1295.9520
Oct	456.20	148.00	675.1760
Nov	0.00	0.00	0.0000
Dec	0.00	0.00	0.0000
Total	4359.4	1505.14	6118.9989

Note: 2010 Total rainfall Amount (A) and maximum Intensity (IM) were taken from NIMET, NWFS, Enugu State Min. of Agriculture, while AIM index is multiplication of both A and IM.

Table.6: Peak Storm Intensity Index (AIM) for year 2009.

Month	TOTAL RAINFALL AMOUNT, A (mm)	MAXIMUM RAINFALL INTENSITY, IM ($mmhr^{-1}$)	PEAK STORM INTENSITY INDEX, AIM (cm^2hr^{-1}) = A x IM
Jan	66.00	131.91	87.0606
Feb	0.60	30.00	0.1800
March	36.40	173.33	63.0921
April	378.40	160.00	605.4400
May	776.00	166.67	1293.3592
June	432.20	176.92	764.6482
July	736.60	165.52	1219.2203
Aug	506.80	157.11	796.2335
Sept	541.60	170.37	922.7239
Oct	756.00	205.45	1553.2020
Nov	106.20	138.46	147.0445
Dec	0.00	0.00	0.0000
Total	4336.8	1675.74	7452.2043

Note: 2009 Total rainfall Amount (A) and maximum Intensity (IM) were taken from NIMET, NWFS, Enugu State Min. of Agriculture, while AIM index is multiplication of both A and IM.

Table.7: Kinetic Energy of Rainfall for year 2014.

Month	RAINFALL INTENSITY, I ($mmhr^{-1}$)	KINETIC ENERGY, KE (metric ton ha^{-1}) = $210.3 + 89 \log_{10}(I)$
Jan	304.17	431.2959×10^{-6}
Feb	72.73	375.9913×10^{-6}
March	402.74	442.1450×10^{-6}
April	550.01	454.1956×10^{-6}
May	601.34	457.6399×10^{-6}
June	1030.23	478.4481×10^{-6}
July	481.38	449.0425×10^{-6}
Aug	640.01	460.0518×10^{-6}
Sept	1028.87	478.4036×10^{-6}
Oct	277.63	427.7715×10^{-6}
Nov	372.73	439.1546×10^{-6}
Dec	0.00	210.3000×10^{-6}
Total	5761.84	4673.1439×10^{-6}

Note: 2014 rainfall intensity, I ($mmhr^{-1}$) were taken from NIMET, NWFS, Enugu State Min. of Agriculture.

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

Table.8: Kinetic Energy of Rainfall for year 2013.

Month	RAINFALL INTENSITY, I ($mmhr^{-1}$)	KINETIC ENERGY, KE (metric ton ha^{-1}) = 210.3 + 89 $\log_{10}$(I)
Jan	75.81	377.5933 x 10^{-6}
Feb	0.00	210.3000 x 10^{-6}
March	206.05	416.2460 x 10^{-6}
April	563.75	455.1479 x 10^{-6}
May	751.03	466.2373 x 10^{-6}
June	361.12	437.9353 x 10^{-6}
July	751.68	466.2640 x 10^{-6}
Aug	428.67	444.5569 x 10^{-6}
Sept	1332	488.3805 x 10^{-6}
Oct	536.63	453.2433 x 10^{-6}
Nov	181.18	411.2709 x 10^{-6}
Dec	40	352.8869 x 10^{-6}
Total	5227.92	4980.0623x 10^{-6}

Note: 2013 rainfall intensity, I ($mmhr^{-1}$) were taken from NIMET, NWFS, Enugu State Min. of Agriculture.

Table.9: Kinetic Energy of Rainfall for year 2012.

Month	RAINFALL INTENSITY, I ($mmhr^{-1}$)	KINETIC ENERGY, KE (metric ton ha^{-1})= 210.3 + 89 $\log_{10}$(I)
Jan	44.44	356.9542 x 10^{-6}
Feb	65.00	371.6481 x 10^{-6}
March	44.75	357.2212 x 10^{-6}
April	719.66	464.5819 x 10^{-6}
May	1343.88	488.7276 x 10^{-6}
June	1350.25	488.9056 x 10^{-6}
July	1584.1	495.0822 x 10^{-6}
Aug	788.16	468.0974 x 10^{-6}
Sept	133.13	488.3271 x 10^{-6}
Oct	1631.14	496.3905 x 10^{-6}
Nov	206.82	416.3884 x 10^{-6}
Dec	0.00	210.3000 x 10^{-6}
Total	7911.33	5102.6242x 10^{-6}

Note: 2012 rainfall intensity, I ($mmhr^{-1}$) were taken from NIMET, NWFS, Enugu State Min. of Agriculture.

Table.10: Kinetic Energy of Rainfall for year 2011.

Month	RAINFALL INTENSITY, I ($mmhr^{-1}$)	KINETIC ENERGY, KE (metric ton ha^{-1}) = 210.3 + 89 $\log_{10}$(I)
Jan	0.00	210.3000 x 10^{-6}
Feb	145.45	402.7803 x 10^{-6}
March	129.28	398.2235 x 10^{-6}
April	245.48	423.0100 x 10^{-6}
May	1059.71	479.5428 x 10^{-6}
June	1298.59	487.4015 x 10^{-6}
July	880.77	472.3961 x 10^{-6}
Aug	857.56	471.3637 x 10^{-6}
Sept	1637.42	496.3638 x 10^{-6}
Oct	422.19	443.9695 x 10^{-6}

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

Nov	0.00	210.3000 x 10^{-6}
Dec	0.00	210.3000 x 10^{-6}
Total	6676.45	4714.9512x 10^{-6}

Note: 2011 rainfall intensity, I ($mmhr^{-1}$) were taken from NIMET, NWFS, Enugu State Min. of Agriculture.

Table.11: Kinetic Energy of Rainfall for year 2010.

Month	RAINFALL INTENSITY, I ($mmhr^{-1}$)	KINETIC ENERGY, KE (metric ton ha^{-1}) = 210.3 + 89 $\log_{10}$(I)
Jan	104.24	389.9020 x 10^{-6}
Feb	184.00	411.8672 x 10^{-6}
March	160.00	406.4649 x 10^{-6}
April	930.12	474.4965 x 10^{-6}
May	904.66	473.4285 x 10^{-6}
June	950.59	475.3420 x 10^{-6}
July	1353.60	489.0035 x 10^{-6}
Aug	1594.00	495.3225 x 10^{-6}
Sept	1504.43	493.0886 x 10^{-6}
Oct	826.17	469.9219 x 10^{-6}
Nov	0.00	210.3000 x 10^{-6}
Dec	0.00	210.3000 x 10^{-6}
Total	8511.81	4999.4376x 10^{-6}

Note: 2010 rainfall intensity, I ($mmhr^{-1}$) were taken from NIMET, NWFS, Enugu State Min. of Agriculture.

Table.12: Kinetic Energy of Rainfall for year 2009.

Month	RAINFALL INTENSITY, I ($mmhr^{-1}$)	KINETIC ENERGY, KE (metric ton ha^{-1}) = 210.3 + 89Log_{10} (I)
Jan	189.05	412.9174 x 10^{-6}
Feb	30.00	341.7619 x 10^{-6}
March	173.33	409.5621 x 10^{-6}
April	842.98	470.6962 x 10^{-6}
May	1087.32	480.5396 x 10^{-6}
June	843.42	470.7140 x 10^{-6}
July	1376.43	489.6532 x 10^{-6}
Aug	1294.48	487.2769 x 10^{-6}
Sept	1257.37	486.1555 x 10^{-6}
Oct	1161.64	483.0939 x 10^{-6}
Nov	245.39	423.0011 x 10^{-6}
Dec	0.00	210.3000 x 10^{-6}
Total	8501.41	5165.6718x 10^{-6}

Note: 2009 rainfall intensity, I ($mmhr^{-1}$) were taken from NIMET, NWFS, Enugu State Min. of Agriculture.

Table.13: The Maximum 30 Minutes Intensity Index (EI_{30}).

YEAR	KINETIC ENERGY, E (metric ton ha^{-1})	MAXIMUM 30MINUTES INTENSITY, I_{30}($mmhr^{-1}$)	MAXIMUM 30MINUTES INTENSITY INDEX, EI_{30}=E X I_{30}($mmhr^{-1}$)
2014	4673.1439 x 10^{-6}	139.29	650922.2138 x 10^{-6}
2013	4980.0623 x 10^{-6}	42.55	211901.6509 x 10^{-6}
2012	5102.6242 x 10^{-6}	42.55	217116.6597 x 10^{-6}
2011	4714.9512 x 10^{-6}	123.27	581212.0344 x 10^{-6}
2010	4999.4376 x 10^{-6}	139.17	695771.7308 x 10^{-6}
2009	5165.6718 x 10^{-6}	133.33	688739.0211 x 10^{-6}
Average	4939.3152 x 10^{-6}	103.36	507610.5518 x 10^{-6}

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

Table.14: Yearly Kinetic Energy, Kinetic Energy Greater Than 25, Peak Storm Intensity, and Erosivity.

YEAR	KINETIC ENERGY,KE (metric ton ha^{-1})	KINETIC ENERGY GREATER THAN 25, KE>25(metric ton ha^{-1})	PEAK STORM INTENSITY, AIM(cm^2 hr^{-1})	EROSIVITY, E (metric ton ha^{-1})
2014	4673.1439 x 10^{-6}	4723.2153 x 10^{-6}	3044.6938	650922.2138 x 10^{-6}
2013	4980.0623 x 10^{-6}	4976.9473 x 10^{-6}	4271.4941	211901.6509 x 10^{-6}
2012	5102.6242 x 10^{-6}	5061.3727 x 10^{-6}	2840.6286	217116.6597 x 10^{-6}
2011	4714.9512 x 10^{-6}	4694.3403 x 10^{-6}	4473.3731	581212.0344 x 10^{-6}
2010	4999.4376 x 10^{-6}	4998.2806 x 10^{-6}	6118.9989	695771.7308 x 10^{-6}
2009	5165.6718 x 10^{-6}	5165.6718 x 10^{-6}	7452.2043	688739.0211 x 10^{-6}
AVERAGE	4939.3152 x 10^{-6}	29619.828 x 10^{-6}	4700.2321	507610.5518 x 10^{-6}

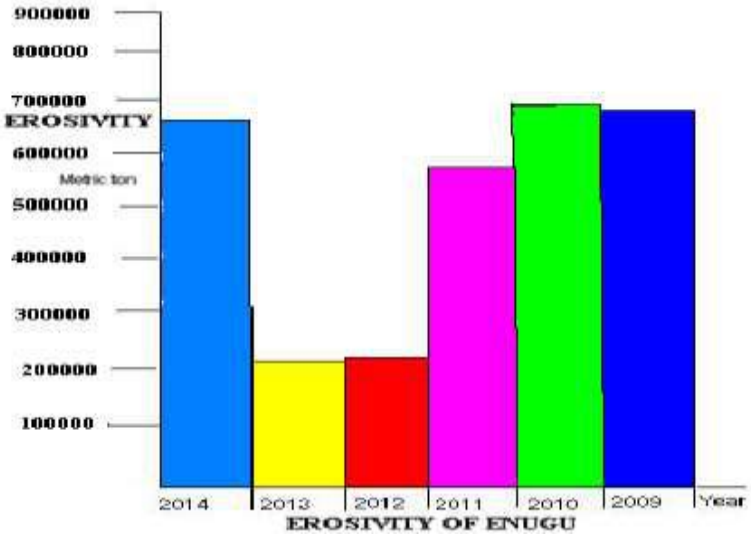


Fig. 1: Erosivity of Enugu.

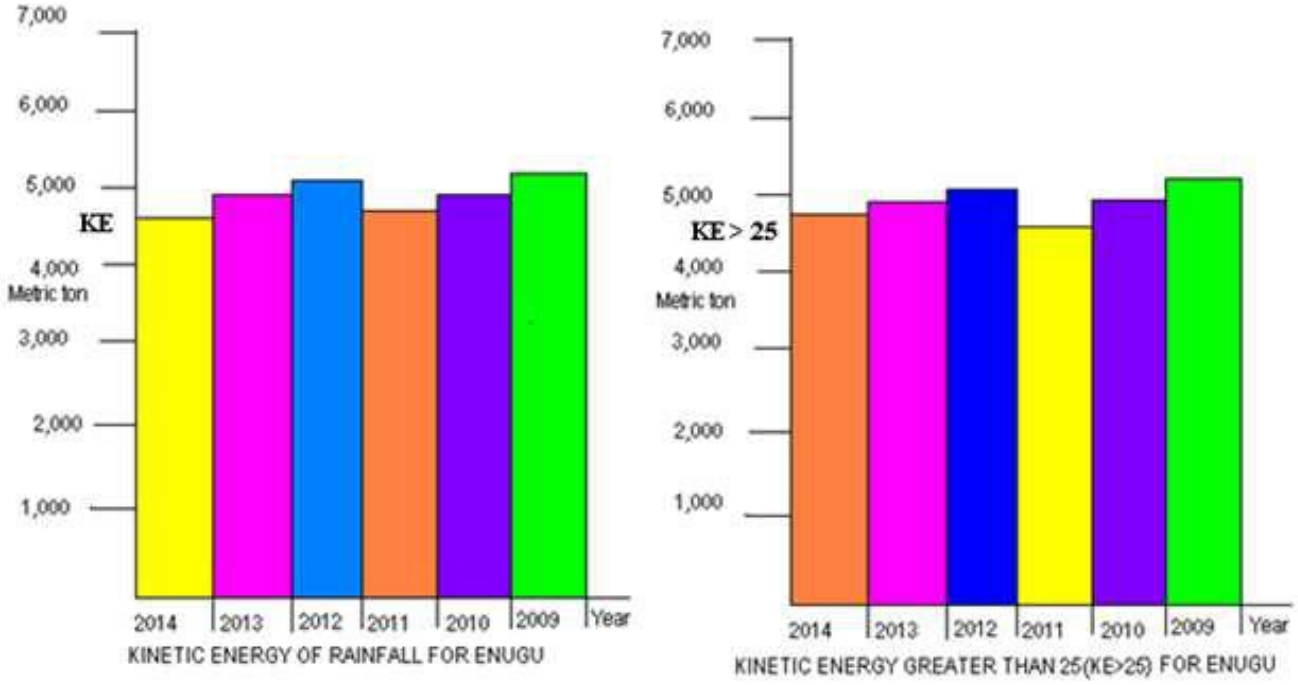


Fig. 2: Kinetic Energy and Kinetic Energy Greater Than 25 index Chart for Enugu Region.

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

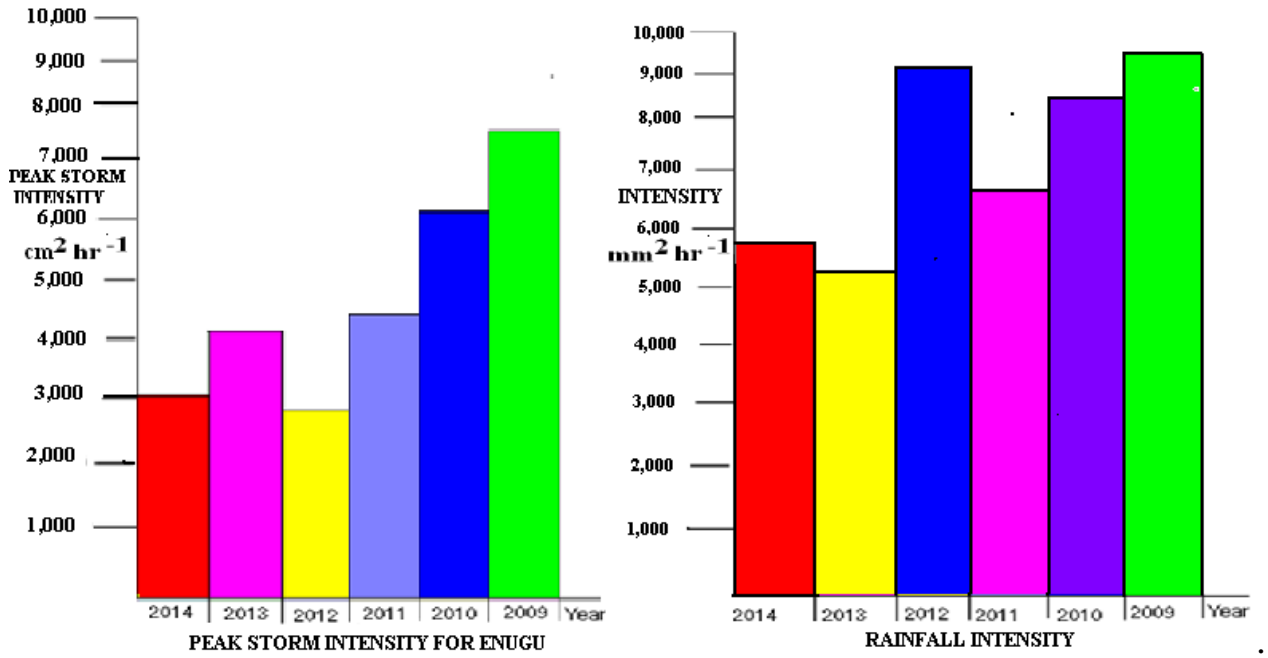


Fig.3: Peak Storm Intensity and Rainfall Intensity Chart for Enugu Region.

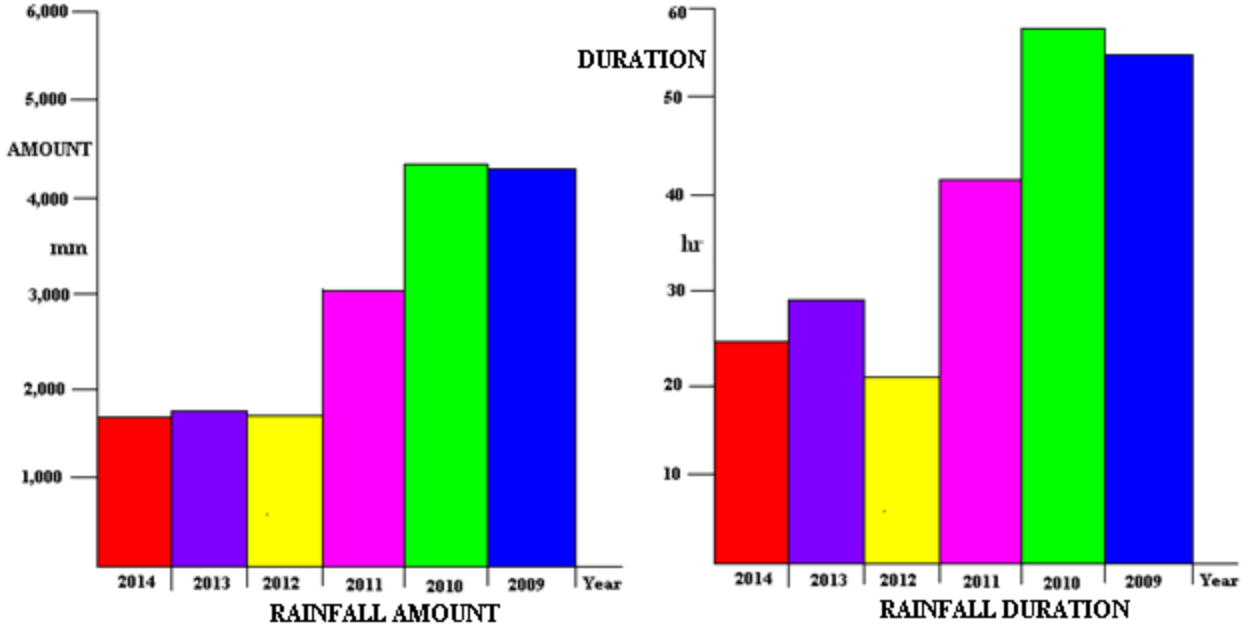


Fig. 4: Rainfall Amount and Rainfall Duration of Enugu.

VI. DISCUSSIONS

From the above calculations, Erosivity (R) in Enugu area is 507610.5518x 10^{-6}. This is equivalent to 0.5076105518and by approximation, it is 0.5. From the results, Erosivity (R) in Enugu area is not very intense since the value is 507610.5518x 10^{-6} or 0.51.Erosive rains were separated from non erosive rains in tables 1 to 6. This method maybe deployed in forecasting future erosive rains and non erosive rains. With the forecast, future erosive rainfalls, preventive measures may be carried out in spotted locations prior predictions. From universal soil loss equation, annual erosion equals the product of all the erosion variables (A = RKLSCP), thus if Erosivity, K is well manage to minimal, erosion will not occur in the controlled locations.

VII. CONCLUSION

The knowledge of Erosivity is essential to understand erosion processes, estimate soil erosion rates, predict future

http://dx.doi.org/10.22161/ijeab/2.1.11 *ISSN: 2456-1878*

erosive rainfall and non-erosive ones and in designing erosion control practices.

VIII. RECOMMENDATION

Conducting subsequent studies on Erosivity in the future years will help monitor any change.

More work and effort should be put in the prevention of erosion rather than remediation of eroded soil.

REFERENCES

[1] Atlas, 2016. Nigeria. World Map. Africa. Nigeria. World Atlas. pp: 1.

[2] Ellison W.D., 1944. Studies of Raindrop Erosion. Agric. Eng. 25. pp: 131.

[3] En. Wikipedia, 2014. Enugu. Article on Enugu. En.m.Wikipedia.org. pp: 1.

[4] En. Wikipedia, 2013. Enugu State Zones.svg.png, maps.pp: 27.

[5] Groisman P.Y., 1994. Rainfall equipments. Article: pp: 36.

[6] Hudson N.W., 1971. Soil Conservation, Bats-ford B.T. Bats Ford limited London. pp: 49-51.

[7] Hudson N.W., 1975. Erosion Control: Progress Report on Experiments, Henderson Research Station .Rhodesian Agric Journal. pp: 54-57.

[8] Lal R., 1976. Soil erosion problems on Alfi soil in western Nigeria and their control. IITA monograph No.1, Ibadan, Nigeria. pp: 208.

[9] Micheal and Ojah, 2005. Soil erosion and remediation. Published article on soil erosion. pp: 19.

[10] Stewart, 1975.Erosion and Soil Properties. Thesis on Erosion. pp: 28.

[11] Wischemeier W.H., 1969. Agricultural Journal on Rainfall Erosive Power of Rainfall.485;pp: 37.

[12] Wischemeier W.H and Smith D., 1978. Predicting Rainfall Erosion Losses: A Guide to Conservation Planning. USDA – ARS Agriculture Handbook No 537, Washington DC. pp: 58.

[13] Yu, 1998. Soil erosion Studies Part I. Article on Soil Erosion. pp: 19&31.

http://dx.doi.org/10.22161/ijeab/2.1.12 *ISSN: 2456-1878*

Optimal Environmental Conditions for Yam Storage in South East (Tropical) Zone of Nigeria

E.A. Udom[1], S.I. Oluka[2], P.C. Eze[3]

[1]Abak Irrigation Project, Cross River Basin Development Authority, Calabar, Nigeria.
[2,3]Department of Agricultural & Bioresource Engineering, Enugu State University of Science & Technology Enugu, Nigeria.

***Abstract*—** *Yam storage methods in Nigeria were studied, evaluated and compared. Among the storage methods studied, evaluated and compared include designed barn, traditional barn, house and pit storages. The parameters taken to assess their performance were tuber weight loss, rotting, sprouting and pest infestation after fourteen (14) weeks of storage. Designed barn storage methods with weight loss of 29.6996kg was compared to other storage methods with weight loss value of 58.199kg (pit storage) 46.800kg (house storage) and 47.8002kg (traditional storage) from the big tuber sizes (1.5 – 1.8kg). From the small tuber size (0.7 – 0.9kg), the weight loss recorded from each storage methods included, designed barn 24.2004kg; pit storage44.8994kg; house storage 43.4994kg and traditional barn 46.6004kg. Rotting was recorded nil from designed barn for both big tubers and small tubers and 10 tubers each were recorded from pit house and traditional barn for big tubers, pit storage recorded 20 tubers and others recorded nil for small tubers. Records on sprouting indicated the following numbers of tubers from each storage methods. From the big tubers set, designed barn had 20 tubers sprouting within 14 weeks duration but pit had 30 tubers while house storage had 20 tubers and traditional barn 30 tubers sprouted. From small sized tubers, records on sprouting indicated the following, designed barn nil, pit storage 80 tubers, house storage 40 tubers and traditional 60 tubers. Data collected from each storage facility were statistically analyzed and compared using Completely Randomized Design (CRD), ANOVA, standard deviation and LSD). Designed storage structure is recommended for use by yam farmers to alleviate their losses after harvest and to help farmers prolong the life span of their produce for future use as food, planting materials, industrial use and commercial uses.*

***Keywords*—Barn, weight loss, rotting, sprouting, storage, yam.**

I. INTRODUCTION

Yam is an important staple food crop in tropical Africa, the Pacific and the Caribbean (Adesuji, 1982).In Nigeria the most widely available or most prevalent species are the white yam (Dioscorearotundata) and the yellow yam (Dioscoreacayenensis). But the most common species worldwide is the water yam (Dioscoreaalata).Most Nigerians consider yam as the best of all staple foods. It is noteworthy to mention that yam is not only the most preferred staple food in the country on the basis of taste and texture, it is also the most widely acceptable food served at important occasions (Terry *et al.*, 1983). Yam tuber is prepared for consumption in a variety of ways. These include boiling, pounding, frying and baking. The prepared yam is normally consumed with soup, meat, stew, fish or green vegetables (Mabel, 1999). Yam plays an important role in social and religious festivals. In fact in the yam growing area, yam is a vital integral part of the cultural heritage for many people (Coursey, 1975). In Nigeria, the yam festival marks the earliest date on which new yam may be harvested or eaten. It ensures that the crop is ushered in formally and that its consumption does not occur until the community gives thanks to God and celebrates the event. Yam is normally cultivated as an annual crop, and is required in good condition for germination and propagation, as well as in good texturally sound state throughout the year for good food preparation. Conservative estimate indicate that about 15% of yam produced do not reach the market mainly because of post-harvest losses which occurs as a variety of pest, rotting, respiration, sprouting and dehydration (Courtney, 1983, Booth, 1974). These occur due to lack of appropriate storage facilities. There is the need for consideration of suitable design and development of an environment – friendly storage structure for yams. Though scientific storage such as refrigeration (Booth, 1974), curing (Gonzalez and Collazo de River, 1972), chemical treatment (Passam et al., 1976), high temperature treatment (Martin, 1955) and irradiation (Rivera et al., 1974) have been recommended, but none of these measures have been widely adopted due to their complex nature of the technology to the farmers who are currently using traditional methods (Wilson, 1980).To prevent losses, simple and economic yam storage structures are required.

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

There is also the need for consideration of a suitable design and development of environment – friendly storage structure for yams. It is hereby considered that the need for an appropriate and economic yam storage structure had to start with a proper understanding of the available traditional storage facilities in Nigeria with a view to examining their structural and environmental limitations, as well as seek for appropriate improvement. This is the thrust of this study on yam storage methods.

II. MATERIALS AND METHODS

EQUIPMENTS.

Materials and equipment used include Bamboo wood, nails, rope, oil palm leaves (raffia), tape, hammer, weighing balance (with an accuracy of ±0.05kg), thermometer (with an accuracy of ±0.01ºC) and hacksaw. The relative humidity was observed and recorded from CRBDA meteorological station. Also, the air velocity of the environment was observed and recorded from the Nigerian Meteorological Agency, Uyo, Akwa Ibom State.

LOCATION.

This research study is located at Abak Irrigation Project of the Cross River Basin Development Authority, Calabar, which lies within latitude 4º58" and longitude 7º48" with an elevation of 30m above sea level.

EXPERIMENTAL PROCEDURE

Four yam storage facilities were used namely; New Designed/Udom yam storage, Traditional storage, Traditional yam barn, House storage and Pit storage yam barn. The four storage facilities were stored with equal weight of yam tubers (360kg) comprising of two hundred (200) small yam tubers (0.70kg in size) and big size yam tubers of 1.50kg, numbering one hundred (100) tubers. Both small size and big size yam tubers (0.7kg and 1.5kg) weighing 180kg (one hundred and eighty) respectively and numbering two hundred (200) and one hundred (100) tubers were stored per facility and observed through fourteen weeks based on weight loss, rotting, sprouting and pest infestation. The experimental design was completely randomized with four (4) replications. Data collected from each storage facility were statistically analyzed and compared by using ANOVA standard deviation and least significant difference method (LSD).

THE DESIGNED YAM BARN

The Designed yam storage facility structure was constructed having a floor space size of 450 by 300 cm^2 (135,000cm^2). It has a height of 300cm from the ground level with bamboo frame work and bamboo bedding material plastered together by nails and ropes to make it more rigid. A shade is provided at the top of the structure by the use of palm tree leaves leaving adequate space for ventilation (see fig. 1).The yam tubers are arranged in line leaving some space beside each line of tubers for proper air circulation within the structure (see fig. 2). The palm leaves which dried up after some time are replaced by fresh one to ensure adequate protection from sun rays and effective cooling of the storage environment (Courtney, 1967).The effectiveness of this structure is dependent on natural air circulation within the structure, the cooling and provision of shade to the structure to regulate the storage temperature, regulation of relative humidity through natural ventilation of the structure and in addition to the arrangement of the tubers in the structure (see fig. 3)**.** Daily records of temperature of the facility were obtained for fourteen weeks. Weight of tubers were observed and recorded within the fourteen weeks duration. Other records obtained include relative humidity and wind velocity. The general sanitation of the surrounding were regularly maintained to avoid insect attack and disease.

DEAD LOAD FACTOR

A row of (0.9 x 20) kg tubers of yam
= 18 kg (small tubers of 0.9 kg)
Or (1.8 x 10) kg
= 18kg (big tubers of 1.8kg)
Area of designed yam barn (450 x 300) cm^2
= 135,000cm^2
Total weight of yam in kg in the designed yam barn = 360kg

TRADITIONAL YAM BARN

Traditional barn are shed with woven sticks walls and thatched roof (tuber and root crops manual, 1982). They may be in form where tubers are tied on vertical stakes in shaded or un-shaded area (fig 3). Yam tubers of both small and big size of 0.7 and 1.5 totaling 300 tubers (200 tubers of small size of 0.7kg and 100 tubers of big size of 1.5 kg weighing 180kg per set were used in this research work. Records on weight loss, temperature, relative humidity, wind velocity, sprouting and rotting were observed under fourteen (14) weeks.

HOUSE STORAGE

House floor of space of 450 x 300 cm (135,000cm^2) was used to store three hundred (300) yam of both small and big size of 0.7 and 1.5kg and records on weight loss, temperature, relative humidity, wind velocity, sprouting and rotting kept for fourteen (14) weeks of storage.

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

PIT STORAGE

Pit measuring 450 x 300 cm (135,000cm^2) to accommodate three hundred (300) yam tubers which comprised of small size 0.7kg and 1.8kg big sized are store to evaluate weight loss, sprouting and rotting for fourteen (14) weeks duration observed.

III. RESULTS AND DISCUSSION

WEIGHT LOSS

In the four storage structures namely Designed Barn, Pit Storage, House Storage and Traditional Storage, a set of big tubers of yam numbering 100 (hundred) and weighing 180kg was stored in each of the storage structures for 14 (fourteen) weeks. The big tubers weight stored in the Designed Storage structure recorded a decrease from 180kg to 150.3004kg indicated weight loss of 29.6996kg, while Pit Storage recorded a decrease from 180kg to 121.8006kg, indicating a loss of weight of 58.1994kg. House Storage showed a loss of 46.8006kg, while Traditional Barn Storage recorded 47.8002kg loss of weight out of 180kg (Table 1).

Table.1:Big sized (1.5-1.8kg) yam tuber storage weight loss in kg, percentage loss and average temperature in ^oC under different storage methods after 14 weeks.

Storage Structure	Original tuber weight (kg)	Weight after 14 weeks (kg)	Weight loss in kg after 14 weeks	Percentage loss (%)	Average Temperature (^oC)
Designed Barn	180	150.3004	29.6996	16.4998	27.84
Pit Storage Barn	180	121.8006	58.1994	32.333	29.16
House Storage	180	133.1994	46.8006	26.0003	28.92
Traditional Barn	180	1.32.1998	47.8002	26.5557	29.72

From the above records, decrease in weight of big tubers from the four structures were evaluated both in kilogram and percentage (table 1). The records indicated that the Designed Storage structure had the least weight loss of 29.6996kg, in the big tubers set, followed by House Storage which indicated 46.8006kg. The Traditional Barn Storage showed a loss of 47.8002kg and the highest loss in weight was from the Pit Storage which was 58.1994kg.Evaluation in percentage indicated the following percentage in respect of each structure, Designed Structure had 16.4998%, while Pit Storage had 32.333%, House Storage had 26.0003% and Traditional Barn Storage indicated a percentage loss of 26.5557%. From the above records on the four storage structures on big tubers, Designed Storage facility showed the highest efficient storage performance on storing yam tubers which reduced post harvest loss to 16.4998%. This also proves what other researchers work had proven like Booth (1974) and Coursey (1983). Also, the temperature of each storage facility were recorded as for each storage facility. From the data above, the Designed Barn produced the lowest temperature of 27.84^{o}C. This also contributed to its efficiency in storing the yam tubers.

Table.2: Small Sized (0.7-0.9kg) Yam tuber storage weight loss in kg, percentage loss and average temperature under different storage methods for 14 weeks.

Storage Structure	Original tuber weight (kg)	Weight after 14 weeks (kg)	Weight loss in kg after 14 weeks	Percentage loss (%)	Average Temperature in ^oC
Designed Barn	180	155.7996	24.2004	13.4447	27.84
Pit Storage Barn	180	135.8006	44.8994	24.9441	29.16
House Storage	180	136.5006	43.4994	24.1663	28.92
Traditional Barn	180	133.1998	46.6004	25.8891	29.72

Small tubers of yams numbering 200 tubers and weighing 180kg (one hundred and eighty kilogram) in four sets were stored in the four storing structures (table 2). After fourteen (14) weeks, their respective decrease in weight in kilogram and percentage were recorded (table 2). Designed Barn Storage structure showed a decrease in weight of 24.2004kg, which represent 13.4447% of weight loss (table 2), Pit Storage recorded 44.8994kg, that is 24.9441%, while House Storage had a fall in weight of 43.4994kg, which accounted for 24.1663% and Traditional Storage had a weight decrease of 46.6004kg, which is 25.8891% (table 2). From the records on the table 2, the Designed Barn has

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

performed outstandingly different by reducing the loss by 24.2004 which is 13.444% as compared to other storing structures. Also from the big tubers, the Designed Storage structure had significant different values of weight in kg of 29.6996 which is 16.4998% thus proving its storage efficiency in line with other research proposed range for safe storing of yams (Booth, 1994;Noon, 1978; Passam *et al.*, 1974).

Table.3: ANOVA for big sized storage weight loss under different storage for 14 weeks at p<0.05.

Source of variation	**DF**	**SS**	**MS**	**F.cal**	**F.tab5%**
Among treatment	3	23.521	7.840	50.731	0.000
Within treatment	52	8.036	0.555		
Total	55	31.557			

From the above table (table 3), it is indicated that at least one of the storing structures of the big sized yam tuber has a significant difference in weight loss.

Table.4: ANOVA for small sized yam storage weight loss under different storage methods for 14 weeks at p <0.05.

Source of variation	**DF**	**SS**	**MS**	**F.cal**	**F.tab5%**
Among treatment	3	29.846	9.949	75.752	.000
Within treatment	52	6.829	0.131		
Total	55	36.676			

The table 4 of ANOVA for small sized yam tuber also indicates a difference in weight loss from the four storage structures.

Table.5: Weight loss of big sized yam tuber from different storage methods and their standard deviation.

Storage method	**No of weeks**	**Mean (unit)**	**Standard Deviation**	**Standard Error 0.0055**
Designed Barn Storage	14	2.1214	.40984	0.10953
Pit Barn Storage	14	4.1571	.40328	0.10778
House Storage	14	3.3429	35456	0.09476
Traditional Barn Storage	14	3.4143	.26270	0.07021
Total	56	3.2589	.81660	0.10912

Table 5 indicates 2.1214kg mean weight loss from the designed barn which is the least when compare to pit storage mean weight loss of 4.1571. House storage mean weight loss of 3.3429kg and mean weight loss of traditional storage of 3.4143kg and is better to use the designed barn in storing yam tubers for future use.

Table.6: Mean weight loss of small seized yam tuber for different storage methods standard deviation, and standard error

Storage Method	**No of weeks**	**Mean (unit)**	**Standard Deviation**	**Standard Error 0.0055**
Designed Barn Storage	14	1.7286	.37092	.09913
Pit Barn Storage	14	3.2071	.23027	.06154
House Storage	14	3.1071	.48431	.12944
Traditional Barn Storage	14	3.3286	.43928	.11740
Total	56	2.8429	.75747	.10122

Table.7: Comparison of weight loss in big and small sized tuber yam under different storage methods

Tuber Sized	**Designed Storage**	**Pit Storage**	**House Storage**	**Traditional Barn**
Big size	2.12±0.41	4.16±0.40^{abc}	3.34±0.35 ^{a}	3.41 ± 0.26 ^{a}
Small size	1.734 ±0.31	3.21.±0.35 ^{a}	3.11±0.48^{a}	3.33±0.44 ^{a}

(a) P < 0.05, significantly different from designed storage

(b) P < 0.05, significantly different from house storage

(c) P < 0.05, significantly different from traditional barn, values reported as means -+ standard deviation.

BIG SIZED YAM TUBER

http://dx.doi.org/10.22161/ijeab/2.1.10 *ISSN: 2456-1878*

Weight loss from designed storage was significantly difference from the one obtained from other storage methods (table 7). That of Pit Storage was significantly different from House Storage method (P=0.000, P<0.5) and Traditional Barn (P=0.00, p<0.05). Pit Storage produced the highest weight loss (table 9).

Weight loss due to Pit Storage, House Storage and Traditional Storage were all significantly higher than that of Designed Storage (table 7). No significance difference in weight loss was observed between House Storage and Traditional Storage (P=0.142, P>0.05), House Storage and Pit Storage(P=0.148, P>0.05) although, Traditional Barn recorded the highest weight loss (table 7).

SMALL SIZED YAM TUBER

Table.8: LSD for big sized yam tuber weight loss under different storage methods at 0.05 level.

(I) Storage Method	(J) Storage Method	Mean difference (I-J)	Standard Error	Significance
Designed Storage	Pit Storage	-2.03571*	.13697	.000
	House Storage	-1.22143*	.13697	.000
	Traditional Barn	-1.29286*	.13697	.000
Pit Storage	Designed Storage	2.03571*	.13697	.000
	House Storage	.81442*	.13697	.000
	Traditional Barn	.74286*	.13697	.000
House Storage	Designed Storage	1.22143*	.13697	.000
	Pit Storage	-.81429*	.13697	.000
	Traditional Barn	-07143ns	.13697	.000
Traditional Barn	Designed Storage	1.29286*	.13697	.000
	Pit Storage	-.74286*	.13697	.000
	House Storage	-07143ns	.13697	.000

Using the LSD to evaluate the big sized yam tuber weight loss from the different storage methods at 0.05 levels indicated that designed storage is significantly difference from Pit, House and Traditional Barn (table 8). Also Pit Storage showed significant difference from Designed, House and Traditional Barn (table 8). House Storage had no significant difference from Traditional Barn but recorded significant difference from Designed and Pit Storage (table 8). Traditional Barn recorded significant different from Designed and Pit Storage but no significant difference from House Storage (table 8).

Table.9: LSD for small sized yam tuber weight loss under different storage methods at 0.05 level.

Storage Method(I)	Storage method(J)	Mean Difference (I-J)	Standard Error	Significance
Designed Storage	Pit Storage	-1.47857*	.14858	.000
	House Storage	-1.37857*	.14858	.000
	Traditional Barn	-1.60000*	.14858	.000
Pit Storage	Designed Storage	1.47857*	.14858	.000
	House Storage	.10000 Ns	.14858	.504
	Traditional Barn	.12143 Ns	.14858	.418
House Storage	Designed Storage	1.37857*	.14858	.000
	Pit Storage	-.10000 Ns	.14858	.504
	Traditional Storage	-.22143 Ns	.14858	.412
Traditional Barn	Designed storage	1.60000*	.14858	.000
	Pit Storage	.12143 Ns	.14858	.418
	House Storage	.22143 Ns	.14858	.412

*****: The mean difference is significant at the 0.05 level, **Ns**: Not significant difference.

http://dx.doi.org/10.22161/ijeab/2.1.12 *ISSN: 2456-1878*

From table 9, using the LSD to evaluate the small sized yam tuber at 0.05, significant difference recorded that Designed Storage was significant different from Pit, House and Traditional Barn. While Pit Storage indicated significant difference from the Designed Storage but indicated no significant difference from House and Traditional Barn (table 9). Also Traditional Barn recorded significant difference from the Designed Storage but no significant difference from Pit and House Storage (table 9).To further compare and evaluate the effectiveness of each structure on the big sized yam, a graph of weigh loss versus number of weeks of storage from the four storing structure namely: - Designed Barn, Pit Storage, House Storage and Traditional Barn was plotted. From the graph it is recorded that the Designed Barn had the least weight loss (fig 1). This further confirms the effectiveness of the Designed Barn in storing yam tubers. Another graph, fig. 2 also showed weight loss versus number of weeks for small size tuber yam which indicated the values of weight loss from a Designed Barn as the least compared to other storing structures.

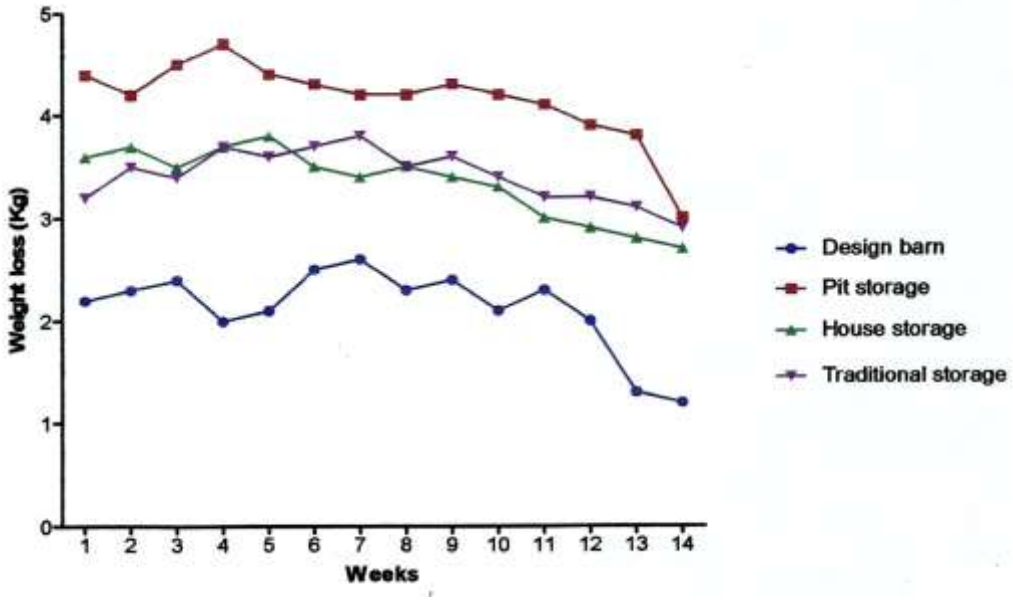


Fig.1: Weight loss versus number of weeks for big sized tuber yam under four storing methods.

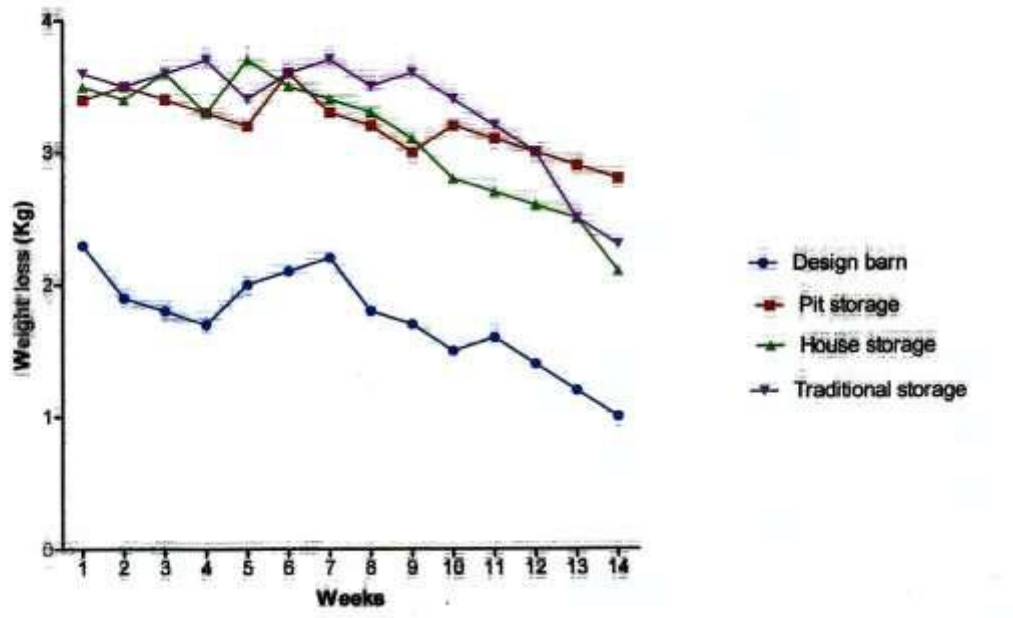


Fig.2: Weight loss versus number of weeks for small sized tuber yams under four storing methods.

http://dx.doi.org/10.22161/ijeab/2.1.12 *ISSN: 2456-1878*

ROTTING AND SPROUTING

Record on rotting from the four storing structures namely Designed Barn, Pit Storage, House and Traditional Barn indicated the following numbers of tubers that rotted from the big sized yam tuber; Nil tubers from Designed Barn, 10 tubers from Pit, House and Traditional Barn respectively (table 10). While sprouting was recorded on 20 tubers from the Designed Barn, 30 tubers from Pit Storage, 20 tubers from House Storage and 30 tubers from Traditional Barn (table 10).From the records on small sized yam tuber quality, the following records on rotting were observed; Nil from the Designed Barn, 20 tubers from the Pit Storage, Nil from the House Storage and also Nil from the Traditional Storage (table 11). Still on the small sized yam table observation on sprouting indicated thus; Nil for Designed Barn, 80 tubers from Pit Storage, House Storage had 40 tubers and Traditional Barn had 60 tubers (table 11). Evaluating on rotting on both big and small sized yam tubers, it is on record that no tuber got rotten from the Designed Barn (table 10 and table 11). This was attributed to sufficient spacing and shading within the structure and between the yam tubers which were placed individually on the shelves of the structure (fig. 3). While it was recorded in other storage structure on big sized tuber (table 10) with Pit Storage recording 20 tubers but none was recorded from other storage structures on small sized yam tuber quality (table 11). Sprouting was occurred in all the storage facilities on big sized yam tuber but less in the Designed and House Storage Barn (table 10), while Pit and Traditional Barns, recorded the same with highest number of sprouting tubers (table 10). Observation on small sized yam tuber on sprouting indicated the highest number from Pit Storage, followed by Traditional Barn (table 11).

Table.10: Effect of storage on big sized yam tubers quality under different storage structures.

S/N	Storage Structure	No. of Tubers Stored	No. of Tubers Rotting	No. of Tubers Sprouting
1	Designed Barn	100	Nil	20
2	Pit Storage	100	10	30
3	House Storage	100	10	20
4	Traditional Barn	100	10	30

Table.11:Effect of storage on small sized yam tuber quality under different storage structures

S/N	Storage Structure	No. of Tubers Stored	No. of Tubers Rotting	No. of Tubers Sprouting
1	Designed Barn	200	0	0
2	Pit Storage	200	20	80
3	House Storage	200	0	40
4	Traditional Barn	200	0	60

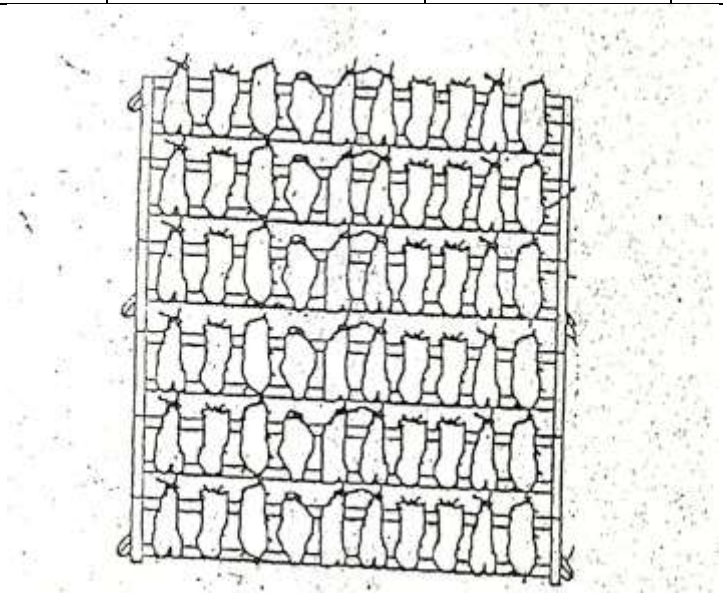


Fig. 3: Arrangement of tubers in designed barn.

http://dx.doi.org/10.22161/ijeab/2.1.12 *ISSN: 2456-1878*

IV. CONCLUSION

Based on the results of the study, the designed barn structure is very economical since it requires local materials for construction. The storage structure is also suitable for both small and large scale farmers in rural areas. It also alleviates the problems of deterioration of yam tubers and increases the financial benefits of yam farmers as well as provides good quality planting materials for farmers in Nigeria.

REFERENCES

[1] Adesuji S. A. (1982). Application of high technology to improvement of yam storage in Miege J and Lyonga S. N (ed) Yams Ignames Oxford, England et Clarendon press 312-319.

[2] Booth, R. H. (1974). Post harvest deterioration of topical root crops; losses and their control. Tropical science 16, pp49-63.

[3] Coursey D. G (1983). Post harvest losses in perishable foods in the developing world in Liebermann, M, ed. Post harvest physiology and crop protection. New York, U. S. A. plenum publishing.

[4] E. R. Terry, E.V. Doku, O. B. Arene N. M. Mahunga (1983). Tropical Root Crops. Production and Uses in Africa pp32.

[5] Matrin, W.J. (1955). Effect of Storage Temperature on Development of Internal Cork in Sweet Potato Roots Plant. Diseases Report 39, pp 620.

[6] Noon, R. A. (1978) Storage and market Diseases of Yams. Tropical Science 20 pp.1777 -188.

[7] Oji, M. A.(1999) Foods from Roots and other Crops with Recipes for variety of Dishes. New Africa Publishing Co Ltd Offong Close Akwakumo Orlu Road P. O. Box 1178, Owerri Imo State – Nigeria pp 16-22.

[8] Passam H. C. Wickham, L.D and Wilson L. A. (1982). Long Term Storage of Yam Tubers (*DioscoreaAlata L*) Tropical Science 24, 99-110.

[9] Tuber and Root Crops production manual series No. 9. (1982). pp 175-179.

http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*

Correlation of Emerging Substances and Physiological Groups of Microorganisms in Surface Water of River Moraca

Radonjic Drazana

Faculty of Science, Study group of Biology, George Washington Boulevard, Podgorica, Montenegro

***Abstract*—** *The article focuses on the reliable screening analyses of water quality of river Morača section near Podgorica, Montenegro. Sampling of screening analyses of surface water samples from the locality Vukovci, the lower course of the riverMorača during 2012 and 2013. The water samples were analysed by GC-MS. The compounds occurring most frequently in the analysed water samples were phthalates, PAHsdetergents, personal care products, flame retardants, , and corrosive residues, benzoate, pesticides, decane and the additive residues. Dibutyl phthalate, diethyl phthalate, dioctyl phthalate which are on the NORMAN list of emerging substances, and di(2-ethylhexyl) phthalate, which is on the list of the WFD priority substances, were detected in all the examined samples. A large group of terpenes, such as nerol, citronellol, menthol, ionone, and compounds as camphor, ethyl citrate or methyl jasmonate that could be found in cosmetics, personal care products or home cleaning products were determined in river samples. The presence of hormones in all the surface water samples indicates human or animal faecal pollution, while the detected caffeine in all samples confirms an anthropogenic impact.A significant number of separated organic components spaces were not defined, which is acause for performance of microbiological analysis in the presence of physiological groups of microorganisms. The identified compounds can be associated with the presence of specific physiological groups of microorganisms at the site, which can in many ways reduce environmental stress due to their functional and significant role in ecosystem.*

Keywords—water quality, emerging substances, the Morača, gas chromatography, physiological groups of microorganisms, environmental stress.

I. INTRODUCTION

In the natural aquatic environment diverse physical, chemical and biological processes occur and directly affect the content, transformation and movement of different constituents in water. A significant number of chemicals that can be found in water may have destructive impact on the environment and human health, often due to low level of knowledge and awareness, as well as lack of understanding of impacts and the toxicological implications [1].Generally, substances of concern tend to precipitate to sediment, which can represent a different level of problem, as sediment particles are often resistant to biodegradation, and most of all, have the high ability to bioaccumulate chemical substances. Emerging substances, present another level of concern, as low dose and pseudo-persistence can produce a very strong chemical and ecological stress in a long period of time, which can completely and irreversibly change the balance in the ecosystem as well as in the environment [2].

The importance of the low doses should be emphasized, especially for emerging substances (endocrine disruptive substances –EDCs), nano to pikogram (ppb toppt, respectively) concentrations, which mimic function and cycle of hormone like substances. For the purpose of emerging substances identification at the locality Vukovci (42° 27’ 81.5’’N , 19° 12’ 34.5’’E)for the first time in the Republic of Montenegro, a screening study was conducted on 3 samples of River Morača surface water in 2 separate sampling campaigns.

Water samples from the locality were taken in November 2012. and in August of 2013. Analyses were performed on gas chromatographer coupled with mass spectrometer (GC-MS), obtaining qualitative data about the chemical composition of samples, providing a range of substance groups varying from priority and hazardous, to emerging and benign. A screening analysis is a analytical process consisting of extraction, isolation and possible identification of a compound or group of compounds in a sample with the minimum number of steps and the minimal manipulation of the sample[3].

http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*

Suspected chemical that have been identified in surface water samples belong to emerging and priority groups of substances - detergents, personal care products, flame retardants, insecticides and pesticides, benzoate, pesticides, higher alkanes, additive residues were found. The screening analyses have shown a significant number of unidentified organic substances, which was the reason for microbiological analysis of water samples in the presence of physiological microorganism groups.

The surface water samples from the river Morača in locality Vukovci observed a significant presence of lipolytic bacteria: 4 900 per ml of sample, and the presence of 20.000 colonies of proteolytic bacteria per ml of sample. These data show that the bacterial population is responsible for the transformation of the most organic micropolutants in environment. During the summer sampling a significantly smaller amount of physiological groups of microorganisms was determined - the amount of proteolytic bacteria was 130 bacterium per ml of sample, and lipolytic 17 bacteria per ml of sample.

II. MATERIALSAND METHODS

Location Vukovci is a part of sedimentation zone of the lower flow of River Morača, and in this part of the flow river has the characteristics of a typical lowland river. Surface water samples for screening and microbiological analyses have been collected simultaneously in two separate campaigns in November of 2012 and August of 2013. Samples have been taken from both sides of the River Morača. During the screening analyses several groups of priority and emerging substances have been identified.

Sampling was carried out at the location Vukovci, on both sides of the river. Sampling for microbiological and chemical analysis was carried out in the littoral segment of the river and sampled in pre-sterilized dark glass bottles.

Sampling bottles were washed and dried, then sterilized at 190 °C in a dry sterilizer for one hour. During the sampling, grab sampling procedure as prescribed by Water Act, 27/2007 of Montenegro was followed in full.A disposable sterile rubber stopper is carefully removed and the bottle is opened, with one hand holding the cap and the other hand grabbing the water sample, taking into account that the cap is not contaminated.

After sampling the bottle is tightly closed with a sterile cap. Sampling bottles for chemical analyses were rinsed with surface water three times before submerging for sampling, so the glass surface is chemically harmonized with sample. Sampling for microbiological analysis was done by quickly submerging prepared bottles, so the contamination of bottle is avoided. Samples were transported to the laboratory in portable fridge.

The screening analyses were performed on gas chromatograph Agilent 7890N coupled with mass spectrometry detector Agilent 5975 at the Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University for Technology in Bratislava, Slovakia. Gas chromatography coupled with mass spectrometry analyses were performed on capillary column DB-FFAP 30 m x 250 mm I.D., 0.25 mm, in scan acquisitionmode. Carrier gas was helium with flow 1 ml/min, oven program 40 °C, 10min holding time; rate 2 °C /min to 230 °C, and splitlessinjector. Samples of surface water were prepared with liquid liquid extraction and evaporated in Kuderna Danish apparatus.

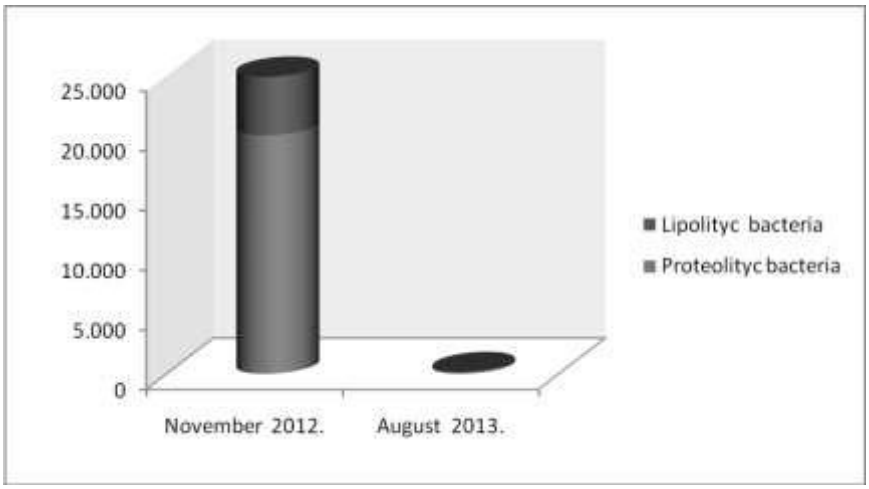
Liquid extraction was performed with different extraction solvents, polar and non-polar solvent, dichloromethane and pentane, respectively. Dichloromethane has shown to be a better choice for selected type of sample, in regard of efficiency and simplicity of liquid liquid extraction, as well as obtained chromatogram quality and mass fragments separation.

The microbiological analyses were performed in Hydrobiological Institute of Montenegro, Department of Biology. For the purpose of analyses the microbiological culture media were used and the ingredients for substrates used in this study are a product of the Institute for Immunology and Virology "Torlak" Belgrade, BioLive-Milano (Italy) and Seminem, Sarajevo (BiH). Substrates were prepared as specified by the manufacturer and sterilized in an autoclave for 15 to 20 minutes at 120 ° C under a pressure of 1.5 atmospheres.

III. RESULTS AND DISCUSSION

During the research conducted in the summer period gathered results for proteolyc and lipolytic bacteria showed lower number where proteolyc bacteria were represented in 130 bacteria per ml in a sample, and lipolytic bacteria 17 per ml in a sample. The obtained results are shown in Graphic 1.

http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*



Graphic.1: Presence of proteolyc and lipolytic bacteria during winter and summer sampling

According to [4] the obtained results, due to the low level of water and high sludge thickness in the summer period, it can be concluded that the water condition results in overweight of coliform and bacteria of fecal origin, as well as intensive anaerobic process of organic substances decomposition in the sludge.

During the study in the November of 2012, significantly high content of bacteria was observed that the water samples from the river Moračanear locality Vukovci, lipolytic as well as proteolytic, 4,900 per ml of sample and 20,000 colonies of bacteria per ml of sample, respectively. Following study analysis conducted in Decemberthe presence of lipolytic and proteolytic bacteria was also detected. The amplitudes oflipolytic bacteria distribution was observed during seasonal changes, winter to summer period [5]during the year. Studying the quality of Čerava, the author draws attention on predominance of proteolytic bacteria compared to lipolytic. If literature data is compared to conducted research, the resemblances are evident.

The conclusion is that it could be a result of great amount of organic substances in water, which determines their distribution and development, meaning that we could assume that it depends on number of phytoplankton and macrophytes as well as organic alochtone nature. Proteolyc and lipolytic organisms are organisms performing the reduction and decomposition of a chemical compound to simpler forms, by utilizing the energy for their growth[6].Conducting the bacteriological analysis of the river Koselska water quality, it was determined that heterotrophic bacteria from every sample contained minimal qualities during the spring period, where maximal was determined in September, or late summer.

In November of 2012 during the first champagne 304 compounds have been detected, 183 of those were not identified. The identified substances with quality match index (QMI) higher than 60% are shown in Table 1.

Table 1: Identified chemical components in water samples from the river Morače locality Vukovcianalyzed by GC-MS

#	Compound name (CAS)	QMI	Library	Samples
1	Benzene, methyl-	94	WILEY	1
2	Disulfide, dimethyl	95	WILEY	1
3	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	98	WILEY	1
4	2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl-	67	NIST	1
5	1-Butanol, 3-methyl- (impure)	80	WILEY	1
6	1-Pentanol	83	WILEY	1
7	2,4-Dithiapentane	61	NIST	1
8	2-Pentanol, 4-methyl-	76	NIST	1
9	Nonane, 1-chloro-	80	NIST	1
10	Benzeneethanol	60	NIST	1

http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*

11	Isopropyl myristate	64	NIST	1
12	Hexadecanoic acid, methyl ester	97	WILEY	1
13	Phenol, 2,6-dimethoxy-	60	NIST	1
14	9-Octadecenoic acid (Z)-, methyl ester	97	WILEY	1
15	Octadecanoic acid	91	WILEY	1
16	cis-9-Hexadecenoic acid	67	NIST	1
17	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.alpha.,5.alpha.)-	64	WILEY	1
18	Myristoyl chloride	60	NIST	1
19	Octadecanoic acid, 3-oxo-, methyl ester	61	NIST	1
20	Methane, dichloro-	64	WILEY	2
21	Pentane, 2,2-dimethyl-	75	NIST	2
22	Acetic acid, (1,2-dimethyl-1-propenyl) ester	65	NIST	2
23	Cyclohexane, octyl-	70	NIST	2
24	Phytol	62	NIST	2
25	Octadecane, 1-(ethenyloxy)-	60	NIST	2
26	Cyclohexane, tetradecyl-	64	NIST	2
27	Hexadecanoic acid	99	WILEY	2
28	Hexadecenoic acid, Z-11-;	78	WILEY	2
29	9-Octadecenoic acid (Z)-	97	WILEY	2
30	Octadecanoic acid, 2,3-dihydroxypropyl ester	62	NIST	2
31	2-Propanone, 1-hydroxy-	72	NIST	3
32	3-Furaldehyde	69	NIST	3
33	2-Furanmethanol	75	NIST	3
34	9-Octadecenoic acid	99	WILEY	3
35	9-Octadecenoic acid, (E)-	89	NIST	3
36	17-Octadecynoic acid	66	NIST	3
37	Sulfurous acid, butyl pentadecyl ester	66	NIST	4
38	Pentanal, 2,4-dimethyl-	80	NIST	5
39	3-Pentanol	77	NIST	5
40	1-Octanol, 2-butyl-	70	NIST	6
41	n-Hexane	71	NIST	1,2
42	3-Ethyldibenzothiophene;	78	WILEY	1,2
43	2-Propanol, 1-hydrazino-	64	NIST	1,3
44	1-Chloroundecane	64	NIST	1,3
45	1-Tridecyne	64	NIST	1,3
46	Tridecane, 6-cyclohexyl-	69	NIST	1,4
47	Nonanal		NIST	1,5
48	3-Hexanone, 2,5-dimethyl-	68	NIST	1,6
49	2-Hexanol, (S)-	77	NIST	2,3
50	1,3-Dioxan-4-one, 2-(1,1-dimethylethyl)-6-methyl-	62	NIST	2,3
51	2-Heptanol, acetate	60	NIST	2,3
52	3,3,5,5-Tetramethylcyclohexanol	69	NIST	2,3
53	4-Pyridinol-1-oxide	60	NIST	2,3
54	9,12-Octadecadienoic acid (Z,Z)-	97	WILEY	2,3
55	Dihexylsulfide	74	WILEY	2,4
56	Dodecane, 2,7,10-trimethyl-	77	NIST	2,4
57	17-Octadecynoic acid	64	NIST	2,4
58	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	81	PBM	2,5

http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*

59	Hexanedioic acid, dioctyl ester	60	NIST	4,5
60	1,6-Octadien-3-ol, 3,7-dimethyl-	67	NIST	4,6
61	trans-3-Penten-2-ol	78	NIST	1,2,3
62	2-Hexanol, 2,5-dimethyl-, (S)-	67	NIST	1,2,3
63	1-Decanol	66	NIST	1,2,3
64	2-Methyl-1-undecanol	61	NIST	1,2,3
65	Cyclodecane	71	NIST	1,2,3
66	Eicosane, 7-hexyl-	61	NIST	1,2,4
67	9-Octadecenoic acid (Z)-	99	WILEY	1,2,4
68	Hexadecanoic acid	99	WILEY	1,3,5
69	Dodecane, 2,6,11-trimethyl-	74	NIST	2,3,4
70	2-Hexyl-1-octanol	66	NIST	2,3,4
71	2-Bromotetradecane	65	NIST	2,3,4
72	Octadecane, 3-methyl-	66	NIST	2,3,4
73	Nonadecane, 2-methyl-	65	NIST	2,3,4
74	n-Caproic acid vinyl ester	75	NIST	2,3,6
75	Allopregnane; Pregnane, (5.alpha.)-	70	WILEY	2,4,5
76	1-Decanol, 2-octyl-	60	NIST	2,4,6
77	2-Butanol, 3-methyl-	74	NIST	4,5,6
78	Eicosane, 2-methyl-	73	NIST	1,3,4,5
79	7-Octen-1-ol, 3,7-dimethyl-, (S)-	62	NIST	1,4,5,6
80	7,7-Diethylheptadecane	69	NIST	2,3,5,6
81	Trifluoroacetyl-lavandulol	62	NIST	2,4,5,6
82	8-Azabicyclo[3.2.1]octan-3-amine, 8-methyl-	62	NIST	3,4,5,6
83	Hexadecane, 2,6,10,14-tetramethyl-	73	NIST	1,2,3,4,6
84	Cyclohexane, eicosyl-	68	NIST	1,2,3,5,6
85	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	72	NIST	1,3,4,5,6
86	Oxalic acid, cyclohexylmethyltetradecyl ester	61	NIST	1,3,4,5,6
87	Tetracosane	90	WILEY	1,3,4,5,6
88	Decane, 2-methyl-	81	NIST	2,3,4,5,6
89	Cyclohexane, tetradecyl-	64	NIST	2,3,4,5,6
90	Sulfurous acid, butyl dodecyl ester	71	NIST	2,3,4,5,6
91	Undecane, 3-methyl-	82	NIST	1,2,3,4,5,6
92	1-Octanol	87	NIST	1,2,3,4,5,6
93	Hexadecane	86	NIST	1,2,3,4,5,6
94	Heptadecane	96	WILEY	1,2,3,4,5,6
95	Dodecane, 2,6,10-trimethyl-	79	NIST	1,2,3,4,5,6
96	Octadecane	97	WILEY	1,2,3,4,5,6
97	Nonadecane	91	NIST	1,2,3,4,5,6
98	Eicosane	93	WILEY	1,2,3,4,5,6
99	Disulfide, di-tert-dodecyl	71	NIST	1,2,3,4,5,6
100	Eicosane, 3-methyl-	70	NIST	1,2,3,4,5,6
101	Hexadecane, 2,6,10,14-tetramethyl-	72	WILEY	1,2,3,4,5,6
102	Heneicosane	95	WILEY	1,2,3,4,5,6
103	2,6-Diisopropylnaphthalene	72	NIST	1,2,3,4,5,6
104	1-Tricosanol	64	NIST	1,2,3,4,5,6
105	Eicosane, 2,4-dimethyl-	68	NIST	1,2,3,4,5,6
106	Hydroxylamine, O-decyl-	66	NIST	1,2,3,4,5,6

http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*

107	Heneicosane, 3-methyl-	66	NIST	1,2,3,4,5,6
108	Docosane	91	WILEY	1,2,3,4,5,6
109	1-Heneicosyl formate	70	NIST	1,2,3,4,5,6
110	1-Tricosanol	60	NIST	1,2,3,4,5,6
111	Benzoic acid, 2-hydroxy-, phenylmethyl ester	90	WILEY	1,2,3,4,5,6
112	Tricosane, 2-methyl-	75	NIST	1,2,3,4,5,6
113	Tricosane	81	NIST	1,2,3,4,5,6
114	Heptadecane, 9-hexyl-	61	NIST	1,2,3,4,5,6
115	Cyclohexane, nonadecyl-	78	NIST	1,2,3,4,5,6
116	Heneicosane, 11-(1-ethylpropyl)-	74	NIST	1,2,3,4,5,6
117	Eicosane, 7-hexyl-	69	NIST	1,2,3,4,5,6
118	Octadecanoic acid	99	WILEY	1,2,3,4,5,6
119	Benzophenone	81	I.S.	1,2,3,4,5,6
120	Hexacosane	76	NIST	1,2,3,4,5,6
121	Octacosane	91	WILEY	1,2,3,4,5,6
122	Dibutyl phthalate	85	NIST	1,2,3,4,5,6
123	Diisooctyladipate	71	NIST	1,2,3,4,5,6

More than 96% of the presented literature data from treated waste- and surface waters belong to high-income countries where industrial discharges are supposed to be controlled, e.g. Good Manufacturing Practices and emission regulations in the United States [7].In contrast, there is not enough data available from low- to middle-income countries where several manufacturing facilities are located and less strict regulations are applied.

During the summer research campaigned 63 compounds have been detected, 39 of those were not identified. The identified substances with quality match index (QMI) higher than 70% are shown in Table 2.Emerging Substances in the Aquatic Environment [8].can by Selected by based on Eco toxicological criteria. [9] Separated them on hidrophyleandlipophyle.

During the preparation of water ensamples we are using liquid extraction. [10] shoes to asimportance of organic chemicals in modern societies, pointing to their negative side. Summer screening, suggests significantly less presence of chemical substances, as well as chemical components that were unable to identify trough screening analysis.

Table 2: Identified chemical components in water samples from the river Morače locality Vukovcianalyzed by GC-MS

RT (min)	**compound**	**Quality match**	**Notes**	**Samples**
90.331	Hexadecanoic acid (CAS); Palmitic acid	99	WILEY	2
96.817	9-Octadecenoic acid (Z)- (CAS); Oleic acid;	99	WILEY	3
99.507	9-Octadecenoic acid (Z)- (CAS); Oleic acid;	99	WILEY	1,2,4
92.81	Hexadecanoic acid (CAS); Palmitic acid;	99	WILEY	1,3,5
91.167	Octadecanoic acid	99	WILEY	1,2,3,4,5,6
22.613	dl-Limonene; Cyclohexene, 1-methyl-4-(1-methylethenyl)-	98	WILEY	1
81.201	Hexadecanoic acid, methyl ester (CAS); Methyl palmitate	97	WILEY	1
90.911	9-Octadecenoic acid (Z)-, methyl ester (CAS); Methyl oleate;	97	WILEY	1
97.66	Oleic Acid; 9-Octadecenoic acid (Z)-	97	WILEY	2
102.855	9,12-Octadecadienoic acid (Z,Z)- (CAS); Linoleic	97	WILEY	2,3

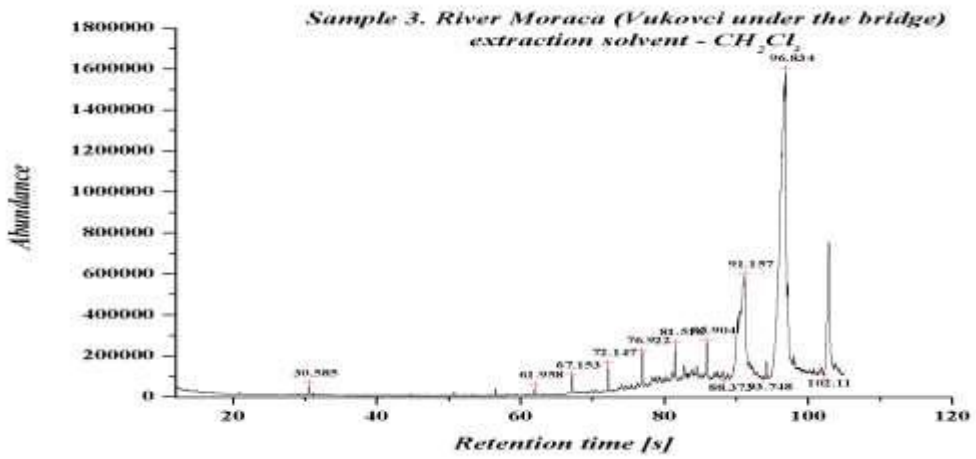
http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*

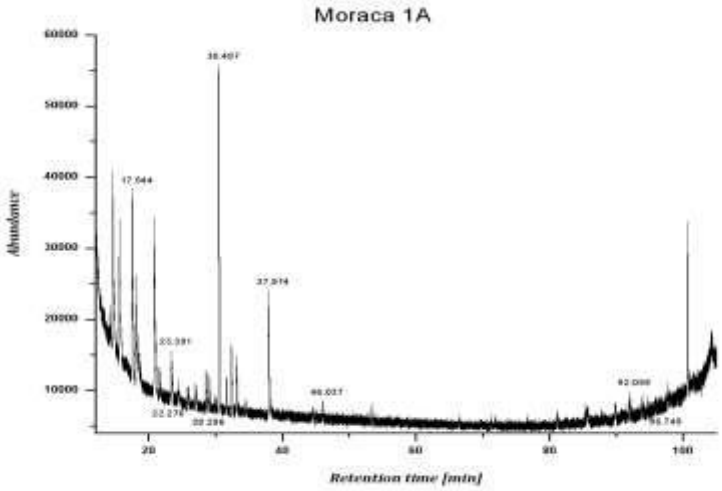
	acid;			
14.369	Disulfide, dimethyl	95	WILEY	1
95.673	Octadecanoic acid (CAS); Stearic acid;	91	WILEY	1
83.963	Benzoic acid, 2-hydroxy-, phenylmethyl ester (CAS); Benzyl salicylate;	90	WILEY	1,2,3,4,5,6
97.129	9-Octadecenoic acid, (E)-	89	NIST	3
46.969	1-Octanol	87	NIST	1,2,3,4,5,6
100.73	Dibutyl phthalate	85	NIST	1,2,3,4,5,6
26.647	1-Pentanol (CAS); Amylol	83	WILEY	1
44.716	Undecane, 3-methyl-	82	NIST	1,2,3,4,5,6
22.607	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-;	81	PBM	2,5
38.266	Decane, 2-methyl-	81	NIST	2,3,4,5,6
23.548	1-Butanol, 3-methyl- (impure)	80	WILEY	1
53.488	Nonane, 1-chloro-	80	NIST	1
60.926	Dodecane, 2,6,10-trimethyl-	79	NIST	1,2,3,4,5,6
93.869	Hexadecenoic acid, Z-11-;	78	WILEY	2
81.9	3-Ethyldibenzothiophene;	78	WILEY	1,2
20.915	trans-3-Penten-2-ol	78	NIST	1,2,3
87.389	Cyclohexane, nonadecyl-	78	NIST	1,2,3,4,5,6
30.065	2-Pentanol, 4-methyl-	76	NIST	1
53.331	2-Furanmethanol	75	NIST	3
43.798	n-Caproic acid vinyl ester	75	NIST	2,3,6
41.142	dihexylsulfide	74	WILEY	2,4
17.101	2-Butanol, 3-methyl-	74	NIST	4,5,6
88.203	Heneicosane, 11-(1-ethylpropyl)-	74	NIST	1,2,3,4,5,6
30.264	2-Propanone, 1-hydroxy-	72	NIST	3
60.735	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- ; (Nerol)	72		1,3,4,5,6
78.332	2,6-Diisopropylnaphthalene	72	NIST	1,2,3,4,5,6
101.884	Sulfurous acid, butyl dodecyl ester	71	NIST	2,3,4,5,6
73.997	Disulfide, di-tert-dodecyl	71	NIST	1,2,3,4,5,6
104.383	Diisooctyladipate	71	NIST	1,2,3,4,5,6
50.169	Cyclohexane, octyl-	70	NIST	2
74.823	1-Octanol, 2-butyl-	70	NIST	6
93.365	Allopregnane; Pregnane, (5.alpha.)-	70	WILEY	2,4,5
82.792	1-Heneicosyl formate	70	NIST	1,2,3,4,5,6
41.669	3-Furaldehyde	69	NIST	3
82.815	Tridecane, 6-cyclohexyl-	69	NIST	1,4
54.069	3,3,5,5-Tetramethylcyclohexanol	69	NIST	2,3
81.116	7,7-Diethylheptadecane	69	NIST	2,3,5,6
23.043	2-Oxabicyclo[2.2.2]octane, 1,3,3-trimethyl- ;Eucalyptol	67	NIST	1
46.421	1,6-Octadien-3-ol, 3,7-dimethyl-	67	NIST	4,6

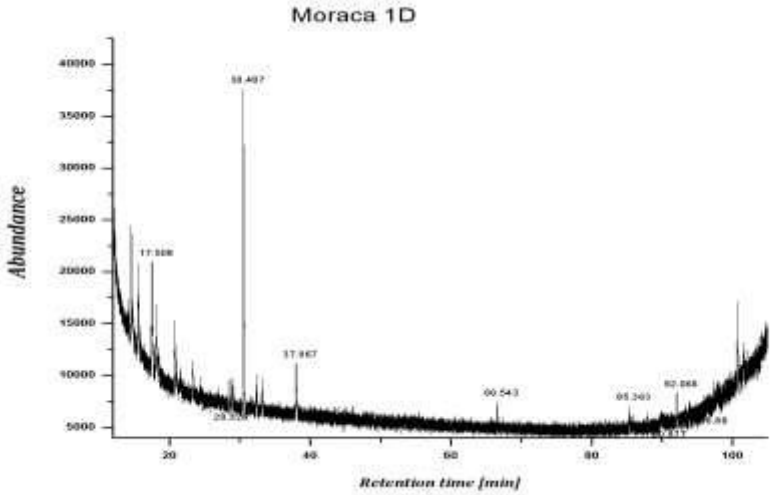
http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*

103.74	17-Octadecynoic acid	66	NIST	3
96.316	Sulfurous acid, butyl pentadecyl ester	66	NIST	4
58.105	1-Decanol	66	NIST	1,2,3
65.481	Octadecane, 3-methyl-	66	NIST	2,3,4
79.368	Hydroxylamine, O-decyl-	66	NIST	1,2,3,4,5,6
31.664	Acetic acid, (1,2-dimethyl-1-propenyl) ester	65	NIST	2
65.136	2-Bromotetradecane	65	NIST	2,3,4
72.967	Isopropyl myristate	64	NIST	1
97.762	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.alpha.,5.alpha.)-; Pinocamphone;	64	WILEY	1
13.09	Methane, dichloro- (CAS); Dichloromethane; R 30; Freon 30; Narkotil;	64	WILEY	2
72.921	Cyclohexane, tetradecyl-	64	NIST	2
49.205	2-Propanol, 1-hydrazino-	64	NIST	1,3
59.371	1-Chloroundecane	64	NIST	1,3
61.381	1-Tridecyne	64	NIST	1,3
104.062	17-Octadecynoic acid	64	NIST	2,4
78.66	1-Tricosanol	64	NIST	1,2,3,4,5,6
68.95	Phytol	62	NIST	2
103.822	Octadecanoic acid, 2,3-dihydroxypropyl ester	62	NIST	2
45.182	1,3-Dioxan-4-one, 2-(1,1-dimethylethyl)-6-methyl-	62	NIST	2,3
58.919	7-Octen-1-ol, 3,7-dimethyl-, (S)-	62		1,4,5,6
63.287	Trifluoroacetyl-lavandulol	62	NIST	2,4,5,6
72.951	8-Azabicyclo[3.2.1]octan-3-amine, 8-methyl-	62	NIST	3,4,5,6
28.949	2,4-Dithiapentane; Formaldehyde dimethyl mercaptal	61	NIST	1
104.534	Octadecanoic acid, 3-oxo-, methyl ester	61	NIST	1
59.669	2-Methyl-1-undecanol	61	NIST	1,2,3
85.038	Oxalic acid, cyclohexylmethyltetradecyl ester	61	NIST	1,3,4,5,6
66.51	Benzeneethanol (CAS); Phenethyl alcohol	60	NIST	1
83.477	Phenol, 2,6-dimethoxy-	60	NIST	1
98.516	Myristoyl chloride	60	NIST	1
72.528	Octadecane, 1-(ethenyloxy)-	60	NIST	2
45.569	2-Heptanol, acetate	60	NIST	2,3
56.085	4-Pyridinol-1-oxide	60	NIST	2,3
103.78	Hexanedioic acid, dioctyl ester	60	NIST	4,5
92.915	1-Decanol, 2-octyl-	60	NIST	2,4,6
83.162	1-Tricosanol	60	NIST	1,2,3,4,5,6

http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*







http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*

The screening analysis of water samples is indicating the present of certain emerging substances: phenolic and benzene derivates, detergents, personal care products, irritants, benzoate, pesticides, isohexadecane, Flammable substances and residues corrosives.

The reduction of emerging species is evident during August of 2013. Compared to the November of 2012. This can certainly be explained by the difference in atmospheric and water temperature, the significant reduction of river flows divergence of aquatic life and etc. During the summer and winter period the river Morača on the site of Vukovci is significantly different, by water flow and volume, which is certainly reflected onto the water quality and present of aquatic life, which can be observed in Picture 1.

Picture.1: Locality Vukovci, taken by author during the winter and summer sampling

The persistence of the chemicals identified as emerging substances, during the screening analysis conducted in the August of 2013, indicates the consistent input of certain chemicals in surface, their persistency and potency for deposition in ecosystem, and, if necessary, reactivation during optimal period.

In literature source [12]it is emphasised that the fate and content of pharmaceuticals and other emerging substances in surface and ground water can be associated with the content of coliform bacteria in water. The presence of bacteria in water shows evidence of organic influence on water quality[13].Microorganisms have the potency for adapting to new conditions and existing organic pollutants due to the relevant mutations that will spread through the population. The process is known as adaptation, characterized by longer and less reproducible initial period, before degradation can be observed. After the adaptation period the aquatic population of specific location will be able to breaks down a substance without the lengthy initial phase [14].

IV. CONCLUSIONS

The presence of physiological groups of microorganisms can be a significant indicator of organic pollution in surface water caused by chemical substances introduced into the water body from various sources. The identification of physiological groups of microorganisms in the study of locality Vukovci certainly can be correlated with the presence of emerging substances or their transformation metabolites in water.

The surface watersensitive to natural and antropogenic impacts occuring daily, which can accelerate, decelerate or pospone the transformation processes – (bio)degradation, adsorption, absorption, photolysis, hydrolysis, oxidation/reduction and etc.The significance of these processes is reflected in normal functioning of an ecosystem, natural river ecosystem. Every chaneg of chemical content in aquatic system is causing the corresponding reaction. The microorganisms are adapting to changes so the impact on natural ecosystem can be neutralized. The toxicity, persistancy and biodegradation properties of chemical entities introduced to the ecosystem (naturaly or antropogenicaly) have the most important influence onto the microorganisms and their ability to adapt to changed conditions.

Microorganisms and their activity can be a crucial indicator for a change or instability of an aquatic ecosystem as well as a powerfull mechanism of its recovery. Taking into account that the microorganisms are the best natural source of remediation, we can conclude that their presence is constant with the presence of emerging substances in selected location Vukovci.

http://dx.doi.org/10.22161/ijeab/2.1.13 *ISSN: 2456-1878*

REFERENCES

[1] Đukić, D., Jermecev, B. T. (2003): Mikrobiološkabiotehnologija."Deret" Belgrade,Vol. 503. pg.25-27

[2] Miloradov – Vojinović, M., Miloradov, M.,Sekulić-Turk, M., Radonić, J., Stošić, M., (2012): Low doses effects of Emerging substances, pseudopersistancy and hazard concequences to aquatic enviromentt and public heslth,2. International Conference "Ecology of urban areas 2012", Zrenjanin, 2012

[3] Mun˜ oz-Olivas,R. (2004) Trends in Analytical Chemistry, Vol. 23, No. 3, 203-2016, Elsevier B.V. doi:10.1016/S0165-9936(04)00318-8

[4] Radonjić, D.(2007): Utvrđivanjekvalitetavodenanekimlokacijama Skadarskogjezerai Rijeka Moračei Bojane-Magistarski rad

[5] Lokovska, L. (2003a): Bakteriološkaanalizarijeke Čerave, 32. Konferencija o aktuelnim problemimakorišćenja I zaštitevoda, Voda 2003. Zlatibor, 2003. pg. 305-309

[6] Lokovska, L. (2003b): BakteriološkakontrolakvalitetavodeKoselskereke, 32. Konferencija o aktuelnimproblemimakorišćenja I zaštitevoda, Voda 2003. Zlatibor 2003. pg. 309-314.

[7] Velagaleti, R., Burns, P.K., Gill, M. &Prothro, J. (2002). Impact of current good manufacturing practices and emission regulations and guidances on the discharge of pharmaceutical chemicals into the environment from manufacturing, use, and disposal. Environmental Health Perspectives 110(3), pg. 213-220

[8] Abdel-El-Haleem, D. (2003) Acinetobacter: environmental and biotechnological applications. African Journal of Biotechnology Vol. 2(4), pg. 71–74

[9] Ternes TA (2006). Consumption and Occurrence. In Human Pharmaceuticals, Hormones and Fragrances: The challenge of micro pollutants in urban water management , Ternes TA, Joss A (Eds.). IWA Publishing, London, UK, pp. 15-54

[10] Richardson S. D. Water analysis: emerging contaminants and current issues. *Analytical Chemistry*. 2007;79(12):4295–4323. doi: 10.1021/ac070719q. [PubMed] [Cross Ref]

[11] Al-Ahmad, A., Al-Ahmad,MW.,Schon, G., Daschner, F.D., Kummerer, K. (2000) Role of Acinetobacter for biodegradability of quaternary ammonium compounds. Bulletin of Environmental Contamination and Toxicology Vol. 64 pg. 764–770

[12] Daneshvar,A. (2012): Source, Occurrence, and Fate of Pharmaceuticals in Natural Waters-Doctoral Thesis

[13] Petrovic, O., Knežević, P., Simeunović, J., Rončević, S., Ivanović, D. (2009): IzvorišteŠtrand–mikrobiološkikvalitetpodzemnevode I sanitarnazaštita; Vodaisanitarnatehnika, 2009, Vol. 39 (5) pg. 9-14

[14] Abrahamsson, K., Klick, S. (1991) Degradation of halogenated phenols inanoxic natural marine sediments. Marine Pollution. BulletinVol. 22 pg. 227–233.

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*

Determination of Pesticide Residues in curry leaf in different markets of Andhra pradesh and Telangana, India

Priyadarshini G , Shashi Vemuri , Narendra Reddy C., Swarupa rani S

Department of Entomology and AINP on Pesticide Residues, PJTSAU, Hyderabad, Telangana state, India

***Abstract*— *Studies were conducted for determining the residues of commonly used pesticides in curry leaf samples collected every month from different markets of AP and Telangana India during 2014 and 2015. Samples collected from eight selected markets every month were analysed using QuEChERS method on LC-MS/MS.During 2014, only in the December month all the market samples contained pesticide residues whereas in the November month there were no pesticide residues from all the eight markets. During 2015, in April and December months, all the market samples showed pesticide residues, whereas in November month only two market samples had pesticide residues The most commonly detected pesticide residues were of Profenophos, Ethion, Cyfluthrin, Bifenthrin, Chlorpyriphos, Triazophos, Phorate,Methyl-parathion, cypermethrin, Fenpropathrin, Monocrotophos, Acetamaprid, Methamidophos, Acephate, Allethrin, alpha cypermethrin, Fipronil, Carbendazim, Deltamethrin, Malathion, quinalphos etc indicating that, curry leaf samples contained detectable level of the pesticides residues for which Maximum Residue Limits (MRL) are not fixed. As there are no MRLs for curry leaves, it should be considered as most important to fix MRLs to ensure food safety and consumer health and to create awareness among the farmers about the application dose, method of application and Pre Harvest Intervals.The mismanagement or non-availability of proper information about the pesticide application can lead to contamination of pesticide residues in curry leaf. The findings of this study provided important data about contamination of pesticide residues in curry leaf sold in different local markets of AP and Telangana states and hence, it is essential to conduct monitoring studies in other curry leaf growing agro climatic regions, which may serve as basis for future policy about the standards and quality control of pesticides.*

***Keywords*— *Curry leaf, QuEChERS method, Chlorpyriphos, Cypermethrin, Monocrotophos* .**

I. INTRODUCTION

Curry leaf (*Murraya koenigii*) is a leafy spice ,belongs to the Rutaceae family, is native to India, Sri Lanka, Bangladesh and the Andaman Islands.Its leaves are widely used in Indian cookery for flavouring foodstuffs. The major constituent responsible for the aroma and flavor is due to the presence of essential oils used in the soap industry.(Salikutty and Peter, 2008), it has anti carcinogenic properties due to the presence of carbazole alkaloids,(Khanum et al.,2000).Traditionally curry leaf is used in Ayurvedic medicines for treating many diseases. Oxidative stress related diseases are treated by extensive use of synthetic antioxidants which in turn causes unwanted side effects, hence there is increasing interest of using naturally occurring antioxidants(Maxwell, 1995). Curry leaves can be used as antioxidants as they contain the antioxidants tocopherol,b-carotene and lutein (Palaniswamy,2001). As a rich source of antioxidants curry leaf showed highest antioxidant and free radical scavenging activity (Mylarappa et al.,2008). The phyto chemical constituents of Murraya koenigii are also useful in waste water treatment to reduce the effect of harmful compounds (Sharmila et al.,2013). Curry leaf is now grown throughout India and attacked and damaged by number of pests and diseases at various stages of its growth. As a part of crop protection and for increasing crop yields,curry leaf farmers are using wide range of chemicals leaving residues in the plant parts consumed as food (Agnihotri 1999), which enters food chain directly or indirectly. European union, the major importers of curry leaf have sent a red alert message that the residues in curry leaves are much more than the permissible limits, which created a panic among the exporters.Since there is a need to analyse the pesticides used by farmers in the market samples at different locations and to create awareness among the consumers, farmers and extension workers and also to suggest them the proper dosages, waiting periods etc. these studies were taken up to

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*

know the type of pesticides used by the farmers and their residues in samples at market.

II. MATERIALS AND METHODS

Market samples of curry leaf were collected from different markets in AP and Telangana every month from 2014 to 2015 Curry leaf sample of 1 kg (1/4 kg each, randomly from four different vendors in the market were collected from these eight markets. Samples were extracted for pesticide residues following the validated QuEChERS method utilizing LC-MS/MS

Sample extraction procedure curry leaf samples were analyzed for pesticide residues following the AOAC official method 2007.01 (QuEChERS) after validation of the method in the laboratory. The samples were collected from different markets . Each sample was homogenized separately with robot coupe blixer and homogenized 15 ±0.1g sample was taken in 50 ml centrifuge tube and 30±0.1 ml acetonitrile was added to sample tube. The sample was homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher. 3±0.1 g sodium chloride was added to sample, mixed thoroughly by shaking gently followed by centrifugation for 3 min at 2500-3000 rpm to separate the organic layer. The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube and added with 9±0.1 g anhydrous sodium sulphate to remove the moisture content. 8 ml of extract was taken in to 15 ml tube, containing 0.4±0.01 g PSA sorbent (for dispersive solid phase d-SPE cleanup),1.2±0.01 g anhydrous magnesium sulphate and 0.05 g of GCB (Graphatised Carbon Black) , AOAC official method 2007.01 suggests that , it is desirable to add 50 mg of GCB per milliliter of extract for any commodities with higher pigments such as green leafy vegetables. The sample tube was vortexed for 30 sec then followed by centrifugation for 5 min at 2500-3000rpm. The extract of about 1 ml (0.5 g sample) was taken for analysis on LCMS/MS under standard operational conditions(Table-1).Certified Reference Materials (CRM) of different pesticides having purity ranging from 95.10to 99.99 per cent were stored in a freezer at low temperature, with light and moisture excluded. Solvents used in the study were all glass distilled before use. Sodium sulphate, sodium chloride and magnesium sulphate were activated in hot air oven at 450 °C for 5 h. A weighed amount of analytical grade material of each pesticide was dissolved in a minimum quantity of distilled acetone and diluted with methanol to obtain a stock solution of 1000 mg kg-1. The intermediate standards and working standards of 0.5, 0.25,0.1, 0.05, 0.025 and 0.01 mg kg-1 were prepared by suitably diluting the stock solution in methanol and used as standard check in analysis, linearity and recovery studies(Table-2).

METHOD VALIDATION

The analytical method for estimation of residues of pesticides in curry leaves has been validated by conducting recovery studies using control samples. 15g of sample was taken in 50 ml centrifuge tubes in three replicates, each were spiked with pesticide mixture at the required fortification levels ie.LOQ, 5x LOQ and 10x LOQ, adding an appropriate volume of working standard. This mixture was then shaken to attain a proper homogeneity of pesticides in the samples. The tubes containing fortified samples were left open for a while, just to allow the evaporation of excess solvent. Sample extraction procedure was followed as given above.

III. RESULTS AND DISCUSSION

Samples collected from eight different markets every month during 2014 and 2015 were analysed and the results are presented hereunder. It is observed that in some samples there were no pesticide residues. During 2014, only in the December month all the market samples contained pesticide residues whereas in the November month there were no pesticide residues from all the eight markets. During 2015, in April and December months, all the market samples showed pesticide residues, whereas in November month only two market samples had pesticide residues. Number of markets detected with pesticide residues month-wise in curry leaf samples during 2014-15 are depicted in figures (1-9).

Mehidipatnam rythubazar In the year 2014, pesticides were not detected during the months of February, May, July, August and November; whereas in the other months notable number of pesticides i.e.,15 insecticides were detected. Of the 12 curry leaf samples analysed, four (33.33%) samples were highly contaminated with ethion while one (8.33%) sample was least contaminated with acephate, dimethote, methamidophos, methyl parathion, phorate, quinalphos, triazophos, fenpropathrin, permethrin and fipronil. Residue levels of triazophos was high (4.330 mg kg^{-1}) followed by profenophos (3.352 mg kg^{-1}) while methyl parathion was least (0.050 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 and number of pesticides detected month wise during 2014-15 presented in figure 1. During 2015, pesticides were not detected during the months of July and August; whereas in the other months notable number of pesticidesi.e.,20 insecticides and three fungicides were detected. Of the 12 curry leaf samples analysed, seven (58.33%) samples were highly

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*

contaminated with cypermethrin and acetamiprid while one (8.33%) samples were least contaminated with anilophos, phorate, phosphamidon, quinalphos, lambda cyhalothrin, spiromesifen and spirotetramat. Residue levels of acetamiprid was high (5.468 mg kg^{-1}) followed by profenophos (4.728 mg kg^{-1}) while spirotetramat was least (0.056 mg kg^{-1}). Besides insecticides, among the 12 curry leaf samples analysed during 2015, three (25%) samples were highly contaminated with tebuconazole while one (8.33%) sample was least contaminated with carbendazim and trifloxystrobin. Residue levels of tebuconazole was high (2.722 mg kg^{-1}) followed by carbendazim (0.365 mg kg^{-1}) while trifloxystrobin was least (0.183 mg kg^{-1}).

Erragadda rythubazar

In the year 2014, pesticides were not detected during the months of, September, October and November; whereas in the other months notable number of pesticides i.e.,11 insecticides and one fungicide were detected. Among the 12 curry leaf samples analysed, residue levels of profenophos was high (5.728 mg kg^{-1}) followed by acetamiprid (4.468 mg kg^{-1}) while thiamethoxam was least (0.068 mg kg^{-1}). Five (41.67%) samples were highly contaminated with cypermethrin while one (8.33%) sample was least contaminated with triazophos, cyfluthrin and thiamethoxam. Of the 12 curry leaf samples analysed during 2014, 8.33 per cent samples were contaminated with fungicide tebuconazole with residue concentration of 3.293 mg kg^{-1}. Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 and number of pesticides detected month wise during 2014-15 is presented in figure.2. During 2015, pesticides were not detected during the months of, September and October; whereas in the other months notable number of pesticides i.e., 24 insecticides, one fungicide and one herbicide were detected. Of the 12 curry leaf samples analysed, six (50.00%) samples were highly contaminated with chlorpyriphos while one (8.33%) sample was least contaminated with chlorpyriphos-methyl, methyl parathion, phorate, triazophos, allethrin, alpha-cypermethrin, cyfluthrin, cypermethrin, permethrin, spiromesifen, spirotetramat, acetamiprid and abamectin. Residue levels of profenophos was high (25.690 mg kg^{-1}) followed by acetamiprid (11.98 mg kg^{-1}) while allethrin was least (0.046 mg kg^{-1}). Of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with herbicide pendimethalin with residue concentration of 0.28 mg kg^{-1}. 16.67 per cent samples were contaminated with fungicide tebuconazole with residue range of 0.38-10.29 mg kg^{-1}.

L. B. Nagar rythubazar Pesticides were not detected during the months of, July, August and November of the year 2014; whereas in the other months considerable number of insecticides i.e., 13 were detected. Of the 12 curry leaf samples analysed, two (16.67%) samples were highly contaminated with chlorpyriphos, monocrotophos, triazophos, cypermethrin and acetamiprid while one (8.33%) sample was least contaminated with acephate, chlorpyriphos-methyl, ethion, methamidophos, profenophos, bifenthrin, fenpropathrin and lambda-cyhalothrin. Residue levels of acephate was high (8.179 mg kg^{-1}) followed by triazophos (2.47 mg kg^{-1}) while chlorpyriphos-methyl was least (0.103 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 and number of pesticides detected month wise during 2014-15 is presented in figure.3. Pesticides during 2015, not detected during the month of November; whereas in the other months notable number of pesticides i.e., 16 insecticides and one fungicide were detected. Of the 12 curry leaf samples analysed, five (41.67%) samples were highly contaminated with ethion while one (8.33%) sample was least contaminated with acephate, quinalphos, triazophos and alpha-cypermethrin. Residue levels of profenophos was high (15.439 mg kg^{-1}) followed by cypermethrin (10.81 mg kg^{-1}) while methyl parathion was least (0.053 mg kg^{-1}). Of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with fungicide carbendazim with residue concentration of 0.05 mg kg^{-1}.

Nalogonda rythubazar

In the year 2014, pesticides were not detected during the months of, January, February, March, May, August, September, October and November; whereas in the other months notable number of insecticides i.e., five were detected. Of the 12 curry leaf samples analysed, one (8.33%) sample was contaminated with chlorpyriphos, methamidophos, alpha-cypermethrin, cyfluthrin and deltamethrin. Residue levels of cyfluthrin was high (0.479 mg kg^{-1}) followed by methamidophos (0.256 mg kg^{-1}) while chlorpyriphos was least (0.036 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014, and number of pesticides detected month wise during 2014-15 are presented in figure. 4.

During 2015, pesticides were not detected during the months of, March, May, August, September, October and November; whereas in the other months notable number of pesticides i.e., 11 insecticides and one herbicide were detected. Of the 12 curry leaf samples analysed, three (25%) samples were highly contaminated with profenophos

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*

and bifenthrin while one (8.33%) sample was least contaminated with chlorpyriphos, ethion, monocrotophos, phosphomidon, deltamethrin and fenpropathrin. Residue levels of acetamiprid was high (1.136 mg kg^{-1}) followed by profenophos (1.088 mg kg^{-1}) while phosphamidon was least (0.064 mg kg^{-1}). Besides insecticides, of the 12 curry leaf samples analysed during 2015, 16.67 per cent samples were contaminated with herbicide atrazine with residue range of 0.048-0.628 mg kg^{-1}.

Warangal rythubazar

In the year 2014, pesticides were not detected during the months of, January, February, March, May, June, August and November; whereas in the other months notable number of insecticides i.e., eight were detected. Of the 12 curry leaf samples analysed, three (25%) samples were highly contaminated with cyfluthrin, while one (8.33%) was least contaminated with methyl parathion, profenophos, triazophos and fenpropathrin. Residue levels of cyfluthrin was high (3.934 mg kg^{-1}) followed by phorate (0.548 mg kg^{-1}) while methyl parathion was least (0.053 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 are presented in table 4.16, and number of pesticides detected month wise during 2014-15 is presented in figure 4.6. During 2015, pesticides were not detected during the months of, March, August and November; whereas in the other months notable number of pesticides i.e., ten insecticides and one fungicide were detected. Of the 12 curry leaf samples analysed, six (50%) samples were highly contaminated with chlorpyriphos while one (8.33%) sample was least contaminated with acephate, chlorpyriphos-methyl, dimethoate, monocrotophos, bifenthrin and cypermethrin. Residue levels of ethion was high (2.136 mg kg^{-1}) followed by chlorpyriphos (0.918 mg kg^{-1}) while cypermethrin was least (0.052 mg kg^{-1}). Besides insecticides, of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with fungicide carbendazim with residue concentration of 0.05 mg kg^{-1}. Number of samples contaminated, per cent contamination and residue range of all pesticides during 2015 are presented in fig.5

Guntur rythubazar

In the year 2014, pesticides were not detected during the months of, February, May, July, August and November; whereas in the other months notable number of insecticides i.e., 11 were detected. Of the 12 curry leaf samples analysed, three (25%) samples were highly contaminated with ethion, while one (8.33%) sample was least contaminated with acephate, chlorpyriphos, methamidophos and monocrotophos. Residue levels of cyfluthrin was high (12.654 mg kg^{-1}) followed by cypermethrin (5.510 mg kg^{-1}) while methamidophos was least (0.054 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 are presented in table, and number of pesticides detected month wise during 2014-15 is presented in figure During 2015, pesticides were not detected during the months of, March, July, August and November; whereas in the other months notable number of pesticides i.e., 21 insecticides and three fungicides were detected. Of the 12 curry leaf samples analysed, six (50%) samples were highly contaminated with acetamiprid while one (8.33%) sample was least contaminated with chlorpyriphos-methyl, diazinon, dichlorvos, dimethoate, ethion, methamidophos, triazophos, cyfluthrin, cypermethrin, lambda-cyhalothrin, imidacloprid, carbofuran and abamectin. Residue levels of acephate and were high (8.179 mg kg^{-1}) followed by acetamiprid (8.159 mg kg^{-1}) while imidacloprid was least (0.056 mg kg^{-1}). Besides insecticides, of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with fungicides like myclobutanil (0.459 mg kg^{-1}), tebuconazole (1.824 mg kg^{-1}) and trifloxystrobin (0.332 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range of all pesticides during 2015 are presented in fig.6

Nellore rythubazar

In the year 2014, pesticides were not detected during the months of, March, April, May, June, August, September, October and November; whereas in the other months notable number of insecticides i.e., ten were detected. Of the 12 curry leaf samples analysed, two (16.67%) samples were highly contaminated with cyfluthrin, while one (8.33%) was least contaminated with acephate, chlorpyriphos, dimethoate, ethion, methamidophos, monocrotophos, profenophos, bifenthrin and fenpropathrin. Residue levels of cyfluthrin was high (5.428 mg kg^{-1}) followed by profenophos (4.940 mg kg^{-1}) while methamidophos was least (0.171 mg kg^{-1}). Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 are presented in table 4.20, and number of pesticides detected month wise during 2014-15 is presented in figure 4.8. During 2015, pesticides were not detected during the months of, February, August, September, October and November; whereas in the other months notable number of pesticides i.e., 15 insecticides, three fungicides and one herbicide were detected. Of the 12 curry leaf samples analysed, four (33.33%) samples were highly contaminated with monocrotophos and profenophos while one (8.33%) sample was least contaminated with anilophos, dichlorvas, dicofol, dimethoate, cypermethrin,

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*

fenpropathrin, lambda-cyhalothrin and carbofuran. Residue levels of acetamiprid was high (6.748 mg kg^{-1}) followed by monocrotophos (5.98 mg kg^{-1}) while chlorpyriphos was least (0.051 mg kg^{-1}).Besides insecticides, of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with fungicides like carbendazim (0.08 mg kg^{-1}), tebuconazole (0.2 mg kg^{-1}) and trifloxystrobin (0.332 mg kg^{-1}). In addition to these 16.67 per cent of samples were contaminated with one herbicide namely, pendimethalin with residue range of 0.052-0.072 mg kg^{-1}. Number of samples contaminated, per cent contamination and residue range of all pesticides during 2015 are presented in fig.7

Vijayawada rythubazar

In the year 2014, pesticides were not detected during the months of, May, August and November; whereas in the other months notable number of pesticides i.e., 16 insecticides and one fungicide were detected. Of the 12 curry leaf samples analysed, three (25%) samples were highly contaminated with ethion and cyfluthrin while one (8.33%) sample was least contaminated with acephate, chlorpyriphos, triazophos, allethrin, alpha-cypermethrin, cypermethrin, deltamethrin, fenpropathrin, lambda-cyhalothrin, acetamiprid and imidacloprid. Residue levels of profenophos was high (21.546 mg kg^{-1}) followed by cypermethrin (10.810 mg kg^{-1}) while chlorpyriphos was least (0.02 mg kg^{-1}).

Besides insecticides, of the 12 curry leaf samples analysed during 2014, 8.33 per cent samples were contaminated with fungicide metalaxyl with residue concentration of 0.137 mg kg^{-1}. Number of samples contaminated, per cent contamination and residue range of all insecticides during 2014 is presented in table 4.22, and number of pesticides detected month wise during 2014-15 is presented in figure 4.9. During 2015, pesticides were not detected during the months of, January, May, June, July and November; whereas in the other months notable number of pesticides i.e., 17 insecticides, two fungicides and one herbicide were detected. Of the 12 curry leaf samples analysed, five (41.67%) samples were highly contaminated with bifenthrin while one (8.33%) samples were least contaminated with anilophos, chlorpyriphos-methyl, phosphomidon, profenophos, cypermethrin, imidacloprid and abamectin. Residue levels of acetamiprid was high (11.976 mg kg^{-1}) followed by monocrotophos (10.292 mg kg^{-1}) while thiamethoxam was least (0.068 mg kg^{-1}). Besides insecticides, of the 12 curry leaf samples analysed during 2015, 8.33 per cent samples were contaminated with fungicides like carbendazim (0.256 mg kg^{-1}), and tebuconazole (0.056 mg kg^{-1}). Besides, 16.67 per cent of samples were contaminated with herbicide namely, pendimethalin with residue range of 0.072-0.280 mg kg^{-1}. Number of samples contaminated, per cent contamination and residue range of all pesticides during 2015 is presented in 8

Results of market samples are indicative of indiscriminate and over use of insecticides by the curry leaf growers Fig.9. It also indicates that farmers are neither adopting good agricultural practices (GAP) nor observing safe waiting period. Thus, constant monitoring from time-to-time is essential for maintaining up-to-date information on pesticide residues and guidelines for the manufacturers and users. The results are in line with the work done by Beena kumari (2007) who reported that, of the 60 market samples analysed, 4-100 per cent contamination with low but measurable amounts of residues of four major chemical groups i.e., organochlorine, organophosphate, pyrethroid and carbamate pesticide was recorded. Residues of cypermethrin, chlorpyriphos and permethrin, each in two samples of brinjal, cabbage and cauliflower, exceeded their respective MRL values thereby showing 10 per cent samples with residues above maximum residue limits. Fifty vegetable samples in Kolar district of Karnataka were analysed and found that of all the samples contaminated, the organochlorines (97%) dominated followed by organophosphates (83%) and pyrethroids (60%). However, 58 per cent of the samples were found to contain the residues of these insecticides above their respective maximum residue limits (MRL). The results obtained in the present investigations are in agreement with earlier reports by Fytianos *et al.* (1985) Presence of organochlorine compounds in food commodities reported by many researchers like Kumari *et al.* (1996), Kumari *et al.* (2003), Kaphalia *et al.* (1990) revealed wide spread contamination due to these pesticides although their use has been either banned or restricted during the last one decade Hence, the present research will not only serve as reference document but also be helpful in taking necessary and timely preventive measures to mitigate such problems. As reproted by Swarupa et al., (2016) the increase in frequency and magnitude of residues in the curry leaf could be attributed to indiscriminate and over use of pesticides by farmers despite efforts by various concerned agencies. It has been found that the farmers are neither following recommended waiting periods nor abide by good agricultural practices (GAP). (Bhanti et al.,2004). Therefore an effective way of educating the farmers via training and electronic media is advised particularly in view of the export potential of the crop. A periodical monitoring studies of pesticide residues may be extended

to different agro climatic regions to know actual status of contamination and to strengthen the confidence of consumer in quality of food as well as food quality control authorities for future policies.

REFERENCES

[1] Agnihotri N P (1999) Pesticide safety evaluation and monitoring. All India Coordinated Research Project (AICRP) on Pesticide Residues, Division of Agricultural Chemicals, Indian Agricu tural Research Institute,NewDelhi,pp.132–142

[2] Beena Kumari. 2007. Monitoring of pesticide residues in vegetables of Hisar market. *Haryana Journal of Horticultural Sciences*. 36 (1 and 2): 175-179.

[3] Bhanti,M., Shukla, G. and Taneja, A. (2004) Contamination levels of organochlorine pesticides and farmers' knowledge, perception, practices in rural India–A case study,Bull Environ Contam Toxicol,73, 787-793.

[4] Fytianos, K., Vasilikiotis, G., Weil, L., Kavlendis, E and Laskaridis, N. 1985. Preliminary study of organochlorine compounds in milk products, human milk and vegetable. *Bulletin of Environmental Contamination and Toxicology*. 34: 504–508.

[5] Kaphalia, B. S., Takroo, R., Mehrotra, S., Nigam, U and Seth, T. D. 1990. Organochlorine pesticide residues in different indian cereals, pulses, vegetables, spices, fruits, milk, butter, desi ghee and edible oil. *Journal of AOAC.* 73: 509-512.

[6] Khanum,F., Anilakumar, K.R.,Sudarshana Krishna, K. R., Viswanathan, K. R.,& Santhanam, K.(2000). Anti carcinogenic effects of curry leaves in dimethyl hydrazine- treated rats. Plant Foods for Human Nutrition, 55, 347–355.

[7] Kumari B., Singh, R., Madan, V. K., Kumar, R., Kathpal, T. S and Singh, J. 2003. Magnitude of pesticidal contamination in winter vegetables from Hisar, Haryana. *Environmental Monitoring Assessment.* 87: 311-318.

[8] Kumari, B., Singh, R., Madan, V. K., Kumar, R and Kathpal, T. S. 1996. DDT and HCH compounds in soils, ponds and drinking water of Haryana, India. *Bulletin of Environmental Contamination and Toxicology*. 57: 787-793.

[9] Salikutty Joseph and K. V. Peter, Curry leaf (Murraya koenigii), perennial, nutritious, leafy vegetable , Economic Botany 2008 , 39 (1): 68-73.

[10] Sharmila S, Jeyanthi Rebecca L and MdSaduzzaman(2013) Biodegradation of domestic effluent using different solvent extracts of *Murrayakoenigii, J. Chem. Pharm. Res., 2013, 5(2):279-282*

[11] Swarupa,S., Shashi Vemuri and Venkateswara Reddy, V. (2016). Determination of Pesticide Residues in curry leaf in local markets of Hyderabad, India, International Journal of Environment Agriculture and Biotechnology 1(3):386-392.

Table.1: LC MS/MS Operating Parameters

LC-MS/MS	***SHIMADZU LCMS/MS - 8040.***		
Detector	*Mass Spectrophotometer*		
Column	*Kinetex, 2.6µ, C18 Column, 100 x3.0.*		
Column oven temperature	*40°C*		
Nebulizing gas	*Nitrogen*		
Nebulizing gas flow	*2.0 litres/min*		
Pump mode/ flow	*Gradient / 0.4 ml/ min*		
Solvents	*A:Ammonium Formate in Water (10Mm)* *B: Ammonium Formate in Methanol(10Mm)*		
LC programme	*Time*	*solvent*	*Conc*
	0.01	*B Conc*	*35%*
	2.00	*B Conc*	*35%*
	7.00	*B Conc*	*60%*
	9.00	*B Conc*	*60%*
	14.00	*B Conc*	*95%*
	17.00	*B Conc*	*85%*
	19.00	*B Conc*	*70%*

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*

	21.00 *24.00*	*B Conc* *B Conc*	*35%* *35%*
Total Time Programme	*24 min*		

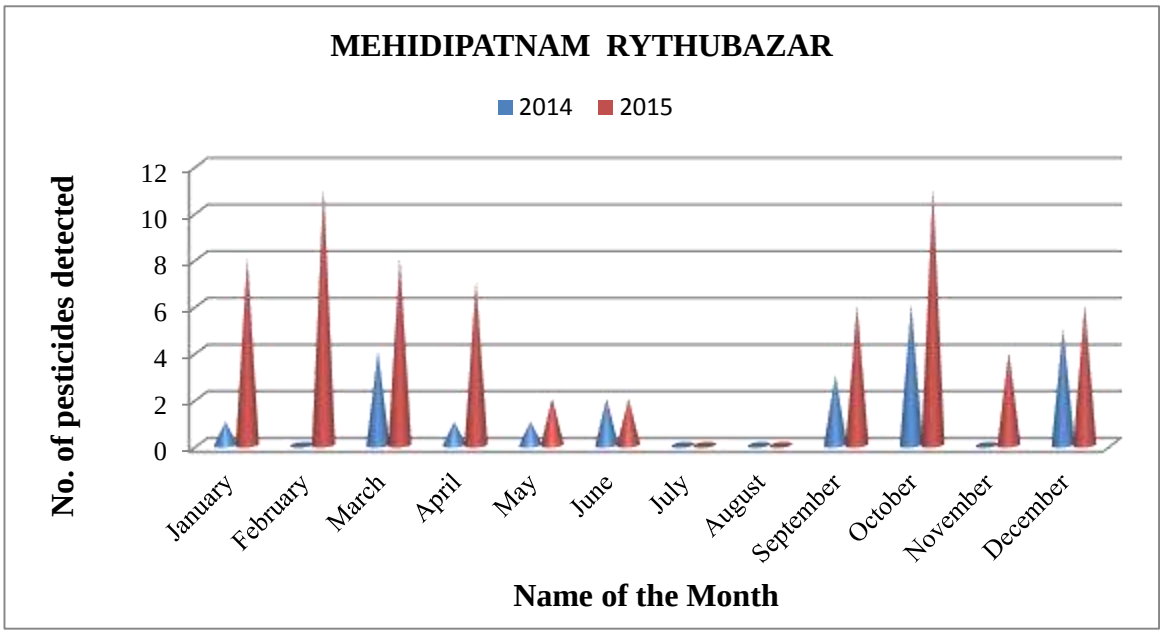


Fig.1: Number of pesticide residues detected in Mehidipatnam rythubazar during 2014 and 2015

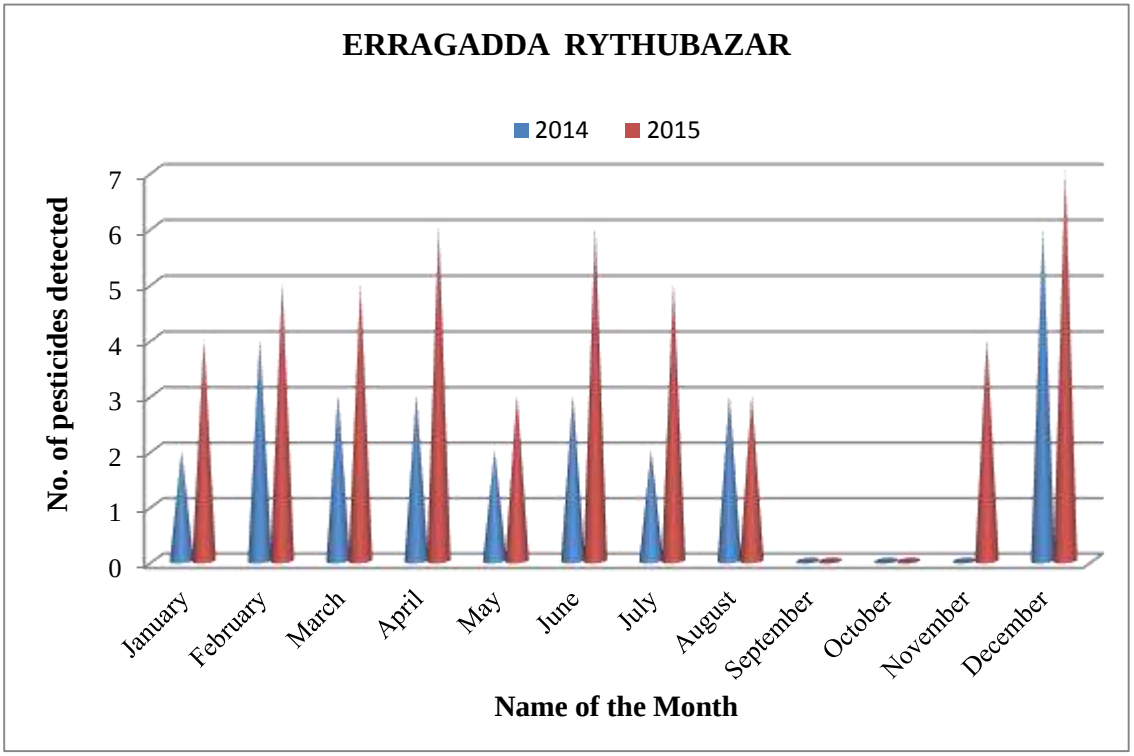


Fig.2: Number of pesticide residues detected in Erragadda rythubazar during 2014 and 2015

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*

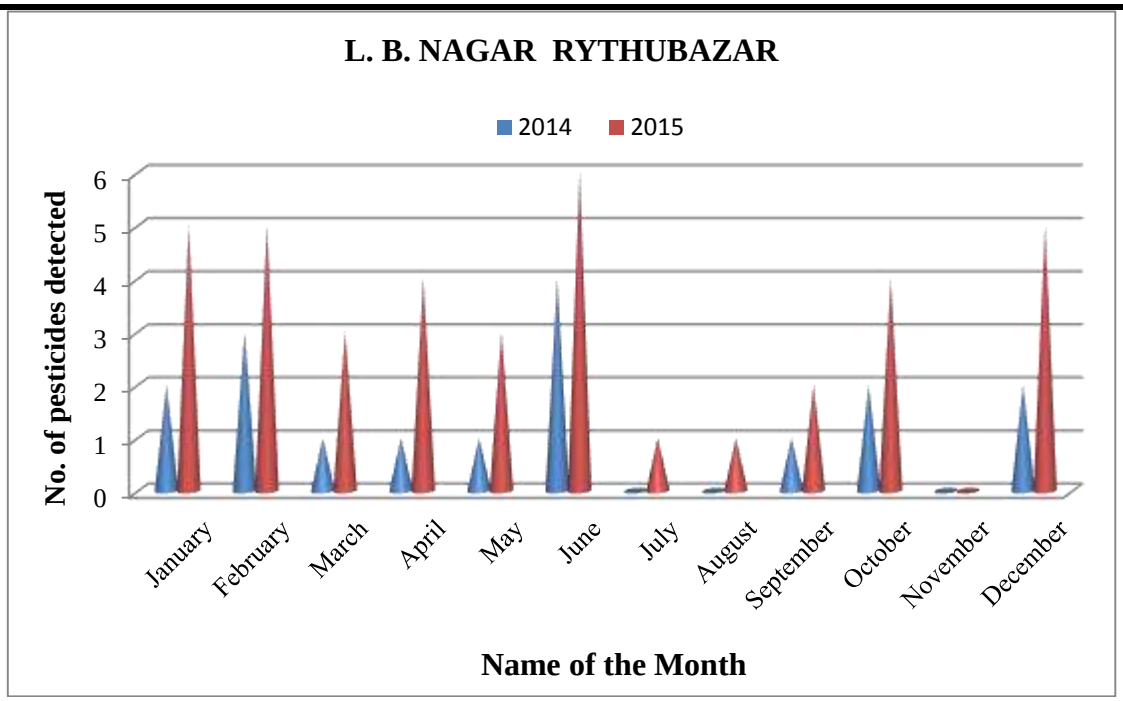


Fig.3: Number of pesticide residues detected in L. B. Nagar rythubazar during 2014 and 2015

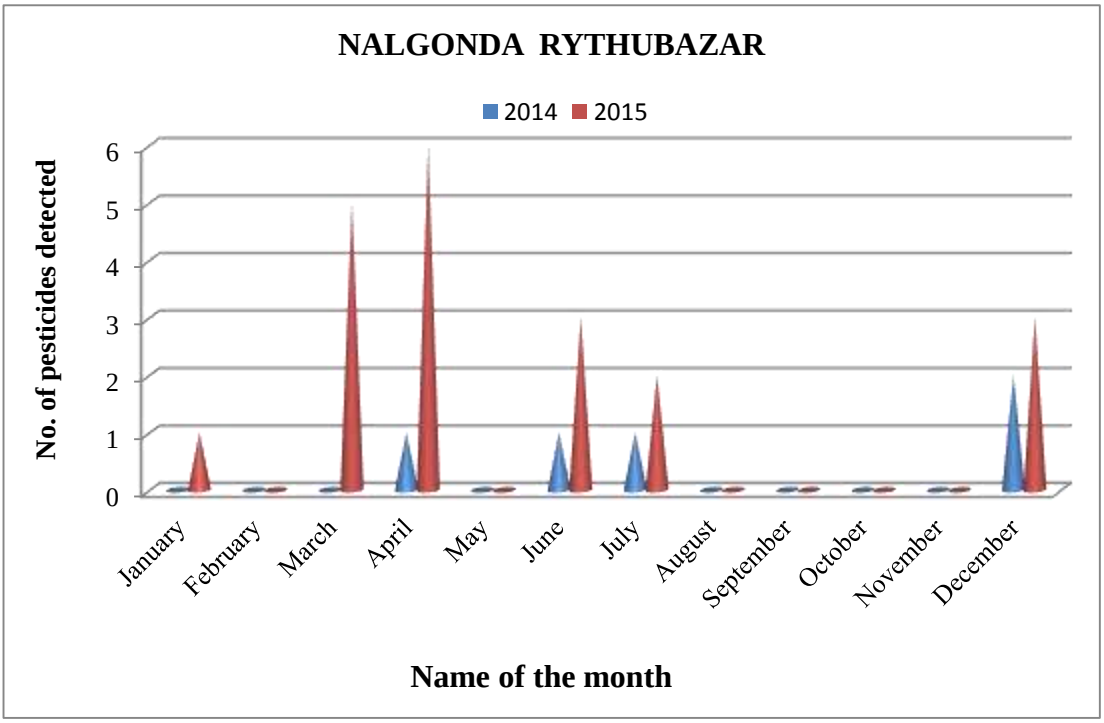


Fig.4: Number of pesticide residues detected in Nalgonda rythubazar during 2014 and 2015

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*

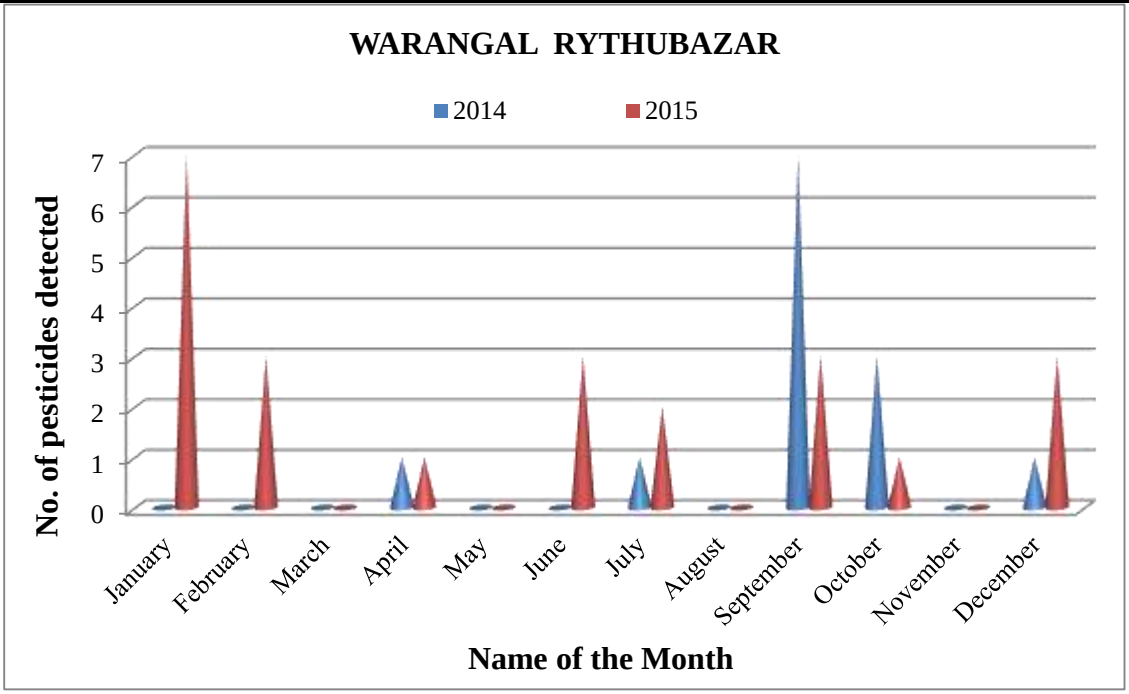


Fig.5: Number of pesticide residues detected in Warangal rythubazar during 2014 and 2015

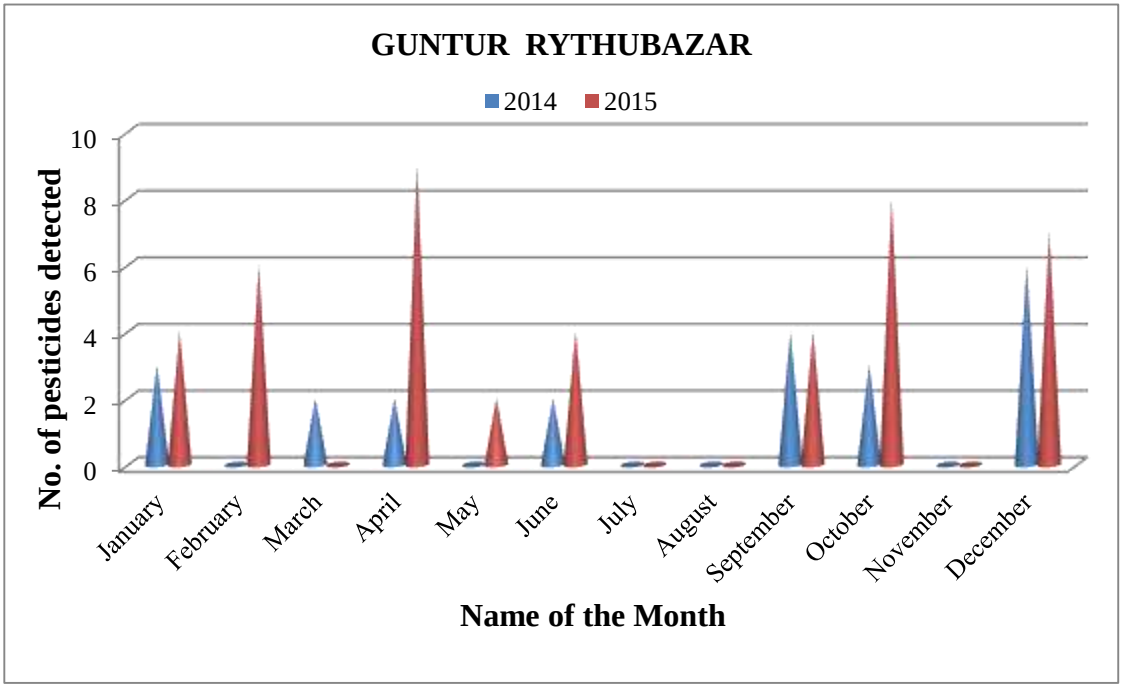


Fig.6. Number of pesticide residues detected in Guntur rythubazar during 2014 and 2015

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*

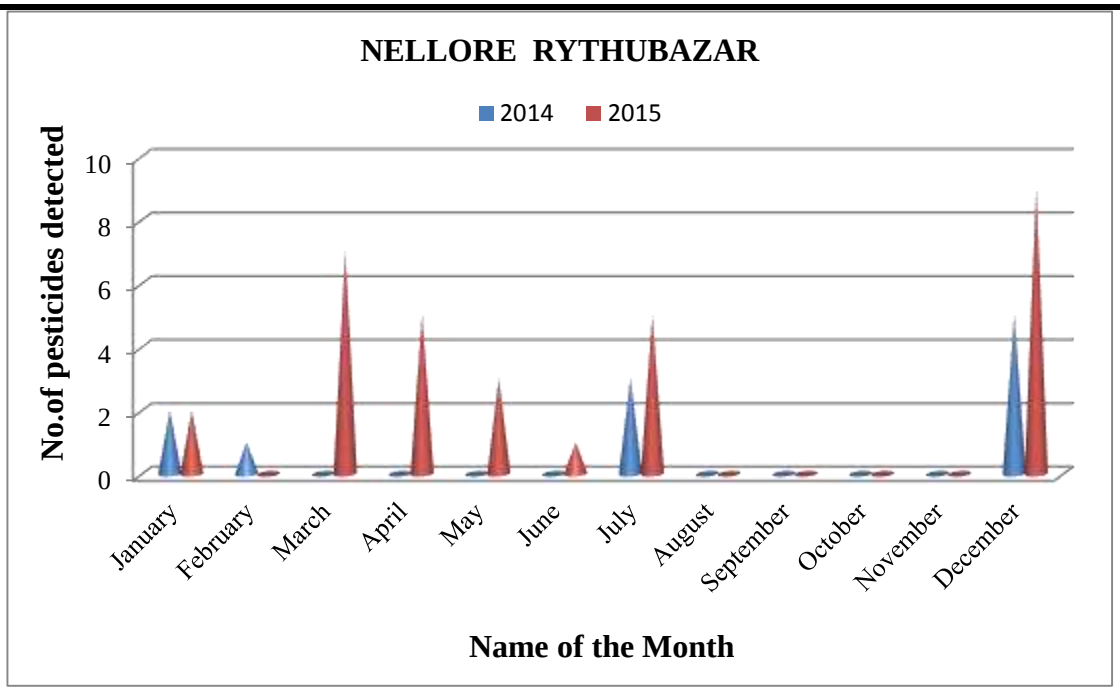


Fig.7: Number of pesticide residues detected in Nellore rythubazar during 2014 and 2015

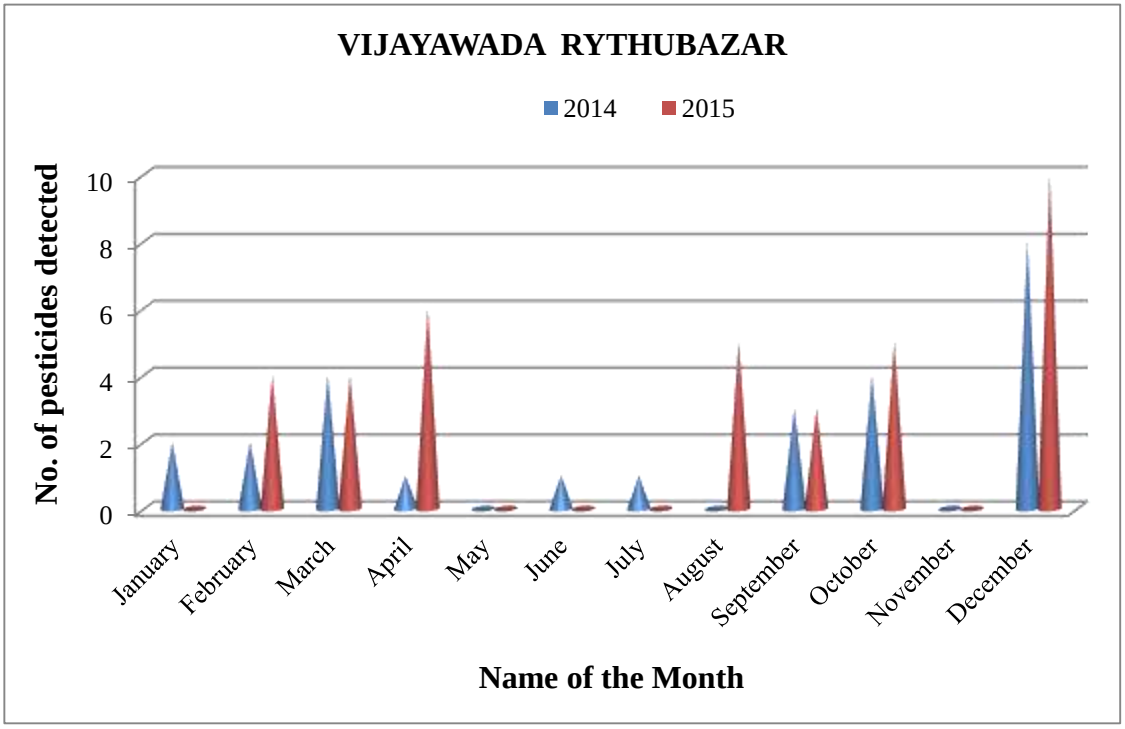
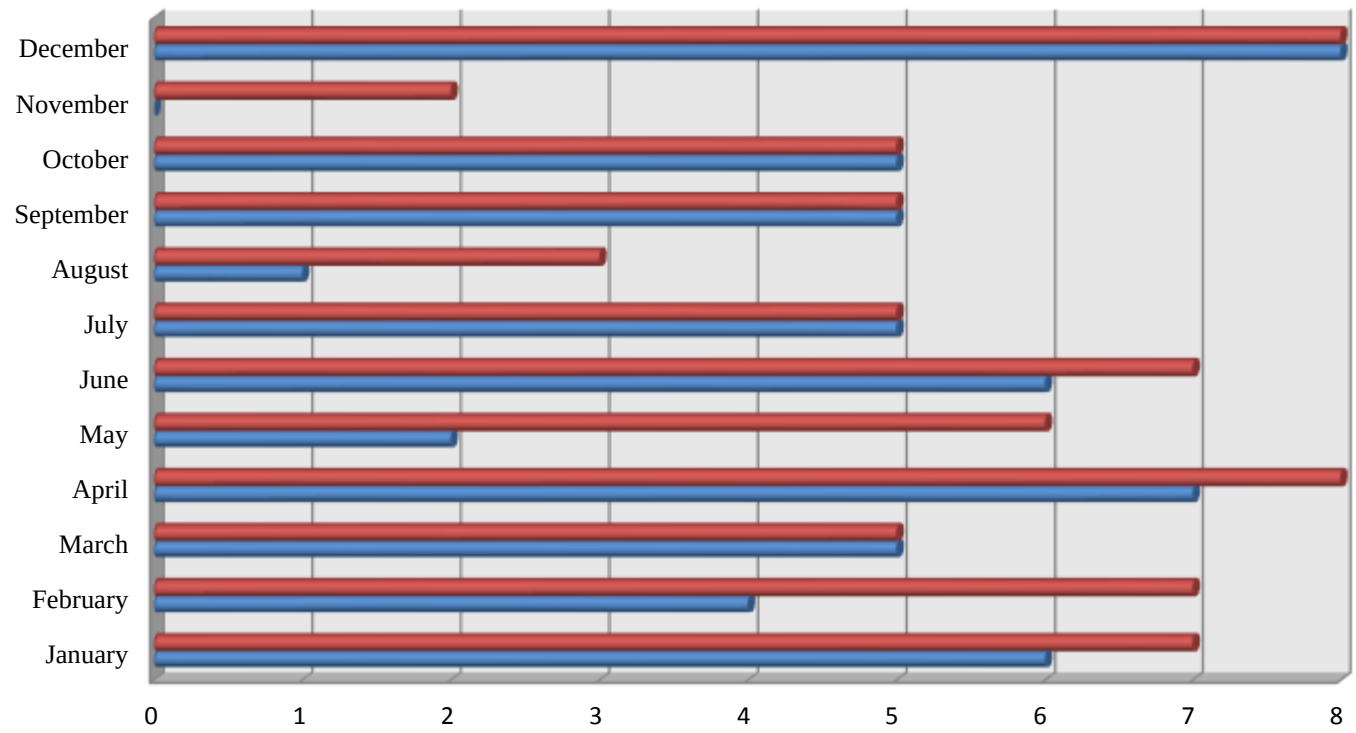


Fig.8: Number of pesticide residues detected in Vijayawada rythubazar during 2014 and 2015

http://dx.doi.org/10.22161/ijeab/2.1.14 *ISSN: 2456-1878*



	January	February	March	April	May	June	July	August	September	October	November	December
2015	7	7	5	8	6	7	5	3	5	5	2	8
2014	6	4	5	7	2	6	5	1	5	5	0	8

Fig.9: Number of markets detected with pesticide residues month-wise in curry leaf samples during 2014-15

http://dx.doi.org/10.22161/ijeab/2.1.15 *ISSN: 2456-1878*

Mycoflora associated with cocoa (*Theobroma cacao*) pods in Cameroon and antifungal effect of plant extracts

Yaouba Aoudou*, Ze Medjap Abel Second

Phytopathology Laboratory, Department of Plant Protection, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 222 Dschang, Cameroon

***Abstract*—** *Mycoflora associated with the pod rot disease of cocoa (Theobroma cacao) and evaluation of the in vitro efficacy of aqueous and ethanolic extracts of A. conyzoïdes and Chromolaena odorata against the pathogenic fungi, C. gloeosporioides and B. theobromae, isolated from cocoa pods were investigated. After isolation, the fungal species were exposed to various concentrations (5 ; 10 ; 15 ; 20 mg/ml) of aqueous, and ethanolic (1.25 ; 2.5 ; 5 ; 10 mg/ml) extracts. Results obtained showed some variations in isolation frequency of fungi from cocoa pods of each locality. Aspergillus, Colletotrichum, Botryodiplodia, Trichoderma and Verticillium were the most common genera that colonized the cocoa pods from Akonolinga and Tonga with different incidences. Colletotrichum gloeosporioides was present (48.84%) in pods collected in Tonga and in those from Akonolinga (41.46%), followed by Botryodiplodia theobromae which was present on 20.93% and 29.27% respectively. All the used concentrations of extracts of both plants significantly reduced the growth of the fungal pathogens. For ethanolic extracts, Ageratum conyzoïdes completely (100%) inhibited the growth of both fungi at 10 mg/ml and for Chromolaena odorata, total (100%) inhibition was observed on B. theobromae at 5 mg/ml while C. gloeosporioides was completely inhibited at 10 mg/ml. In the case of aqueous extracts, Chromolaena odorata, completely (100%) inhibited the growth of B. theobromae and C. gloeosporioides at 20 mg/ml. Similarly, Ageratum conyzoïdes completely suppressed the growth of B. theobromae at 20 mg/ml, however, this dose was obtained as an inhibition of 78% of C. gloeosporioides. Further investigation of the isolation of active antifungal compound should be done.*

Keywords*— *A. conyzoides, C. odorata, antifungal effect, Cocoa pods, mycoflora, plant extracts.

I. INTRODUCTION

Theobroma cacao (Cacao tree and cocoa tree), is a small (4 to 8 m) tall evergreen tree in the family Malvaceae (Juan *et al*., 2008) native to the deep tropical regions of Central and South America. Its seeds, cocoa beans, are used to make cocoa mass, cocoa powder and chocolate (Copetti *et al*., 2010). The fruit or cocoa pod is ovoid shape, 15 to 30 cm long and 8 to 10 cm wide, ripening yellow to orange. Cacao is grown both by large agro industrial plantations and small producers, the bulk of production coming from millions of farmers who have a few trees each (Henderson, 2007). In cocoa orchards in Cameroon, cacao pods are threatened by the surge in fungal diseases such as the brown rot caused by species of *Phytophthora* genus (Assoumou, 1997). This disease can cause yield losses between 60 to 100% in field (Luter and Akrofi, 1993, Berry and Cilas, 1994, Opoku *et al*., 2000), when conditions favor the development of disease. Other diseases such as black rot, and Witch's broom respectively caused by *Botryodiplodia theobroma, Moniliophtora roreri* and *Roniophtora perniciosa* take more and more scale (Koné, 1999 ; Koumé, 2006). Over the years there have been reports of fungal attack on cocoa pods rendering the seeds (beans) unfit for human consumption. Fungi such as *Phytophthora palmivora*, *P. capsici*, *P. kevea* causative agents of (black pod rot), *Lasiodiplodia* spp (*Lasiodiplodia* pod rot) *Macrophoma* spp *(Macrophoma* pod rot), *Phytophthora citropthora* and *P. megakarya* (*Phytophthora* pod rot) have been reported to cause depletion of pods/seeds value in the field (APS, 2011).

Although chemical control was developed by the research scientists, the dissemination of this method to the farmers was little successful. The requirements of the international market in terms of bean quality, environmental constrains, health issues for the consumers (Anonyme, 2006), are numbers of constraints that do not facilitate the development of the chemical control method. Face with this distrust increased with respect to these chemicals, there is a renewed interest in methods such as varietal resistance, use of biofongicide. On one hand, this study aims to analyze the fungi associated with decay of cocoa pods of 3 varieties collected from 2 localities in Cameroon: one located in the central region, belonging to the agroecological area with bimodal rainfall and the other

located in the Western region of country, in the upland area with single rainfall mode. On the other hand, this study also aims the evaluation of antifungal activity of some local plant species in order to offer an alternative of biocontrol.

II. MATERIALS AND METHODS

Sample collection and pathogen identification

One hundred matured infected cocoa pods were obtained from the field at different locations in Akonolinga (Central Region) and Tonga (Western Region) of Cameroon and transported to the Phytopathology Laboratory of the University of Dschang for analyses. The two Local Areas are the major producers of cocoa of the country. Cocoa beans (about 5mm in diameter) from the symptomatic and asymptomatic cocoa pods were removed following surface sterilisation with 70% ethanol for 10secs, blotted dry with sterile paper towel, and plated onto chloramphenicol-amended Potato Dextrose Agar (PDA). The V6 and V8 culture media were also used to promote the *Phythophtora* highlighting. After 3-5 days of incubation at 28˚C microbial growth was assessed microscopically. Cultures of the isolates were transferred to a new culture medium plated on Petri dishes, from where axenic cultures were obtained (Gevens *et al*., 2008). Identification of the isolates was based on morphological characteristics, described in the 1998 illustrated genera of fungi by Barnett and Hunter (1998) and with literature on the identification of pathogenic fungi by Dugan (2006).

Plant extracts

Aerial parts (leaves and stem) of *Chromolaena odorata* and *Ageratum conyzoïdes* L were collected in June 2016 from the locality of Akonolinga, Centre region of Cameroon**.** Their identification were confirmed through consultation in the Herbarium of the Department of Plant Biology, University of Dschang. Plant parts collected were washed three times with running tap water and rinsed with distilled sterile water. They were separately air-dried at room temperature and ground in a mortar. One hundred grams of the resulted dried powder were macerated in 500 ml of distilled water or ethanol and mixed thoroughly. For aqueous extract the mixture was allowed to rest for 48 hours and the supernatant passed through whatman's N°. 1 filter paper to obtain the extract. With regards to ethanolic extract, after maceration for 4 hours in a warring blender (Warring International, New Hartford, CT, USA), the macerate was passed through Whatman's N°. 1 filter paper and evaporated using a Rota vapour at 40°C water bath temperature (Heidolph) (Keuete *et al*., 2015). Extracts were preserved aseptically in a brown bottle at 4°C until further use (Souza *et al*., 1995).

In vitro antifungal activity of plant extracts

The antifungal effect of plant extracts were evaluated on *C. gloeosporioides* et *B. theobromae*, isolated from cocoa pods. The *in vitro* antifungal activity was assessed according to the agar dilution method (Sharma and Trivedi, 2002) on PDA (Difco). Plant extracts were dissolved in dimethylsulphoxide (DMSO) and diluted to give serial dilutions that were incorporated into growth medium. Concentrations of 1.25 ; 2.5 ; 5 and 10 mg/ml for ethanol extracts and 5, 10, 15, 20 mg/ml for aqueous extracts were used. PDA medium supplemented with different concentrations of the extracts were inoculated with 6-mm diameter (plugs) of the test pathogen cut from the margin of 7-day-old cultures. The plates were incubated in duplicates over a period of 10 days for *C. gloeosporioides* and *B. theobromae* at 20 ± 2°C. The radial mycelia growth was measured daily and the fungi toxicity was expressed as percentage inhibition of radial mycelia growth. In order to distinguish between fungicidal and fungi-static activity of the selected plant extract against the test pathogen, the mycelia plugs that did not show any growth were transferred to a freshly poured PDA plate and incubated for 7 days at 20 ± 2°C to observe the recovery of growth. The fungicidal effect was classified as an absence of growth whereas any observed growth was classified as fungi-static.

Statistical analysis

Data collected on percentage inhibition and lesion area were subjected to analysis of variance (ANOVA) using SPSS software version 17. The mean values were separated using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

III. RESULTS AND DISCUSSION

Mycoflora associated with cocoa pods

The fungal species listed in Figure1 could be regarded as common post-harvest decay agents of various studied fruits. Through this investigation at 20 ± 2°C 6 fungal species attributed to six genera were isolated. *Aspergillus, Colletotrichum, Botryodiplodia, Trichoderma* and *Verticillium* were the most common genera that colonized the cocoa pods from Akonolinga and Tonga with different incidences. The most frequent fungi were *Colletotrichum gloeosporioides* 48.84% of pods collected in Tonga and 41.46% in those from Akonolinga, followed by *Botryodiplodia theobromae* which was present in 29.27% of cocoa pods from Akonolinga and 20.93% in those of Tonga. Figures 2 and 3 shows the macro and microscopic characters of *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides*.

In these two production areas, fungal biodiversity affecting the cocoa pods vary qualitatively and quantitatively. The

isolations made on the different pods collected showed a predominance of three fungal species including *Colletotrichum gloeosporioide, Botryodiplodia theobromae* and *Trichoderma sp*. Other species such as *Fusarium oxysporum, Aspergillus niger* and *Verticillium sp*. appear at low frequency. Similar results have been reported by Evans *et al*. (2003) and Rubini *et al*. (2005) showing that soils under cocoa tree and pods are sites of preliferation of indigenous microorganisms potentially antagonistic of *Phytophtora* such as *Trichoderma sp., Colletotrichum gloeosporioide, Fusarium oxysporum* and *Botryodiplodia theobromae* occupying the same ecological niche. The high proliferation of *Colletotrichum gloeosporioide, Botryodiplodia theobromae* and *Trichoderma sp* in these two cocoa ecosystems could justify the scarcity of *Phytophthora megakarya*, causal agent of brown rot. Similar results have been achieved using isolates of *Trichoderma sp.* and *Stromaticum sp*. to fight against the brown rot of cacao tree (Krauss and Soberanis, 2002).

Antifungal effect of plant extracts

Effect of ethanol extracts

Antifungal effects of ethanol extracts of *Chromolaena odorata* and *Ageratum conyzoïdes* L. on fungal growth are presented on Table 1. There were significant differences in the mycelia growth inhibition of plant extract-supplemented samples compared with the negative control (ANOVA and Duncan Multiple Range Test, $P < 0.05$). The effect of extracts with increasing concentrations showed a gradual inhibition of the growth of *C. gloeosporioides* and *B. theobromae*. It was noted that ethanolic extracts of *Ageratum conyzoïdes* completely (100%) inhibited the growth of both fungi at 10 mg/ml. With the ethanolic extracts of *Chromolaena odorata*, 100% inhibition was observed for *B. theobromae* at the dose of 5 mg/ml while *C. gloeosporioides* was completely inhibited at 10 mg/ml.

Effect of aqueous extracts

Antifungal effects of aqueous extracts of *Chromolaena odorata* and *Ageratum conyzoïdes* L. on fungal growth are presented on Table 2. Generaly there are significant differences in the mycelia growth inhibition of plant extract-supplemented samples compared with the negative control (ANOVA and Duncan Multiple Range Test, $P < 0.05$). Aqueous extracts of *Chromolaena odorata*, completely (100%) inhibited the growth of *B. theobromae* and *C. gloeosporioides* at the dose of 20 mg/ml. Similarly aqueous extracts of *Ageratum conyzoïdes* completely inhibited the growth of *B. theobromae* at 20 mg/ml, however this dose was obtained as an inhibition of 78% of *C. gloeosporioides*.

Aqueous and ethanolic extracts of *C. odorata* and *A. conyzoides* showed fungicidal effect at concentrations 20 mg/ml and 10 mg/ml respectively.

The growth inhibition percentages of different fungi by plant extracts proved to be dependent on the concentration, the type of extract and the plant tested. Results obtained from *Ageratum conyzoïdes* extracts are in agreement with previous studies that showed the antifungal activities of this plant against devastating pathogen on variety of economic plants (Mughal *et al*. 1996; Bajwa *et al*., 2001; Sidra and Uzma, 2012). Similarly, the results achieved with leaves extract of *Ageratum conyzoïdes* are similar to those obtained by Tsapi (2000) and Megatche (2011) which showed that these extracts inhibit the development of *Phytophtora megakarya* (responsible for the brown rot of cocoa) and *P. colocasiae* (causative agent of late blight of taro). A wide range of allelochemicals including alkaloids, flavonoids, chromenes, benzofurans and terpe-noids have been isolated from *A. conyzoides* (Okunade, 2002). According to Tran *et al*. (2004), three phenolic compounds were identified in the leaf, stem and root of *A. conyzoides* including gallic acid, coumallic acid and protocatechuic acid and catechin were found only in the stem. Three additional allelochemicals were also found in the leaf consisting of p-coumaric acid, sinapic acid and benzoic acid. The greater number of allelochemicals found might result in the stronger inhibitory activity.

Also, results obtained with *C. odorata* extract are similar to those reported by (Ngono *et al*., 2006) which showed that this extract inhibit the development of yeast, filamentous fungi and that of several multicellular dermatophyte fungi. Kra *et al*. (2009) showed the effect of the leaf extract of *C. odorata* in vitro on two isolates of *F. oxysporum*, causing symptoms of *Fusarium* wilt. A qualitative chemical analysis of the extract and fractions showed the presence of biologically active constituents such as some coumarins, flavonoids, phenols, tannins and sterols, this could justify the antifungal activity.

IV. CONCLUSION

For the two areas investigated, the fungal biodiversity appeared to be highly variable both qualitatively and quantitatively. *Aspergillus, Colletotrichum, Botryodiplodia, Trichoderma* and *Verticillium* were the most common genera that colonized the cocoa pods from Akonolinga and Tonga with different incidences. This study suggests that *A. conyzoides* and *C. odorata* have fungitoxic chemicals against *B. theobromae* and *C. gloeosporioides*, cocoa rot causing pathogen. Ethanolic and aqueous extracts of *A. conyzoides* and *C. odorata* greatly reduced the fungal growth, which can be used for the disease management. Further investigation on the isolation of active antifungal compounds should be done

http://dx.doi.org/10.22161/ijeab/2.1.15 *ISSN: 2456-1878*

and the isolated antifungal compounds should be checked against other pathogenic fungi to control the different diseases.

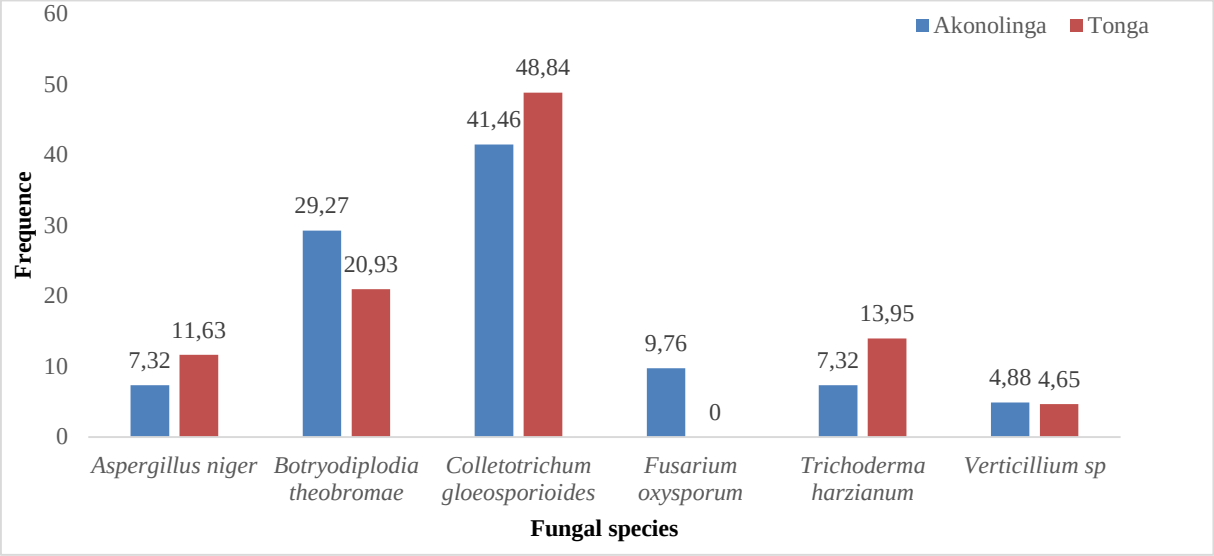


Fig.1: Frequencies of the different fungal species identified with respect to the locality

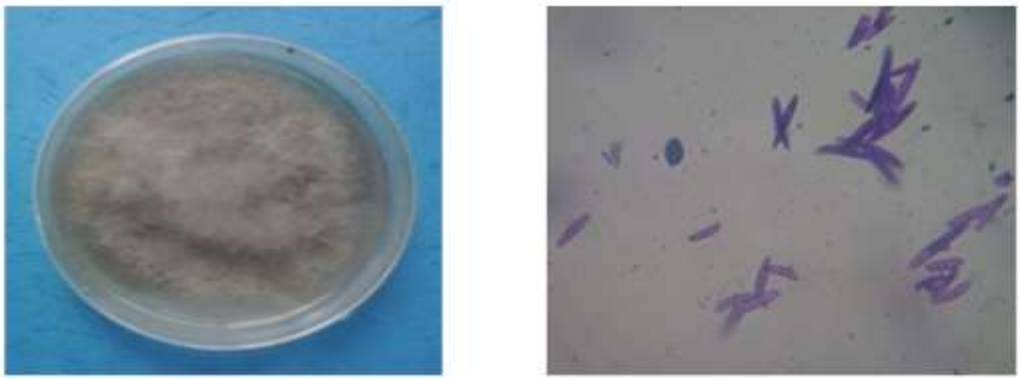


Fig.2: Botryodiplodia theobromae, axenic culture and conidia

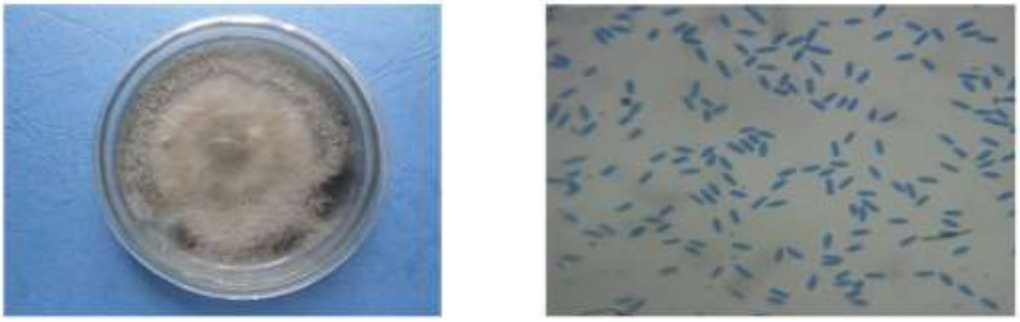


Fig.3: Collectotrichum gloeosporioides, axenic culture and conidia

Table.1: Inhibition Percentage (%) of radial growth of fungal pathogens by ethanol plant extracts

Ethanolic extracts	Concentration	*C. gloeosporioides*	*B. theobromae*
A. conyzoides	T-	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
	1.25 mg/ml	$39.01 \pm 7,40^{b}$	53.72 ± 5.65^{b}
	2.5 mg/ml	$39.41 \pm 11,34^{b}$	40.98 ± 4.75^{b}
	5 mg/ml	$49.02 \pm 18,68^{b}$	48.24 ± 14.01^{b}

	10 mg/ml	100.0 ± 0,00[a]	100.00 ± 0.00[a]
	T+	100.0 ± 0,00[a]	98.60 ± 0.03[a]
C. odorata	T-	0.00 ± 0.00[c]	0.00 ± 0.00[e]
	1.25 mg/ml	62.94 ± 11,93[b]	58.63 ± 5.65[d]
	2.5 mg/ml	69.61 ± 14,04[b]	76.67 ± 4.33[c]
	5 mg/ml	91.17 ± 8,54[a]	100.00 ± 0.00[a]
	10 mg/ml	100.0 ± 0,00[a]	100.00 ± 0.00a
	T+	100.0 ± 0,00[a]	83.33 ± 1.22[b]

Values in the same row followed by different letters are significantly different ($P \leq 0.05$).
T- = Negative control (Distilled water) ; T+ = Positive control (Mancozeb).

Table. 2: Inhibition Percentage (%) of radial growth of fungal pathogens by aqueous plant extracts

Aqueous extract	**Concentration**	***C. gloeosporioides***	***B. theobromae***
A. conyzoides	T-	0.00 ± 0.00[e]*	0.00 ± 000[c]
	5 mg/ml	35.29 ± 16.38[d]	26.47 ± 14.70[b]
	10 mg/ml	45.69 ± 8.93[cd]	32.94 ± 12.0 [b]
	15 mg/ml	59.61 ± 5.30[c]	84.90 ± 16.64[a]
	20 mg/ml	78.63 ± 3.40[b]	100.00 ± 0.00[a]
	T+	100.00 ± 0.00[a]	100.00 ± 0.00[a]
C. odorata	T-	0.00 ± 0.00[e]	0.00 ± 0.00 [e]
	5 mg/ml	32.16 ± 4,34 [d]	19.01 ± 5.89 [d]
	10 mg/ml	52.94 ± 4.70 [c]	54.31 ± 8.67 [c]
	15 mg/ml	72.15 ± 8.34 [b]	67.45 ± 1.22 [b]
	20 mg/ml	100.00 ± 0.00[a]	100.00 ± 0.00[a]
	T+	91.76 ± 7.34 [a]	100.00 ± 0.00[a]

Values in the same row followed by different letters are significantly different ($P \leq 0.05$).
T- = negative control (Distilled water); T+ = positive control (Mancozeb).

REFERENCES

[1] Anonyme, 2006. New EU regulation on maximum residue levels of pesticides in food : minimising the impact on the cocoa sector. *In*: proceedings of the 15th International Cocoa Research Conference. 9-10 October 2006. San Jose (Costa Rica). pp. 1565-1571.

[2] APS, 2011. Diseases of *Theobroma cacao* in Florida. American Phytopathogical Society, Minnesota, USA. p. 415

[3] Assoumou, J., 1977. Agriculture d'exportation et bataille du développement en Afrique tropicale: Economie *du cacao*. Ed. Jean Pierre De large. Paris. p 350.

[4] Bajwa, R., Akhtar, N. and Javaid, A., 2001. Antifungal activity of allelopathic plant extracts. Effect of aqueous extracts of three allelopathic *Asteraceous* species on growth of aspergilla. Pak. J. Biol. Sci. 4: 503-507.

[5] Barnett, H.L., Hunter, B.B., 1998. Illustrated genera of imperfect fungi 4th edition, APS press, St. Paul Minnesota. p. 32

[6] Berry, D., Cilas, C., 1994. Etude génétique de la réaction à la pourriture brune des cabosses chez des cacaoyers (*Theobroma cacao* L.). Thèse de Doctorat d'Etat. Université de Paris-Sud, centre d'Orsay, 153p.

[7] Copetti, M., Lamanka, B., Frisvad, J., Pereira, J., Taniwaki, M., 2011. Mycobiota of cocoa. from farm and chocolate. Food Microbiol. 28:1499-1504.

[8] Dugan, F.M., 2006. The identification of fungi. APS press, St Paul Minnesota. p. 50.

[9] Evans, H.C., Holmes, K.A. and Thomas, S.E., 2003. Endophytes and mycoparasites associated with an indigenous forest tree, *Theobroma gileri*, in Ecuador and preliminary assessment of their potential as biocontrol agents of cocoa diseases. *Mycological Progress* 2: 149-160.

[10] Gevens, A.J., Donahoo, K.H.L., Hausbeck, M.K., 2008. Characterization of *Phytophthora capsici* causing foliar and pod blight of snap bean in Michigan. Plant Dis. 92(2):198-320.

[11] Henderson, J.S., 2007. Chemical and archaeological evidence for the earliest *Cacao* beverage. Proceed. Nat. Acad. Sci. 104:18937-18940.

[12] Juan, C., Motamayor, P.L., Jay, W.I., Da Silva, E.M., Reg, L., David, N.K., Steven, B.J., Raymond, J.S., 2008. Geographic and Genetic population differentiation of the Amazonian chocolate tree (*Theobroma cacao)* PLos ONE 3(10):1371-1376.

[13] Keuete Kamdoum, E., Tsopmbeng Noumbo, G., Yaouba, A., Djeugap F.J., And Serferbe Signaboubo, 2015. Antifungal potential of some plant extracts against three post-harvest fungal pathogens of avocado (*Persea americana* Mill.) fruits. Int. J. of Multi. Res. and Dev. Vol : 2, Issue:4, 148-152 e-ISSN: 2349-4182

[14] Koné, Y.R., 1999. Etude de la structure actuelle des populations de *Phytophthora* spp., agents de la pourriture brune des cabosses du cacaoyer (*Theobroma cacao* L.) en Côte d'Ivoire. Mémoire de Diplôme d'Agronomie Approfondie, Option Défense des cultures, Ecole Supérieure d4agronomie, Yamoussoukro.111p

[15] Kouamé, K.D., 2006. Structure et dynamique des populations de *Phytophthora* spp., agents de la pourriture brune des cabosses du cacaoyer (*Theobroma cacao* L.) en Côte d'Ivoire. Mémoire de DEA. UFR biosciences. Université de Cocody, Abidjan, Cote d'Ivoire. 74p

[16] Krauss, U., Soberanis, W., 2002. Effect of fertilization and biocontrol application frequency on cocoa pod diseases. Biological Control 24: 82-89.

[17] Luter bacher, M.L., Akrofi, A.Y., 1993. The current status and distribution of *Phytophthora megakarya* in Ghana. *In: Proceedings of the 11th International Cocoa Reseach Conference*, Yamoussoukrou, Côte d'Ivoire, pp.57-58.

[18] Megatche, 2011. Efficacité *in vitro* des extraits de plantes sur la croissance et le développement de *Phytophthora colocasiae*, agent causal de la flétrissure des feuilles de taro (*Colocasia esculenta*). Mémoire de Master II en phytopathologie. Université de Dschang, 164 p.

[19] Mughal, M.A., Khan, T.Z. and Nasir, M.A., 1996. Antifungal activity of some plant extracts. Pak. J. Phytopathol. 8: 46-48.

[20] Ngono Ngane, A., Ebelle Etame, R., Ndifor, F., Biyiti, L., Amvam Zollo, P.H., Bouchet, P., 2006. Antifungal Activity of *C. odorata* (L.) King & Robinson (Asteraceae) of Cameroon. *Chemotherapy*. 2006; 52(2):103-6.

[21] Okunade, A.L., 2002. *Ageratum conyzoides* L. (*Asteraceae)* Fitoterapia. 73: 1-16.

[22] Opoku, I.Y., Appiah A.A., Akrofi A.Y., Owusu G.K., 2000. *Phytophthora megakarya*: a potential threat to the cocao industry in ghana. J. Agri. Sc. 33: 237-248.

[23] Rubini, M.R., Silva-Ribeiro, R.T., Pomella, A.W.V., Maki, C.S., Araujo, W.L., Dos Santos, D.R., and Avevedo, J.L., 2005. Diversity of en dophytic fungal community of cocoa (*Theobroma coca* L.) and biological control of *Crinipellis perniciosa*, causal agent of Withches'Broom Disease. *Int. J Biol Sci*. 1:24-33.

[24] Sharma, N. and Trivedi, P.C., 2002. Screening of leaf extracts of some plants for their nematicidal and fungicidal properties against *Meloidogyne incognita* and *Fusarium oxysporum*. Asian J. Exp. Sci. 16: 21-28.

[25] Sidra Javed, and Uzma Bashir, 2012. Antifungal activity of different extracts of *Ageratum conyzoïdes* for the management of *Fusarium solani*. African Journal of Biotechnology Vol. 11(49), pp. 11022-11029.

[26] Souza, C., Koumaglo, K., Gbeassor, M., 1995. Evaluation des propriétés antimicrobiennes des extraits aqueux totaux de quelques plantes médicinales. Pharma. Med. Trad. Afr. 103-112.

[27] Tran, D.X., Shinkichi, T., Hong, N.H., Khanh, T.D., Min, C.I., 2004. Assessment of phytotoxic action of *Ageratum conyzoides* L. (billy goat weed) on weeds. Crop Prot. 23: 915-922.

[28] Tsapi, 2000. Etude de la composition chimique et évaluation *in-vitro* de l'activité antifongiques des huiles essentielles de *Cupressus lusitanica* Mill. Sur quelques isolats de *Phytophthora megakarya*, *agent causal* de la pourriture brune des cabosses du cacaoyer (*Theobroma cacao* L.*)*. Mémoire de maîtrise de biochimie. Université de Dschang, 1-59 p.

http://dx.doi.org/10.22161/ijeab/2.1.16 *ISSN: 2456-1878*

Carbon Trading and India's Road Map

Dr. Debesh Bhowmik

Retired principal and Former Associate Editor-Arthabeekshan-the journal of Bengal Economic Association

Abstract— *In this article, the author describes the concept of carbon trading , its global market ,mechanism of global trading, international organization, EUETS, relationship between REDD and carbon market in relation with agreements of Paris convention. The 10 myths of REDD+ and carbon market are additional features which can explore future research. The paper highlighted India's roadmap for carbon market potentiality in 2020.*

Keywords— Carbon trading, global carbon market, REDD, India's road map.

JEL-F18,O44,Q56

I. WHAT IS CARBON TRADING

Carbon trading is the buying and selling of a new, artificially-created commodity – the right to emit carbon dioxide. Unlike trading in other commodities like crude oil or bananas, carbon trading is not a voluntary exchange between producers and those who want to consume or sell on the goods. Instead, it results from action by governments to create this new commodity – the right to emit carbon – and then to limit the availability of this right in order to create scarcity and therefore a market for it.

Carbon trading is one of a number of different approaches that have been developed and adopted by governments as a means of controlling the amount of carbon dioxide that is emitted into the atmosphere and reducing this amount over time. It is based on the broader approach, purportedly to control the emission of pollutants, known as 'cap and trade'. Cap and trade is often referred to as a market-based mechanism and contrasted with a different set of tools available to governments to influence behaviours, those which come under the umbrella of direct regulation or standard setting. However, this contrasting of market-based and non-market-based approach is sometimes unhelpful. It ignores the fact that market mechanisms do not operate in a vacuum. Instead, they always take place in a social and economic environment underpinned by various government laws and regulations and often require these laws in order to be effective. Carbon trading is a case in point. Carbon markets are directly created by government regulation.

Perhaps a more useful distinction for the purposes of this report is that between direct and indirect mechanisms. Carbon trading can be classed as an indirect tool as it is supposed to achieve its purpose of reducing emissions indirectly by affecting the price of those emissions. This in turn affects the behaviour of 'actors' in the market, i.e. those responsible for producing the emissions, by creating an incentive for them to save money by reducing their emissions and hence change their behaviour. In contrast, government regulation and standard setting are direct interventions to change behaviour, not reliant on intermediate mechanisms such as prices. Taxation is an indirect mechanism as it aims to change behaviour through affecting the price of a good, service or activity. However, it is arguably less indirect than trading as governments fix the price with a tax whereas with trading the price is determined by the market.

The carbon trade is an idea that came about in response to the Kyoto Protocol. The Kyoto Protocol is an agreement under which industrialized countries will reduce their greenhouse gas emissions between the years 2008 to 2012 to levels that are 5.2% lower than those of 1990.

The idea behind carbon trading is quite similar to the trading of securities or commodities in a market place. Carbon would be given an economic value, allowing people, companies or nations to trade it. If a nation bought carbon, it would be buying the rights to burn it, and a nation selling carbon would be giving up its rights to burn it. The value of the carbon would be based on the ability of the country owning the carbon to store it or to prevent it from being released into the atmosphere. A market would be created to facilitate the buying and selling of the rights to emit greenhouse gases. The industrialized nations for which reducing emissions is a daunting task could buy the emission rights from another nation whose industries do not produce as much of these gases. The market for carbon is possible because the goal of the Kyoto Protocol is to reduce emissions as a collective.

On the one hand, the idea of carbon trade seems like a win-win situation: greenhouse gas emissions may be reduced while some countries reap economic benefit. On the other hand, critics of the idea suspect that some countries will exploit the trading system and the consequences will be negative. While the proposal of carbon trade does have its merits, debate over this type of market is inevitable since it

http://dx.doi.org/10.22161/ijeab/2.1.16 *ISSN: 2456-1878*

involves finding a compromise between profit, equality and ecological concerns.

The carbon market is one of the most effective policies for tackling climate change. It inspires operational excellence and incentivizes business investments in low-carbon technologies. Not only is the market expected to save over 2 billion tones of CO_2 emissions by the end of 2012, but the development of the current global carbon market, now worth over US$140 billion, has catapulted climate change to the forefront of business decisions. But while it exhibits real environmental and economic impact, and helps achieve climate change goals, it remains vulnerable to external factors.

II. GLOBAL CARBON TRADING

Emission trading is considered an important market-based instrument to control emissions and is an essential element of the 1997 Kyoto Protocol. The EU Emissions Trading System (EU ETS) is the largest existing cap-and-trade system in the world and commenced operations in 2005. It covers about 2Gt of CO_2 emissions at more than 10,000 installations across the 27 EU member states. Following the EU ETS, an increasing number of world regions are currently introducing cap-and-trade systems that establish a price for greenhouse gas emissions. These include New Zealand, Australia, the Regional Greenhouse Gas Initiative (RGGI) of ten US-States in northeastern USA, California, the Western Climate Initiative (eight US-State and two Canadian Provinces), and the Midwestern Regional Greenhouse Gas Reduction Accord (nine US-States and one Canadian Province). In Japan, the cities of Tokyo and Hiroshima as well as the Kyoto prefecture intend to introduce mandatory emissions trading systems (Point Carbon, 2008). This development is underlined by the establishment of the International Carbon Action Partnership (ICAP) by several EU member states, the European Commission, California and other WCI members, several RGGI member states, New Zealand, and Japan (as an observer). ICAP sets up an expert forum to support the implementation and linking of emissions trading systems (ETS).

The Doha climate summit was no landmark event, but governments adopted an extension of the

Kyoto Protocol, set milestones in the lead up to a 2015 agreement. The most significant outcome from Doha was the adoption of the second commitment period of the Kyoto Protocol. Europe and a handful of others, amounting to less than 15% of global emissions, effectively put their existing national targets under the Kyoto framework. In doing so, they maintain the institutions and mechanisms established by the Protocol through to the end of 2020. However, only those developed countries which have taken on KP2 targets are eligible to use credits from Clean Development Mechanism (CDM) projects after 2012.

III. INTERNATIONAL CARBON ACTION PARTNERSHIP

The International Carbon Action Partnership (ICAP) constitutes an expert forum that explores design issues and linkages of regional emissions trading systems. ICAP investigates the relevant issues and proposes solutions where barriers are identified. The work of ICAP focuses on the three pillars of technical dialogue, ETS knowledge sharing and capacity building activities. ICAP's objectives are:

- Share best practices and learning from each other's experience of ETS
- Help policymakers recognize ETS design compatibility issues and opportunities for the establishment of an ETS at an early stage
- Facilitate future linking of trading programs
- Highlight the key role of emissions trading as an effective climate policy response
- Build and strengthen partnerships amongst governments

The 'ICAP Political Declaration' (ICAP, 2007) states:

"The International Carbon Action Partnership (ICAP) will create an international forum of governments and public authorities that are engaged in the process of designing or implementing carbon markets. ICAP will establish an expert forum to discuss relevant questions on the design, compatibility and potential linkage of regional carbon markets. The forum will convene regularly and define a work program, including joint research and studies. It will identify barriers, including barriers posed by applicable state, federal and national laws, and it will identify solutions with the view to developing recommendations for consideration by each of the signatories hereto. ICAP aims to support the United Nations process on climate change by facilitating working relationships among governments and public authorities engaged in developing and implementing programs to combat climate change."

In particular, in the formal linking scenario ICAP could evolve to become the international clearinghouse for a carbon market established by linking domestic ETS.In order to deal with the uncertainty on the evolution of carbon markets and thus the future role of ICAP identifies critical design issues that are relevant in the global trading, formal linking, mixed approach and indirect linking scenario, respectively.

http://dx.doi.org/10.22161/ijeab/2.1.16 *ISSN: 2456-1878*

IV. MECHANISM OF TRADING

1. Global trading

A global emissions trading system building on the Kyoto approach can be established from the top-down as follows: an international treaty establishes national emission targets for all Annex-I (and possible other) countries for specified periods post-2012. From an economic point of view a global trading system is a first-best policy instrument that will ensure that the costs of achieving given reduction targets are minimized. Within this overarching framework, governments can devolve responsibility for allowance trading to the private sector by establishing domestic ETS and linking these to the domestic ETS of other regions. Thus governments will only have to
engage in international emissions trading on behalf of sectors that are not covered under a linked domestic ETS.

2. Formal linking of domestic ETS

If post-2012 negotiations within the UNFCCC do not lead to a global cap-and-trade consensus, nations and regions can establish domestic carbon markets and link these, thus constructing an international carbon market bottom-up (Tangen and Hasselknippe, 2005; Victor, 2007; Pizer, 2007). A major advantage of this approach is that if no agreement on a global trading system is achieved within UNFCCC negotiations by 2009, linking offers an opportunity to keep and build political momentum for constructing a global carbon market in the mid- to long term.

In principle, linking regional trading systems will enhance the efficiency of reduction efforts, increase liquidity of carbon markets, and reduce competitiveness concerns that could arise from different allowance price levels across systems (Edenhofer et al,2007). Unlike the global trading approach, however, the linking of regional trading systems does not allow controlling global emissions. Most of the issues arising when negotiating a global trading system remain important when linking bottom-up (e.g. defining a global policy target, and agreeing on burden sharing rules). However, these issues are negotiated only between the linking partners. Again, developing countries can participate in international emissions trading through credit schemes.

3. Indirect linking

Even if there is no agreement on formally linking regional emissions trading schemes, there will still be indirect linkages if national and regional domestic ETS accept credits from the same credit schemes like CDM. There will be some convergence in ETS price levels due to indirect linking. The levels of price convergence will depend on the supply curve of credits, import restrictions for credits, marginal abatements cost (MAC) curves and cap levels in the regional

ETS. However, this mechanism cannot guarantee that allowance prices across domestic ETS are completely equalized. More specifically, the degree of convergence of ETS allowance prices should be higher, the larger the available amount of credits and the less restrictive the limits for the import of credits into the ETS. In the indirect linking scenario all ETS that enable the use of a certain credit type need to agree on its design features. This particularly concerns monitoring and verification and the additional requirements that ensure emission reductions take solely place

due to the financing obtained from the credit scheme.

4. Mixed approach

Finally, mixed approach is conceivable containing elements of each of the stylized three approaches outlined above. If, for example, UNFCCC negotiations evolve towards agreement on a multilateral climate policy architecture by 2009, but not all major emitters are willing to join a global cap-and-trade system immediately, the treaty may comprise a provision that enables reluctant countries or possibly subnational regions to join this scheme later. It is conceivable that the acceding regions would join the international trading system with their full economy or with some sectors only – that is, only their domestic ETS may be integrated into the global trading structure. It is also conceivable that developing countries gradually join such a trading system with specific sectors only, e.g. starting with the electricity sector.

Clean Development Mechanism

"Climate Change, Carbon markets and the CDM: A call to action" was released in September 2012. The report built the case for restoring faith in CDM, made 51 recommendations for addressing the shortcoming of CDM, improving performance and responding to future challenges and opportunities to keep it relevant to mitigation efforts. It urged nations to intervene to address the crisis in the carbon market and substantially increase level of mitigation ambition. However, the report did not result into any action at the UNFCCC conference in Doha in December 2012. The UNFCCC secretariat also launched The CDM Loan Scheme in 2012 to boost CDM project development in LDCs. The Scheme provides interest-free loans for CDM projects in LDCs as well as countries that have fewer than 10 registered CDM projects. The scheme is run jointly by the UNFCCC, the United Nations Environment Programme (UNEP) Risoe Centre and the United Nations Office for Project Services (UNOPS). The loans are utilized to finance

the development of Project Design Documents (PDD), validation by a Designated Operational Entity (DOE), registration of the project with the UNFCCC and the monitoring and verification of Certified Emissions Reductions (CERs). In the first round of solicitation, the scheme received applications from 42 projects in 23 countries in Latin America and the Caribbean, Asia and Africa with the majority of the applications coming from Africa (29). Regional CDM support centres as well as loans for project developers in underrepresented regions also do not change the broader picture.

V. REDD+ AND CARBON MARKET

The world's forests are threatened by an ever-expanding demand for commodities such as soy, timber, palm oil and beef. Every year 13 million hectares of forests are being lost worldwide due to illegal or unsustainable logging and the conversion of forests to agricultural land. Emissions from forest degradation and deforestation account for 18% of global GHG emissions – 5.8 Gt CO_2 – more than the emissions of all EU countries combined. But forests are crucial in the struggle for sustainable development. Proposals to finance REDD+ reach from scaling up public finance, for example through the Green Climate Fund to including REDD+ activities in international carbon markets. A large number of developing countries continue to stress that forest-related activities under the UN Framework Convention on Climate Change (UNFCCC) must primarily be publicly funded. A little over ten years ago, forest conservation was excluded from the Clean Development Mechanism, and the EU decided to ban offset credits from forestry and land use land change activities (LULUCF) in the EU-ETS. There is an inherent high risk that forest offset credits do not represent real emission reductions due to leakage, the impermanence of forest carbon, inflated baselines, problematic additionality testing and difficult MRV. If these artificial credits would be traded in a global compliance market, global emissions would actually rise. However, offsetting is a zero sum game. Even if the credits would represent real emission reductions, allowing REDD projects in an offset mechanism would only shift emission reduction obligations from one country to the other and would not deliver the large long-term emission cuts required to stay below 2 degrees warming. Moreover, costs for the monitoring and implementation of forest carbon projects are high and fraudulent activities related to forest carbon trading have already been reported. REDD+ emission credits must therefore not be included in a global compliance market. Alternative financing options exist and should be prioritized. These include for example a fund-based approach, carbon taxes, levies on international aviation or maritime fuels and financial transaction taxes. A well designed REDD mechanism, in a larger mix of political instruments and financed outside of a compliance carbon market is an opportunity for the protection of forests and the biodiversity of forest ecosystems. However, forests play a vital part for biodiversity and forest-dependent communities around the globe. Therefore it is first and foremost essential that rights and livelihoods of forest dependent peoples are protected. Experience with afforestation and reforestation activities under the Clean Development Mechanism (CDM) has shown that impacts on forest peoples can be excessively negative. Displacements, land grabbing, restriction of traditional use of forests and other violations of indigenous peoples and forest dependent peoples have been reported. The same issues have been reported with voluntary forest carbon activities such as REDD pilot projects and forest conservation projects. A robust and harmonized safeguard framework must therefore be put in place to enable the protection of forest livelihoods, uphold human rights and the conservation of biodiversity. There must be systematical monitoring, reporting and verification of safeguards. Information about these processes to forest dependent peoples must be scaled up considerably.

VI. REDD+ AND CARBON MARKETS:TEN MYTHS EXPLODED

Myth no. 1: 'REDD+ represents a low-cost abatement option, enabling greater and faster emissions cuts than could be achieved for the same total costs with fossil fuel reductions alone. This is essential for stabilizing GHG concentrations at the scale and speed necessary to avoid the most catastrophic effects of climate change.'

Myth no. 2: 'Estimates for the cost of cutting deforestation in half range from US $12 billion to US $35 billion per year. Raising this money will halve deforestation.'

Myth no. 3: 'Carbon trading finance can play an especially important role for REDD+ in the long term by contributing sustainable funding efficiently and on the scale required.'

Myth no. 4: 'Creating an economic value for standing forests will provide the necessary long-term economic incentives for effectively protecting tropical forests and reducing emissions from deforestation.'

Myth no. 5: 'REDD+ is particularly well positioned to benefit from the policy shift from "project" to "sector wide" trading, given the suitability of forestry as a sector-wide mitigation effort.'

Myth no. 6: 'Significant work has already been undertaken on REDD+ methodologies to ensure quality by

http://dx.doi.org/10.22161/ijeab/2.1.16 *ISSN: 2456-1878*

implementing rigorous measurement, reporting and verification requirements and determining reference levels which ensure additionality. As such, REDD+ is poised to be able to contribute rigorous, verifiable credits, fungible with emission reductions from other sources.'

Myth no. 7: 'Concerns about the potential risk of REDD+ supply "flooding" the carbon market can be contained through policy and market design, including the adoption of strict long-term targets with "banking" and, if necessary, limits on the use of REDD+ and other types of credits.'

Myth no. 8: 'For the period 2010–2012, developed countries committed US$4.5 billion for REDD+. The gap between this figure and the estimated annual financing needs for REDD+ is significant.'

Myth no. 9: 'The US acid rain programme is an example of how cap-and-trade and market mechanisms can work to achieve environmental goals at least cost.'

Myth no. 10: 'Concerns about additionality, non-permanence and leakage, which initially kept forests out of carbon markets, have been addressed.'

VII. PARIS CONVENTION, CARBON TRADING AND REDD

Article 6 of the Paris Agreement states a new carbon trading mechanism. It manages to do so with mentioning the wind carbon or trading or market. Carbon offsets are internationally transferred mitigation outcomes. And the new carbon trading mechanism is a mechanism to contribute to the mitigation of GHG emissions and support sustainable development. On 8th December,2015,Brazil and EU put forward a proposal on carbon market such as, "The EU and Brazil have agreed and submitted a ground breaking proposal on rules to governance of the international carbon market at the UN climate talks in Paris. The joint proposal demonstrates a willingness to engage in common and robust rules on accounting for all parties." The final rules of the new trading mechanism have not yet been agreed .It will only start in 2020 at the earliest. That means another five years of negotiating a new carbon market mechanism at the UNFCCC. Once the carbon trading mechanism kicks off, countries generating REDD credits will have no options.[i] Keep the REDD credit to offset the own emission from fossil fuels,[ii] Sell the REDD credits to countries that will use them to offset their emissions from fossil fuels.

Neither of these options reduces global GHG emissions, because in both cases the reduction in emission from forests would be offset against continued emissions from fossil fuels. Rich countries may finance REDD if it creates a loopholes allowing them to continue burning fossil fuels. But it is difficult to see why they would want to finance REDD if it creates burning fossil fuels. At the start of COP21,Norway's PM Erna Solberg announced that Norway wants to include REDD in carbon markets, so that in future Norway can claim to be carbon neutral. We need to dramatically reduce emissions from burning fossil fuels and from deforestation. We can not afford to trade off one against the other. Unfortunately, the Paris Agreement sets the stage for precisely that.

VIII. CARBON MARKET POTENTIAL FOR INDIA IN 2020

Government of India embarked upon its National Action Plan on Climate Change (NAPCC) with 8 missions to ensure energy security, sustainable development, protection of bio-diversity and climate resilience in June 2008. These missions are:

i. National Solar Mission
ii. National Mission for Enhanced Energy Efficiency
iii. National Mission on Sustainable Habitat
iv. National Water Mission
v. National Mission for Sustaining the Himalayan Ecosystem
vi. National Mission for a "Green India"
vii. National Mission for Sustainable Agriculture and
viii. National Mission on Strategic Knowledge for Climate Change.

An expert group was constituted by the Planning Commission to develop a low carbon inclusive growth strategy for India's Twelfth Five Year Plan. This Expert Group on Low Carbon Strategies for Inclusive Growth in its interim report estimated the national emissions reduction potential by 2020 for various sectors under two scenarios namely 8% and 9% annual GDP growth. The sectors covered are power sector, transport, iron & steel, cement, oil & gas, buildings, waste management, other industries and households. The Expert Group has either not considered or considered very limited potential in the following sectors: energy distribution, chemical industries, fugitive emissions from production and consumption of halocarbons and sulphur hexafluoride, construction, solvent use, mining/mineral production and fugitive emissions from fuels (solid, oil and gas). These sectors have been excluded from the analysis.

The regulatory framework, use of market mechanism and incentive mechanism (including price of emission reduction), will significantly influence carbon mitigation potential. This study examines the carbon market potential assuming CDM (or CDM like) framework in terms of baseline and crediting, additionality, etc. As the expert group has assumed a base year of 2007, the analysis has

http://dx.doi.org/10.22161/ijeab/2.1.16 *ISSN: 2456-1878*

first linearly apportioned the estimate to make 2012 as the base year. Two adjustments to account for the characteristics of the CDM were also made to the Expert Group's analysis to quantify the Indian CDM potential in 2020 (Table 1):

Table.1: Estimated CDM potential in 2020 in million ton CO_2

Sectoral Scope	Activity	Emission reduction potential (MtCO2) estimated by the Expert Group	Maximum emission reduction potential (MtCO2)	Emission reduction potential (MtCO2) at Euro 15 / tCO2e	Emission reduction potential (MtCO2) at Euro 10 / tCO2e	Emission reduction potential (MtCO2) at Euro 5 / tCO2e
Energy industries (renewable - / non-renewable sources)	Solar	14	11.72	0.00	0.00	0.00
	Biomass	12	5.82	5.82	4.90	0.00
	Wind	11	8.31	8.31	7.91	0.00
	Fossil fuel switch (Gas Based Combined cycle)	8	5.09	5.09	4.67	3.28
	Supercritical coal power plants	5	0.28	0.28	0.28	0.00
	Hydro	28	4.24	0.16	0.00	0.00
Energy demand	EE commercial building [Heating, Ventilation and Air conditioning, Lighting, Internal Loads and others]	37	0.23	0.23	0.23	0.23
	Lighting	27	26.20	26.20	26.20	0.00
	Fan, TV and AC	12	2.46	2.46	2.46	2.46
Manufacturing industries	Energy Efficiency - Clinker Substitution in Cement[23]	31	0.00	0.00	0.00	0.00
	Energy Efficiency - Fuel Substitution in Cement	5	2.43	2.43	2.43	0.04
	Refrigerators	4	0.74	0.74	0.74	0.74
Metal production	BF-BOF including waste heat projects	17	11.08	11.08	11.08	11.08
	COREX/FINEX-BOF	3	1.54	1.54	1.54	1.54
	DRI-EAF and F-Technology	2	1.23	1.23	1.23	1.23
Transport	Modal shift - Increased freight share of Railways and Non-motorised and public transport	19	1.39	1.39	1.39	1.17
	Fuel efficiency of vehicles	7	0.60	0.60	0.60	0.60
Waste handling and disposal	Landfill gas - (Composting, Solid waste, manure)	37	12.70	12.70	12.70	1.74
	Wastewater					
Afforestation and reforestation	Reforestation/Afforestation	28	0.37	0.12	0.00	0.00
Agriculture	Agriculture	3	3.08	0.00	0.00	0.00
Total		**307**	**99.5**	**80.38**	**78.35**	**24.11**

Source- Interim report of Expert Group on Low Carbon Strategies for Inclusive Growth and PwC analysis

As part of voluntary commitments, India has pledged reducing its emissions intensity of its GDP by 20-25% by 2020 in comparison to the 2005 level. Though restrictions around technologies (HFC23 and N2O abatement in adipic acid production) post true up period has no detrimental impact on the Indian supplies, yet the absence of demand for Indian projects registered after 31 December 2012 has resulted in reduced investment in several other sectors.Figure -1 shows the total investment into CDM projects for each state in the country. Industrialized states also have high renewable energy potential and this has lead to concentration of investments in the states of Gujarat, Andhra Pradesh, Maharashtra, Tamil Nadu and Karnataka. Himachal Pradesh has benefited from large number of

http://dx.doi.org/10.22161/ijeab/2.1.16 *ISSN: 2456-1878*

hydro CDM projects, which is to be expected given its hydro potential, and Delhi because of transport CDM projects. High investment in Madhya Pradesh in on account of coal based supercritical power projects. Arunachal Pradesh, Assam, Bihar, Kerala and West Bengal have seen very limited investments into CDM. Biomass CDM projects in the sample are the most efficient job creator and create more than four times the average jobs (per rupee invested) across all project-types. Wind and hydro projects in the sample create relatively less employment per rupee invested as compared to EE own generation and EE industry projects during the construction phase.

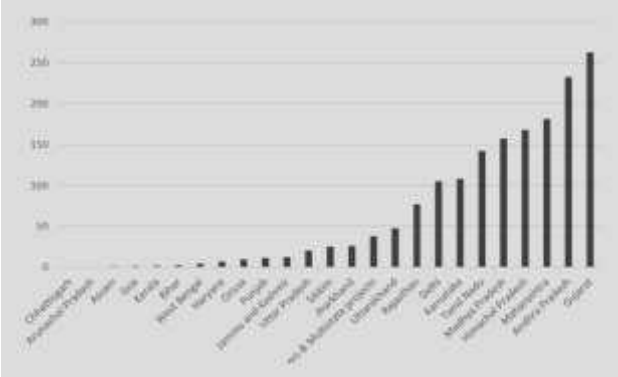


Fig.1:Statewise investment in CDM projects (Rs billion)

Source: BMUB Global Carbon Market Project, New Delhi

The Harnessing demand for Indian projects post-2012 are :

1. Supporting projects through domestic emission trading scheme –
2. Supporting projects through NCEF and CSR funds of large companies
3. Developing standardized baselines
4. Developing sustainable development impact reporting
5. 5.Evaluating and highlighting the benefits of CDM projects focusing on sustainable development Impacts
6. 6. Constituting a high level Multi Stakeholder Advisory Group for Climate Change issues
7. like Loss and Damage, Equity, Sustainable Development, Gender etc
8. 7. Developing NAMAs
9. 8. Developing the capacity for national emission reduction reporting and develop credible and robust reporting frameworks for corporate carbon reporting–

But there are several causes of delays of maturing projects which are as follows:

i. One of the major reasons for delays in registration of CDM projects is on account of lack of acceptable guidelines for setting benchmark, lack of institutional capacity, frequent revisions to CDM EB guidelines and lengthy validation cycle
ii. The delay in registration of CDM projects was due to the increase in CDM projects from India and limited increase in the number of DOEs
iii. The cement and energy efficiency project-types have higher rejection rate than hydro and wind project-types
iv. Projects in reforestation, EE household, EE in SME, off-grid solar and agriculture project-types face MRV, organizational and financial barriers.
v. HFC 23, N2O and landfill gas(where these is no energy generation) projects risk closure post the withdrawal of market support and fall in CER prices
vi. Goa, Bihar, Jharkhand, Kerala, Jammu & Kashmir, Haryana and North Eastern states have very limited development of CDM projects

The promotion and development of the emission reduction projects will require a combination of the following measures:

i. Demand-side measures: Given the weak demand for CERs and the uncertain time frame for new market mechanisms, all attempts should be made to revive demand in the existing regulatory framework, particularly for projects registered post 2012.
ii. Improving sustainable development impacts: Improving the sustainable development impacts as well as improving communication on the outcomes / impacts of CDM project activities is required for stimulating demand of quality CDM projects and addressing international concerns.
iii. Efficiency of registration: Once there is a revival of demand, measures should be undertaken to remove the barriers in CDM project registration while also improving sustainable development impacts.
iv. Future regulatory mechanisms: Recognizing that CDM is likely to be transitory in nature and new market mechanisms are likely to be more prominent particularly in the post 2020 carbon markets, measures should be undertaken to develop synergies between CDM, NAMAs and other market mechanisms.
v. Supply side measures: Once there is regulatory certainty and robust demand, supply side measures should be undertaken that encourage larger participation of industry in emerging global carbon / CDM market.

http://dx.doi.org/10.22161/ijeab/2.1.16 *ISSN: 2456-1878*

Therefore, the recommendations below are targeted towards:

A. Harnessing demand for Indian projects post 2012;
B. Achieving better sustainable development for CDM projects;
C. Developing synergies between CDM, NAMAs and other market mechanisms; and
D. Encouraging larger participation of industry in carbon market.

IX. CONCLUDING REMARKS

The international carbon market currently faces considerable uncertainties regarding its future architecture. There are a number of options for further development, including a global trading approach building on Kyoto, formal linkages of domestic ETS leading to a global CO2 market, and indirect linkages through credits if domestic ETS remain otherwise unconnected. Also, a mixed approach is conceivable. Regions should share a common understanding on the overall

climate policy goal (e.g., the 2°C target) as well as a burden-sharing rule translating into ETS caps. These two fundamental issues will crucially determine the level of ambition of an ETS as expressed in (a) the emission cap, which in combination with amount and costs of available abatement options of a region crucially determines the allowance price level; and (b) ETS design features also exerting influence on the allowance price level and environmental outcome. For a player with ambitious environmental targets it should be preferable to announce that it will link only under the condition that another system displays a similar level of ambition, thus using the

efficiency and potential reputational benefits from linking as a bargaining chip. Linking to less ambitious regions would undermine the credibility of such announcements. Harmonization of trading systems should start as early as possible in order to enable the option of linking ETS post-2012. For this purpose, ICAP could be a nucleus for such an international clearinghouse.

REFERENCES

[1] Angelsen, Arid., Gierloff, Caroline Wang ,Beltran, Angelica Mendoza & Elzen, Michel den . (2014). REDD Credits in a global carbon market: Options and Impacts. NORDEN.
[2] Anger, N., Dixon, A & Livengood, E.(2009). Interactions of Reduced Deforestation and the Carbon Market. The Role of Market Regulations and Future Commitments. Center for European Economic Research: *Discussion Paper No. 09–001*.
[3] Baron, R.& Bygrave, S.(2002). Towards International Emissions Trading: Design implications for linkages. *IEA/OECD Paper*.
[4] Bhowmik, Debesh. (2017).An Introduction to Climate Change, Synergy Books India Ltd, New Delhi.
[5] Deutsche Gesellschaft fur Internationale Zusammenarbeit.(2014,January).Carbon Market Road Map for India, BMUB Global Carbon Market Project, New Delhi.
[6] Edenhofer, Ottmar, Flachsland, Christian & Marschinski,Robert.(2007,May). Towards a global CO_2 market. Expertise for the Policy Planning Staff in the Federal Foreign Office. Potsdam Institute for Climate Impact Research.
[7] Ellermann, Denny.(2012). Linking Emissions Trading Schemes: Back to the Basics; paper presented on 12 November 2012 at the ZEW Conference "Rise of ETS in Asia"
[8] Ellis, J. & Tirpak, D.(2006). Linking GHG Emission Trading Systems and Markets. *IEA/OECD Paper*.
[9] German Emission Trading Authority.(2013).Linking Different Emissions Trading System: Current State and Future Perspectives, Berlin.
[10] Hausotter, Tobias.,Sibyl, Steuer & Dennis, Tanzler.(2011). Competitiveness and Linking of Emission Trading Systems (*UBA Climate Change 01/2011*) http://www.umweltdaten.de/publikationen/fpdf-l/4051.pdf
[11] Karsenty,A.(2009).'What the (carbon) market cannot do.' CIRARD. http://www.cirad.fr/en/news/all-news-items/articles/2009/just-out/perspective
[12] Moyes, T.E.(2008). Greenhouse gas emission trading in New Zealand: Trailblazing comprehensive cap and trade. *Ecology L.Q. 911*
[13] Murray, B. C., Lubowski,R & Sohngen,B. (2009). *Including International Forest Carbon Incentives* in Climate Policy: Understanding the Economics. Nicholas Institute Report. Durham, NC: Nicholas Institute for Environmental Policy Solutions, Duke University.
[14] Pirard, R.(2008). 'The fight against deforestation (REDD+): economic implications of market-based funding.' Paris: IDDRI.
[15] Pizer, William.(2007). Practical Global Climate Policy. In: Aldy, Joseph E., and Robert N. Stavins (2007): *Architectures for Agreement. Addressing Global Climate Change in the Post-Kyoto World.* Cambridge University Press

[16] Potsdam Institute for Climate Impact Research.(2008).Developing the International Carbon Market: Linking Options for EUETS,NZ
[17] Tangen, Kristian & Hasselknippe, Henrik. (2005, March).Converging Markets. International Environmental Agreements: *Politics, Law and Economics*. Volume 5, No 1.
[18] The Carbon Trust. (2008). Global Carbon Mechanisms: emerging lessons and implications. UK: Carbon Trust, p. 11.
[19] The Munden Project. (2011).REDD+ and forest carbon. http://www.mundenproject.com/forestcarbonreport2.pdf
[20] The RAIN FOREST FOUNDATION. (2011, June). REDD+ and Carbon Market:10 Myths exploded, Green peace.
[21] UNEP. (2010). *Bringing forest carbon projects to the market*, New York.
[22] UNFCCC. (2010b). *Quantified economy-wide emission targets for 2020- Appendix I.* Available from http://unfccc.int/home/items/5264.php
[23] Victor, David.(2007). Fragmented carbon markets and reluctant nations: implications for the design of effective architectures. In: Aldy, Joseph E., and Robert N. Stavins (2007): *Architectures for Agreement. Addressing Global Climate Change in the Post-Kyoto World*. Cambridge University Press
[24] World Bank. (2011,June). *State and Trends of the Carbon Market 2011*. Carbon Finance at the World Bank, Washington DC.
[25] Zetterberg, Lars. (2012). Linking the Emissions Trading Systems in EU and California, *Fores*, Stockholm http://fores.se/assets/780/FORES-California_ETS-web.pdf

http://dx.doi.org/10.22161/ijeab/2.1.17 *ISSN: 2456-1878*

Evaluation of Storage Capacity of Iron Fortified Yogurt by Physico-chemical, Chemical and Microbiological Analysis

Debasmita De[1], Dr. BinataNayak[2]

[1] Junior Research Fellow, Department of Home Science (Food & Nutrition Division)University of Calcutta, Kolkata, West Bengal, India

[2]Assistant Professor, Department of Home Science(Food & Nutrition Division) University of Calcutta, Kolkata-700027, West Bengal, India

Abstract*—Yogurt has gained widespread consumer acceptance. It is excellent source of calcium and protein and other nutrients but it contains very little iron. In this study yogurt was fortified with ammonium ferrous sulfate in three different concentrations (20mg, 30mg, 40mg/kg iron). Yogurt samples were analyzed physico-chemically, chemically and microbiologically at 1st,3rd, 5th day of storage. Physicochemical and chemical result shows that there was significant difference between storage period and different sample concentration. Iron in the fortified samples had no significant effect in Lactobacillus count.The results suggest possibility ofmaking good quality yogurt by fortifying milk with ammonium ferrous sulfate.*

Keywords—Ammonium ferrous sulphate, Chemical, fortification, Microbiological, Physicochemical, Yogurt.

I. INTRODUCTION

Anaemia is a most common world -wide problem in the young children, pregnant woman and adolescent girl. Nutritional anaemia may be defined as the condition that results from the inability of the erythropoietic tissues to maintain a normal haemoglobin concentration on account of inadequate supply of one or more essential nutrients leading to reduction in the total circulating haemoglobin.[1] Most of the anaemias are due to inadequate supply of nutrients like iron, folic acid and vitamin B12, proteins, amino acids, vitamins A, C, and other vitamins of B-complex group i.e., niacin and pantothenic acid are also involved in the maintenance of haemoglobin level.[2]

Globally, anaemia affects 1.62 billion people, which corresponds to 24.8% of the population. The highest prevalence is in preschool-age children (47.4%), and the lowest prevalence is in men (12.7%). However, the population group with the greatest number of individuals affected is pregnant women (41.8%) [3].In women, anaemia may become the underlying cause of maternal mortality and perinatal mortality. Nearly 50 per cent of women of reproductive age and 26 per cent of men in the age group of 15-59 years are anaemic[4].

To reduce the prevalence rate of anaemia three types of measures are taken: 1.Dietary improvement, 2. Supplementation, 3.Food fortification.[1],[5],[6] The iron found in food can be highly bioavailable as in the case with heme iron which is found in red meat . The iron present in other products of vegetable origin contain non-heme iron has disadvantage of interacting with substance in food that inhibits absorption such as tannin and phytates[7]. The best way to prevent problems associated with iron deficiency is through iron fortification of food for whole population or certain group [8].Yogurt has gained widespread consumer acceptance. It is excellent source of calcium and protein,but it contains very little iron. Therefore dairy products are good for iron fortification because they have high nutritive value reach target population and are widely consumed[9],[10],[11]. The ideal iron compound used as fortificants should supply high bioavailability iron, it should not affect the nutritional value or sensory properties of food [12],[13],[14],[15].

The purpose of this study is to prepare iron-fortified yogurt with ammonium ferrous sulfate at three different concentration(20mg,30mg,40mg/kg milk)as it covers respectively 9.52%,14.28% and 19.04% of RDA of iron of an adult woman. And yogurt samples were analyzed chemically and microbiologically during1st,3rd,5th day of storage.

II. MATERIALS & METHODS

The study was designed to prepare Iron fortified yogurt and physicochemical, chemical, microbial analysis of iron fortified yogurt at 1st,3rd,5th day of storage period. Methodologies adopted for this analysis:

1. Preparation of iron fortified yogurt.[6]
2. Physicochemical analysis of iron fortified yogurt
   i) Whey separation by centrifugation method. [16]
   ii) Volume of supernatant by syneresis index. [16]

iii) Determination of Total TitratableAcidity .[17]
iv) Moisture content determination. [18]
v) Total solid content determination. [18]

3. Chemical analysis of iron fortified yogurt.
i) Determination of protein by Lowry method. [19]
ii) Determination of iron by Wong's method. [20]
.4. Microbial analysis of iron fortified yogurt.
i) Enumeration of Lactobacillus count. [21]

Preparation of Iron fortified Yogurt: Locally available AmulTaja toned homogenized pasteurized milk was taken. Milk was fortified with ammonium ferrous sulfate.The milk was divided into four portions. The first portion was not fortified with iron and regarded as control. The rest three portions were fortified with ammonium ferrous sulfate in different concentration respectively 20mg, 30mg, 40mg iron/kg milk. Then milk was inoculated with yogurt culture and filled into plastic cups,covered and kept at room temperature until a firm curd was formed (approximately 6-7 hours). The resultant yogurt was kept in a refrigerator for 5 days at 4°c.[6]

Statistical analysis: This was done by Two way Analysis of Variance (first factor storage period, second factor sample concentration).

III. RESULT & DISCUSSION

3.1 Physicochemical analysis

3.1.1 Volume of whey

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	0.592 ±0.001	0.672 ±0.009	0.866 ±0.02	Between columns=34.85	0.00084
Yogurt (20mg)	0.694 ±0.009	0.86 ±0.06	0.91 ±0.03	Between rows=404.10	0.0005
Yogurt (30mg)	1.293 ±0.05	1.399 ±0.02	1.496 ±0.02		
Yogurt (40mg)	1.475 ±0.01	1.576 ±0.04	1.636 ±0.01		

Inference: Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted.

3.1.2 Volume of supernatant by syneresis index (%)

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	11.85 ±0.03	13.44 ±0.19	17.32 ±0.48	Between columns=38.74	0.00075
Yogurt (20mg)	13.88 ±0.19	17.2 ±1.2	18.2 ±0.66	Between rows=430.30	0.0005
Yogurt (30mg)	25.86 ±1.03	27.98 ±0.48	29.92 ±0.53		
Yogurt (40mg)	29.2 ±0.5	31.52 ±0.81	32.72 ±0.3		

Inference: Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

3.1.3 Total Titratable Acidity (%):

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	0.27 ±0.005	0.29 ±0.005	0.32 ±0.01	Between columns=19.90	0.0021
Yogurt (20mg)	0.27 ±0	0.28 ±0.005	0.29 ±0.005	Between rows=6.18	0.03538
Yogurt (30mg)	0.27 ±0.005	0.30 ±0.005	0.31 ±0.005		
Yogurt (40mg)	0.28 ±0.005	0.32 ±0.01	0.34 ±0.01		

Inference: Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

3.1.4 Moisture(%):

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	86 ±0.7	87.2 ±0.5	88.8 ±1	Between columns=27.06	0.00119
Yogurt (20mg)	87.4 ±0.5	88.8 ±0.27	89.14 ±0.91	Between rows=9.08	0.0158
Yogurt (30mg)	87.6 ±0.9	88.4 ±0.7	89.2 ±0.63		
Yogurt (40mg)	88 ±0.7	89 ±1.7	89.4 ±0.77		

Inference: Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

3.1.5 Total solid(%):

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	14 ±0.7	12.8 ±0.5	11.2 ±1	Between columns=30.09	0.00101
Yogurt (20mg)	12.6 ±0.5	11.2 ±0.27	10.86 ±0.91	Between rows=9.04	0.01593
Yogurt (30mg)	12.4 ±0.9	11.6 ±0.7	10.8 ±0.63		

Yogurt (40mg)	12 ±0.7	11 ±1.7	10.6 ±0.77		

Inference: Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

3.2 Chemical analysis:

3.2.1 Protein (mg/ml):

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	31 ±0.5	31.3 ±0.9	31.4 ±1.2	Between columns=26.99	0.00119
Yogurt (20mg)	31.5 ±0.5	32 ±1	32.3 ±0.7	Between rows=80.98	0.00054
Yogurt (30mg)	32 ±2	32.4 ±0.6	32.5 ±0.5		
Yogurt (40mg)	32.2 ±0.11	32.44 ±0.55	32.56 ±1.05		

Inference: Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

3.2.2 Iron (µg/ml)

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	4 ±0.5	3.5 ±0.2	2.5 ±0.4	Between columns=35.80	0.00081
Yogurt (20mg)	21.06 ±0.40	20.03 ±1.15	19.5 ±0.8	Between rows=13149.72	0.0005
Yogurt (30mg)	31.5 ±0.8	31 ±0.5	30.5 ±0.5		
Yogurt (40mg)	43.5 ±0.5	42.2 ±0.75	41.53 ±0.95		

Inference: Two Way ANOVA shows that there was a significant difference between columns and rows. So null hypothesis is rejected and alternative hypothesis is accepted

3.3 Microbial analysis

3.3.1 Lactobacillus count (cfu/ml) 10^{10}

Sample	1st	3rd	5th	F ratio	P value
Yogurt (NF)	10.2 ±0.3	9.6 ±0.2	9.9 ±0.1	Between columns=1.13	0.40785
Yogurt (20mg)	11.8 ±0.8	11.03 ±0.55	11.3 ±0.3	Between rows=4.44	0.06589
Yogurt (30mg)	9 ±0.5	9.5 ±0.5	11.03 ±1.05		
Yogurt (40mg)	11 ±0.1	10.3 ±0.5	10.7 ±0.3		

Inference: Two WayANOVA shows that there was a non-significant difference between columns and rows. So null hypothesis is accepted.

IV. CONCLUSION

Yogurt is a most important health beneficial nutritious probiotic. It is a product of the lactic acid fermentation of milk. Result shows that during storage period volume of syneresis increased in all samples but this increase is significant in non-fortified sample. There was a steady increase in Total titratable acidity during storage period. The ph was decrasing due to accumulation of lactic acid as a bacterial culture was breaking down lactose in order to obtain energy. This observation is in agreement with the previous study by Nkhata et al[22]. The metabolic enzymatic activity of the yogurt starter culture could be the reason for increases in acidity which could be responsible for decreasing lactobacillus spp. count although statistical analysis shows there was nonsignificant difference in lactobacillus count during storage period and sample concentration. Statistical analysis of moisture, total solid, iron and protein shows that there was a significant difference between sample concentration and storage period. Present study shows all fortified yogurt samples were nutritionally rich and acceptable, suggesting that yogurt is a suitable vehicle for iron fortification.

REFERENCES

[1] Srilakshmi. B,"Nutrition Science",3rd Ed. Chapter10. pp-164-180. ISBN 10: 81-224-2147-4 New Age International (P) Limited. 2008.

[2] Lee GR; Herbert V. In: Lukens J, Paraskevas P, Greer JP, Rodgers GM, editors."Wintrobe's clinical hematology".Baltimore, Maryland USA: William & Wilkins, pp 228-266. **1998**,

[3] WHO, WHO global database on anaemia / Edited by Bruno de Benoist,Erin McLean, Ines Egli and MaryCogswell, **2008**. http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf retrieved on July February 6th, 2014

[4] Ezzati M; Lopus AD; Dogers A; Vander HS; Murray C, *Lancet*, "A Cross-Sectional Study of Hemoglobin Disorders in Pregnant Women Attending Two Urban Hospitals in Eastern Coast of Odisha, India". 360, pp1347-1360,**2002**.

[5] Nayak. Band. Madhavan KNair. Department of Biophysics NINICMR Hyderabad. "In Vitro Bioavailability of Iron from Wheat Flour Fortified with Ascorbic acid,EDTA and SHMP with or without Iron. "Food ChemistryVolume 80,Issue 4,pp.545-550.April 2003

[6] Amira M. El-Kholy, Osman .M, Gouda.A andWafaa A. Ghareeb ,"Fortification of Yoghurt with Iron". World Journal of Dairy & Food Sciences 6 (2): pp159-165, 2011 ISSN 1817-308X IDOSI Publications, 2011

[7] Van den Broek N, Letsky EA. "Etiology of anaemia in pregnancy in south Malawi".Am L ClinNutr. 72 (1Suppl): 247S-256S,2000.

[8] Biswas.T and Nayak .B, "Iron fortification of Green Gram Dhal flour and its iron invitro bioavailability as a preventive measure of anaemia", American International Journal of Research in Formal, Applied and natural science, 9(1), pp. 64-66, 2014.

[9] Sadler A.M., Lacroix D.E., Alford J.A, "Iron content of Baker's and cottage cheese made from fortified skim milks". J. Dairy Sci., 56: 1267-1270,1973.

[10] Tamime A. Y., Robinson R. K,"Yoghurt: Science and Technology". 2nd ed.CRC Press, Boca Raton, FL.1999

[11] Tamime A. Y., Deeth H. C. "Yogurt: technology and biochemistry", Journal of Food Protection 43 939–977,1980.

[12] United States Department of Agriculture.USDA Specifications for Yogurt, Non -fat Yogurt and Low fat Yogurt, Dairy Programs.2001.

[13] Lee W. J., Lucey J. A, "Formation and Physical Properties of Yogurt", Asian-Aust. J.Anim. Sci. Vol. 23, No. 9 : 1127 – 1136,2010.

[14] Zhang D., Mahoney A.W, "Iron fortification of process Cheddar cheese". J.Dairy Sci., 74: 353-358,1991.

[15] Saha.S, Dr. Nayak .B,"Evaluation of Organoleptic Properties and Storage Capacity of Green Gram Dhal Flour (*VignaRadiata*) Frotified with Iron and Its Absorption Promoters ", AIJRFANS 14(1), March-May, 2016, pp. 26-29

[16] Guzman- Gonzalez M., Morais F. and Amigo L,"Influence of skimmed milk concentrate replacement by dairy products in a low-fat set type yoghurt model system. Use of caseinates, coprecipitate and blended dairy powder", J. Sci. Food Agri, 80: 433-438.

[17] Behrad S., Yusof M.Y., Goh K. L.and Baba A. S,"Manipulation of Probiotics Fermentation of Yogurt by Cinnamon and Licorice: Effects on Yogurt Formation and Inhibition of Helicobacter Pylori Growth in vitro". World Academy of Science, Engineering and Technology, 2009; 36: 12-29.

[18] AOAC, 1995," Official Methods of Analysis of the Association of Official Analytical Chemists",VolII 16th ed., AOAC Arlington,VA.

[19] Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.L. Randall," Protein measurement with the Folin Phenol Reagent". J. Biol. Chem., 193: 265-275.1951.

[20] N.Raghuramulu,KM Nair and S. Kalyanasundaram. NIN.ICMR. "A Manual of Laboratory Technique" 77.Page 409.1982

[21] Dr. Pande Sunil S and Gupta Priyanka, " A Promising Functional Dairy Food Using Probiotics With Kalmegh ".Rajiv Gandhi Biotechnology Centre, Rashtrasant Tukdoji Maharaj Nagpur University, L.I.T. Premises, Nagpur-440 033 (M.S.), India. ISSN 2349-8870 Volume: 2 Issue: 3 376-384 Year: 2015.

[22] Nkhata Gilliard Smith ,Ustunol Zeynep, Menevseoglu Ahmed, "Iron Fortification Yogurt and Pasteurised Milk". Journal of nutritional Health and Food Science. Pub: April 13,201

http://dx.doi.org/10.22161/ijeab/2.1.18 *ISSN: 2456-1878*

Comparative Performance of Selected RSJ Bivoltine Silkworm (*Bombyx mori* L.) Breeds under Subtropical region of Jammu

Murali S., Tayal M. K., Anil Dhar

Regional Sericultural Research Station, Central Silk Board, Miran Sahib, Jammu, India

***Abstract*—** *The study was conducted on selected silkworm breeds viz., RSJ 1, RSJ 3, RSJ 4, RSJ 11, RSJ 13, RSJ 14 and RSJ 15 were utilized. Observations on the different morphological and economic traits of silkworm, Bombyx mori L. were taken. The perusal of the data reveals that the fecundity was recorded from 384.00 (RSJ 13) to 493.67 (RSJ 1) and hatching per cent ranged from 86.88 (RSJ 13) to 97.61 (RSJ 3). The larval weight varied in the range of 36.46 g (RSJ 13) to 42.89 g (RSJ 11) whereas, larval duration was observed in the range of 24.00 (RSJ 13) days to 25.03 (RSJ 3) days. The larval length recorded as on 6th day 7.68 cm (RSJ 14) to 8.32 cm (RSJ 11). Single cocoon weight ranged from 1.24 g (RSJ 3) to 1.77 g (RSJ 15). The highest single cocoon weight was RSJ 15 (1.77 g), Single shell weight ranged from 0.22 g to 0.35 g. Maximum shell weight recorded in RSJ 14 (0.35 g), Shell percentage 16.73 per cent (RSJ 13) to 21.12 per cent (RSJ 3). Maximum yield recoded in RSJ 1 (16.01 Kg), was observed significantly superior compared to others. These findings will help the sericulturists in finding suitable breed for getting more economic returns from silkworm rearing. It is observed that RSJ 1 is having highest yield per 10, 000 larvae.*

***Keywords*— Fecundity, Larval duration, Larval length, Cocoon yield, Shell ratio, Bombyx mori L.**

I. INTRODUCTION

The silkworm, *Bombyx mori* L. spins valuable silk fibre, making it one of the most beneficial insects to mankind, and is becoming an attractive multifunctional material for both textile and non-textile uses (Murthy *et al.*, 2013).The practice of silk production involves diverse activities from the cultivation of host plants to silk processing, which engage people of all spectrums. Further, the by-products also find uses ranging from fertilizers in rural areas to pharmaceutical industries (Legay, 1958). Thus, silk production has the potential to make a significant contribution to the economy of many countries where there is surplus labor, low-costs of production and a willingness to adopt new technologies (Hajare *et al*., 2007).

However, rearing of superior silkworm strains that well adapt to the local environment is an important method for enhancing cocoon quality, increasing cocoon yield an improving economic benefit (Nguku *et al*., 2009). Differences in agro-ecologies across regions including significant distinctions in temperature and humidity require a type of silkworm strain which is both hyper silkgeneous and adversity resistant (Basavaraja *et al.*, 2005). Rearing performance in silkworms is also affected by ecological, biochemical, physiological and quantitative characters, which influence growth and development, quantity and quality of silk they produce in different geographical locations (Virk *et al*., 2011; Ramesh *et al*., 2012; Anandakumar and Michael, 2012 and Reddy *et al.*, 2012). The success in silkworm rearing depends on the various factors including successful implementation of technological and managerial tools along with high yielding and best-suited mulberry varieties and silkworm strains (Rajan and Himantharaj, 2005). In addition, the *B. mori* insect is an oligophagous herbivore and depends mainly on the quality of mulberry leaves and environmental conditions for its development (Murthy *et al.*, 2007).

II. METHODOLOGY

The experiment was carried out on seven *B. mori* parental RSJ breeds *viz*., RSJ 1, RSJ 3, RSJ 4, RSJ 11, RSJ 13, RSJ 14 and RSJ 15 maintained at the RSRS, Jammu during spring, 2016 and were incubated for 9-12 days in a neat and clean, disinfected room at 80-85 % Humidity and 24-25ºC Temperature with 18 hrs light till pin head stage, at this stage black - boxing was done to ensure maximum hatching on exposure to bright light. The hatched larvae were reared separately under uniform laboratory conditions as described by Yokoyama (1963) and Krishnaswami (1978). During the entire period of research, same micro-climate and feeding conditions were ensured as per the larval stage. The

experiment analysed for morphological charateristics of egg, larvae and cocoon parameters (Table 1) and biological studies of larvae includes feeding duration (Table 2), hatching percentage, larval weight (Table 5) and total larval duration of different instars were studied, which reflects their variation among the breeds (Table 3) and cocoon characteristics of all breeds were recorded.

All the breeds were reared in three replications by following standard rearing techniques (Krishnaswami, 1978). Three hundred larvae were retained after 2nd moult in each replication. The data pertaining to the morphology/ phenotypic and biological/economic parameters were recorded. During the entire period of research, same micro-climate and feeding conditions were ensured as per the larval stage.

At egg stage: Egg shape, egg colour, hatching percentage and average fecundity per female moth were studied.

At larval stage: Larval colour, markings, larval length and mean weight of 10 larvae on each day of V instar were studied and analyzed for different races.

The statistical analysis was done with the help of software SPSS and weight of larvae was measured with electronic balance.

At cocoon stage: cocoon shape, cocoon colour, cocoon grain and economic characters of cocoon were noted.

III. RESULTS AND DISCUSSION

3.1. Morphological Qualitative Parameters:

Egg: All the RSJ breeds which are selected are ellipsoidal in shape, grey in color and shell color was recorded as white (Table 1). Similar results were obtained by Anita *et al.* (2014).

Larvae: Color of newly hatched larvae was recorded as black and haemolymph color was transparent in all the breeds. The pattern of larvae was observed marked in RSJ 1, RSJ 3, RSJ 11, RSJ 14, RSJ 15 and RSJ 4, RSJ 13 was recorded as plain (Table 1). The results were agreement with work done by Anita *et al.* (2014).

Cocoon: All the RSJ breeds were distinctly white in color. The shape of cocoon was dumbbell and constricted (DC) in RSJ 1, RSJ 11 and RSJ 15 and oval in RSJ 3, RSJ 4, RSJ 13 and RSJ 14 respectively. The build and grain of among all the breeds was recorded hard (RSJ 1, RSJ 11, RSJ 14, RSJ 15), thin (RSJ 3. RSJ 4, RSJ 13) and medium respectively (Table 1). Similar results were obtained by Anita *et al.* (2014).

3.2. Larval biological parameters:

3.2.1. Feeding duration:

First instar: The active period of feeding among all the breeds was recorded three days five hours (Table 2).

Second instar: Two days fourteen hours were recorded as most active period of feeding among all the breeds (Table 2).

Third instar: The active feeding period was recorded among all breeds was three days sixteen hours (Table 2).

Fourth instar: The feeding period was observed among all breeds varying between 4.04 to 4.13 h observed in RSJ 1 and RSJ 15 respectively (Table 2).

Fifth instar: There is variation among breeds was recorded, eight days one hour more active feeding period was recorded in RSJ 4 and less *i.e.* six days observed in RSJ 13 respectively (Table 2).

The total average feeding duration among all the breeds was more in RSJ 11 *i.e.* twenty two days nine hours and less was recorded in RSJ 13 *i.e.* nineteen days fifteen hours respectively (Table 2).

3.2.2. Larval duration:

First instar: The period among all the breeds was recorded as four days five hours respectively (Table 3).

Second instar: Three days fourteen hours were recorded as 2nd stage larval period among all the breeds (Table 3).

Third instar: Four days sixteen hours were recorded as 3rd stage larval period among all the breeds (Table 3).

Fourth instar: The larval period was observed five days thirteen hours among all the breeds except RSJ 1 five days eight hours (Table 3).

Fifth instar: There is variation among breeds was recorded, more days recorded, seven days three hours in RSJ 3 and less *i.e.* six days observed in RSJ 13 respectively (Table 3).

The total average larval duration among all the breeds was more in RSJ 3 *i.e.* Twenty five days three hours and less was recorded in RSJ 13 *i.e.* twenty four days respectively (Table 5) and larval period was ranged from 25.03 – 24.00 days. The results were agreement with Krishnaswami (1978) reported that the larval duration was longer in race M-5 (24.17), but it was non agreement with results reported by Bothikar *et al.* (2014) the larvae reared on S -1635 recorded 19.66 days. Under ideal conditions it has been reported that the total larval duration is 25-30 days for selected JAM breeds (Raina, 2000). The results were justified with work done by Anita *et al.* (2014) reported similar results on JAM breeds, the larval duration which ranges between 23.22 - 26.16 days.

3.2.3. Larval length:

The length of 5th instar larvae was recorded from first day to sixth day results revealed that there is significant differences were observed among all the breeds except in first day (Table 4). During first day the larval length ranges from 4.20 (RSJ 13) to 4.28 cm (RSJ 4), 2nd day it was ranges from 4.60 cm (RSJ 4) to 5.58 cm (RSJ 14), 3rd day

ranges between 6.06 (RSJ 4) to 6.86 cm (RSJ 13), 4th day recorded as 7.02 cm (RSJ 15) to 7.56 cm (RSJ 13), 5th day it was ranges from 7.22 (RSJ 14) to 7.84 cm (RSJ 1) and 6th day recorded as 7.68 cm (RSJ 14) to 8.32 cm (RSJ 11) (Table 4). Similar results were recorded by 6.71 to 7.25 cm (Prabu *et al.*, 2011) and 6.12 to 7.05 cm (Balasundaram *et al.*, 2013). The data shows that there is increase in larval length from day 1 to day 6 of 5th instar. The length of silk worm depends on amount of food it consumes. Length of larvae increases until larvae reaches it's spinning state (6th day). When it reaches spinning stage the larvae reduce in size to one third of its normal length, it is the characteristic feature of a silk worm. This reduction in size increases pressure on silk glands to eject silk from the glands. Hence there is a sudden decrease in length on 6th day onwards. This decrease in length continues until pupal stage. The results also agreement with work done by Venugopal Reddy *et al.* (2015) revealed that the larval length varies from 5.00 to 7.43 cm.

IV. ECONOMIC PARAMETERS

The data pertaining to nine economic traits *viz.*, hatching %, larval duration (h), larval weight (g), cocoon yield per 10,000 larvae by number, cocoon yield per 10,000 larvae by weight, pupation rate, cocoon weight, cocoon shell weight, and cocoon shell ratio of five breeds were presented in Table 5. The perusal of the data reveals that the fecundity was recorded from 384.00 (RSJ 13) to 493.67 (RSJ 1) which shows statistically non significant among all the breeds and hatching per cent ranged from 86.88 (RSJ 13) to 97.61 (RSJ 3) and showing statistically significant among all the breeds where as larval duration shows statistically significant among all the breeds and recorded to a maximum duration of 25.03 days (RSJ 3) and minimum of 24.00 days (RSJ 13) where as larval weight was recorded to a maximum of 42.89 g (RSJ 11) and minimum of 36.46 g (RSJ 13) showing statistically significant among all the breeds. The pupal weight recorded more weight in RSJ 1 and less weight was observed in RSJ 14 having 13.56 g and 11.36 g respectively, whereas pupation rate showing statistically non significant among all the breeds and it was recorded as highest 86.67 per cent (RSJ 1, RSJ 3) and less 82.40 per cent (RSJ 4). With regard to yield per 10,000 larvae by number was recorded the highest (9440.00) in RSJ 1 and lowest in RSJ 4 (8746.67). Yield per 10,000 larvae by weight (kg), ranged to the maximum of 16.01 kg in RSJ 1 and minimum of 9.60 kg in RSJ 4 showing statistically significant among all the breeds with regard both by number and weight basis. The weight of cocoon found to the highest of 16.95 g in RSJ 1 and lowest of 15.18 g in RSJ 14 shows statistically non significant among all the breeds. The cocoon weight ranged from the maximum of 1.77 g (RSJ 15) and minimum of 1.24 g (RSJ 3). The shell weight was maximum (0.35 g) in RSJ 14 and minimum in RSJ 4 (0.22 g) shows statistically significant among all the breeds with respect to both cocoon weight and shell weight. The shell ratio (%) shows statistically significant among all the breeds and was highest in RSJ 3 (21.12) and lowest in RSJ 13 (16.73) respectively.

The highest average fecundity per moth was 522 in Jam 2 and lowest was 355 in Jam 18 reported by Anita *et al.* (2014) these results were non agreement with our results. The lowest hatching percent (93.79 %) was obtained in Jam 11 race and the highest (94.17 %) was obtained in Jam 27 race, with a mean of 93.92 per cent in all studied races Anita *et al.* (2014). It was justified with our results, 86.88 (RSJ 13) to 97.61 (RSJ 3).

The larval weight was agreement with results reported by Bothikar *et al.* (2014) *i.e.* 40.54 g which were reared on S - 1635 and other breeds which on par with silkworm reared on variety M - 5 and breeds having larval weight ranges between 36.46 g (RSJ 13) to 42.89 g (RSJ 11). Similar results were recorded by Pakhale *et al.* (2014), the larval weight was ranges from 33.77-40.67 g.

The larval duration was non agreement with results reported by Pakhale *et al.* (2014), the larval duration ranges from 21.04 - 22.28 days, our results shows that 24.00-25.03 days among the breeds.

The single cocoon weight was not justified with results reported by Bothikar *et al.* (2014) *i.e.*, 1.86 g which were reared on S - 1635 and Rayer (2006) and Chakravorty (2004) reported the highest single cocoon weight on variety V- 1 and also justified with results reported by Pakhale *et al.* (2014) single cocoon weight ranges from 1.76-1.86 g, our results revealed that it ranges between 1.24-1.77 g.

The single shell weight was non agreement with Bothikar *et al.* (2014) report the silkworms which reared on S-1635 but it was similar results with variety M - 5 reported by Bothikar *et al.* (2014). Rayer (2006) and Chakravorty (2004) reported the highest single shell weight on variety V- 1 and also not justified with results reported by by Pakhale *et al.* (2014) single cocoon weight ranges from 0.32 -0.36 g, our results revealed that it ranges between 0.22 (RSJ 4) - 0.35 g (RSJ 14).

The cocoon shell percentage varies between 17.84 to 19.93 per cent reported by Bothikar *et al.* (2014) and it was agreement with our results the shell percentage varies between 16.73 (RSJ 13) - 21.12 per cent (RSJ 3). Similar results Rayer (2006) and Chakravorty (2004) reported the cocoon shell percentage.

http://dx.doi.org/10.22161/ijeab/2.1.18 *ISSN: 2456-1878*

Cocoon yield per 10,000 larvae brushed varied in the range of 18.66 Kg to 16.59 Kg reported by Bothikar *et al.* (2014) and it was non agreement with our results on S - 1635 but similar results which reared on variety M - 5, the yield varies between 9.97- 15.95 Kg.

Varietal differences for studied traits in *B. mori* has been reported by Ahsan *et al*., 2000, Li *et al*., 2001; Furdui *et al*., 2010. Similar studies on varietal diversity have also been sustained by the findings of Reza *et al*.,1993, Mistri and Jayaswal, 1992; Ahsan *et al*., 1999; Umashankara and Subramanya, 2002; Nezhad *et al*., 2009; Nguku *et al*., 2007; Nguku *et al*., 2009; Zannata *et al*., 2009; Pal and Moorthy, 2011).

V. CONCLUSION

The obtained data showed that there are highly significant differences among the breeds for all the studied characters. There is a high positive correlation between economic parameters among all the breeds studied. The differences in obtained results are due to the variability and genotype characters for each individual of every breed.

VI. ACKNOWLEDGEMENTS

The authors wish to thank the authorities of Regional Sericultural Research Station, CSB, Miran Sahib, Jammu - 181101, J & K, India for their encouragement to undertake this work. The authors are also thankful to Dr. Anil Dhar, Scientist-D, for his assistance in conducting effective work and close monitoring during the study. We acknowledge all technical staff who assisted their help in rearing.

REFERENCES

[1] Ahsan M K, Rahman S M and Ali I A. 1999. Inheritance of some quantitative traits in fifteen indigenous varieties of silkworm, *Bombyx mori* L. *Univ. J. Zool. Rajshahi Univ.* **18**: 79-83.

[2] Ahsan M K, Rahman S M and Ali I A. 2000. Variability of some quantitative traits in the hybrids of silkworm, *Bombyx mori* L. *Univ. J. Zool. Rajshahi Univ.* **19**: 20-24.

[3] Anandakumar M D and Michael A S. 2012. Effect of nutritive additive of mulberry and its impact on nutritional components of silkworm, *Bombyx mori* L. *Int. J. Adv. Biotech. Res.* **3**:523-529.

[4] Anita, Tripathi N K, Bhatti A A and Poonam, 2014. Comparative analysis of developmental stages in six different bivoltine races of *Bombyx mori*. *International Journal of Recent Scientific Research,* **5**(11): 1969-1972.

[5] Balasundaram D, Ganesh Prabu P, Selvisabhanayakam, Mathivanan, V and Ramesh V. 2013. Studies on the nutritional supplementation of vitamin C treated MR_2 mulberry leaves fed by V instar larvae of silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae) in relation to feed efficacy and growth rate. *International Journal of Research in Biotechnology and Biochemistry*, **3**(1): 11-18.

[6] Basavaraja H K, Aswath S K, Kumar N S, Reddy N M and Kalpana G V. 2005. Silkworm Breeding and Genetics. Central Silk Board, Bangalore.

[7] Bothikar P A, Jadhav S S and Shinde Y A. 2014. Growth and development of silk worm (*Bombyx mori* L.) on mulberry leaves. *Jr. of Industrial Pollution Control,* **30**(2): 239-241.

[8] Chakravorty, N J. 2004. Studies on different parameters of *B. mori*. *Seridoc*, **20**(2): 21-23.

[9] Furdui E M, Marghitas L A, Dezmirean D, Mihai C M, Bobis O and Pasca I. 2010. Comparative study of biological characters of larvae, crude and dried cocoon in 7 races of Silkworm *Bombyx mori* L. raised in Transylvania area. *Scientific Papers: Animal Science and Biotechnologies*, **43**(1): 490-493.

[10] Hajare T N, Jadhav A D, Venugopalan M, Patil N G, Chaturvedi A and Maji A K. 2007. Evaluation of sericulture for augmenting agricultural income of marginal farmers in semi-arid region of India. *Proceedings of the International Conference on Sericulture Challenges* in the 21st Century and the 3rd BACSA Meeting, September 18-21, 2007. Vratza, Bulgaria.

[11] Krishnaswami S. 1978. New technology of silkworm rearing. Bulletin of the Central Sericulture Research and Training Institute, Mysore, pp. 1-10.

[12] Legay J M. 1958. Recent Advances in Silkworm Nutrition. *Ann. Rev. Entomol.* 3:75-86.

[13] Li M, Yao W, Hou Q, Lin C Q, Chen K P. 2001. Studies of some special characters in the silkworm (*Bombyx mori* L.) germplasm in China. *Sericologia*, **41**: 527-535.

[14] Mistri P K and Jayaswal K P. 1992. Studies on phenotypic correlations between some economic traits of silkworm, *Bombyx mori* L. *Bull. Seric. Res.* **3**: 26-29.

[15] Murthy V N Y, Ramesh H L, Lokesh G, Munirajappa and Yadav, B R D. 2013. Assessment of six mulberry (*Morus*) germplasm varieties through moulting and bioassay parameters by using Crossbreed Silkworms *Bombyx mori* L. for Commercial Exploitation in Kolar

District, Karnataka, India. *Int. Res. J. Biol. Sci.*, **2**: 69-75.

[16] Nezhad M S, Mirhosseini S Z, Gharahveysi S, Mavvajpour M and Seidavi A R. 2009. Analysis of Genetic Divergence for classification of morphological and larval gain characteristics of peanut cocoon silkworm (*Bombyx mori* L.) Germplasm. *American-Eurasian J. Agric. & Environ. Sci.* **6**(5): 600-608.

[17] Nguku E K, Adolkar V V, Raina S K, Mburugu K G, Mugenda O M and Kimbu D M. 2009. Performance of six Bivoltine *Bombyx mori*. (Lepidoptera: Bombyxidae) silkworm strains in Kenya. *The Open Entomology Journal*, **3**: 1-6.

[18] Nguku E K, Muli E M and Raina S K. 2007. Larvae, cocoon and post-cocoon Characteristics of *Bombyx mori L.* (Lepidoptera:bombyxidae) fed on mulberry leaves fortified with Kenyan royal jelly. *J. Appl. Sci. Environ. Manage.* **11**(4): 85-89.

[19] Pal N B and Moorthy S M. 2011. Assessment of variability in larval and cocoon traits in some genotypes of bivoltine silkworm, *Bombyx mori* L. *International Journal of Research in Biological Sciences*, **1**(4): 59-65.

[20] Pakhale S G, Bothikar P A, Lande U L, Shendage S A. 2014. Evaluation of Some Mulberry Varieties for Rearing Performance and Economic Traits of Silkworm (*Bombyx mori* L.). *Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTFT) **8**: 63-65.

[21] Prabu P G, Sabhanayakam S, Mathivanan V and Balasundaram D. 2011. Studies on the Growth Rate of Silkworm, *Bombyx mori* (L.) (Lepidoptera: Bombycidae) Fed with Control and Silver Nanoparticles (AgNps) Treated MR2 Mulberry Leaves. *Int. J. Indust. Entomol.* **22**(2): 39-44.

[22] Rajan R K. and Himantharaj M T. 2005. Silkworm Rearing Technology. Central Silk Board, Bangalore.

[23] Ramesha C, Lakshmi H, Kumari S S, Anuradha C M and Kumar C S. 2012. Nutrigenetic screening strains of the mulberry silkworm, *Bombyx mori*, for nutritional efficiency. *J. Ins. Sci.* **12**:1-18.

[24] Rayer S G., 2006. Stability of improved mulberry varities to shootlet feeding and it's impact on cocoon production. *J. Maharashtra agric. Univ.* **32**(3) : 367-369.

[25] Reddy N M, Kumar S N, Naseema Begum A R, Moorthy S M and Qadri S M H. 2012. Performance of bivoltine silkworm hybrids of *Bombyx mori* L. involving parental foundation crosses of different generation. *Int. J. Res. Zool.* **2**: 1-5.

[26] Reza A M S, Rahman M S and Rahman S M. 1993. Studies on the variation of some larval traits in different breeds of silkworm, *Bombyx mori* L. *Univ. J. Zool. Rajshahi Univ.* **12**: 21-24.

[27] Umashankara M L and Subramanya G. 2002. Correlation between larval weight and cocoon characters in five breeds of silkworm, *Bombyx mori* L. *Geobios*, **29**: 154-157.

[28] Venugopal Reddy B, Divya P, Anitha M. 2015. Quantitative profile Analysis of Mulberry Silkworm, *Bombyx mori.* L. (CSR 2 × CSR 4*). International Letters of Natural Science***, 34**: 34-41.

[29] Virk J S, Kaur L and Singh B. 2011. Evaluation of different strains of mulberry silkworm and eri silkworm for the development of sericulture in Punjab. *Int. J. agric. Sci.* **7**: 266-269.

[30] Yokayama, 1963. Sericulture Annual Review. Entomology, pp. 287-298.

[31] Zanatta D B, Bravo J P, Barbosa J F, Munhoz R E F and Fernandez M A. 2009. Evaluation of economically important traits from sixteen parental strains of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombyxidae). *Neotropical Entomology***, 38**(3): 327-331.

Table.1: Morphological qualitative characteristics of egg, larvae and cocoon parameters of RSJ breeds

Breeds	Egg			Larvae			Cocoon			
	Shape	Shell Colour	Egg colour	Colour of Newly Hatched	Haemoly-mph Colour	Larval pattern	Colour	Shape	Build	Grains
RSJ - 1	E	W	G	B	T	M	W	DC	H	M
RSJ - 3	E	W	G	B	T	M	W	O	T	M
RSJ - 4	E	W	G	B	T	P	W	O	T	M
RSJ - 11	E	W	G	B	T	M	W	DC	H	M

RSJ - 13	E	W	G	B	T	P	W	O	T	M
RSJ - 14	E	W	G	B	T	M	W	O	H	M
RSJ - 15	E	W	G	B	T	M	W	DC	H	M

Note: E - Ellipsoid, W - White, Y - Yellow, G- Grey, SS - Sand Stone, B- Black, T- Transparent, O -Oval, M- Medium, DC - Dumbell Constricted, H – Hard, T- Thin, P - Plain, M - Marked

Table.2: Data showing feeding duration among different stages of RSJ breeds during spring rearing (2016)

Race/Breed	**Different stages (Days: h)**					**Total Avg. duration (Days: h)**
	1st instar	**2nd instar**	**3rd instar**	**4th instar**	**5th instar**	
RSJ -1	3.05±0.00	2.14±0.00	3.16±0.00	4.04±0.04	6.01±0.02	20.14±1.72
RSJ -3	3.05±0.00	2.14±0.00	3.16±0.00	4.08±0.00	7.03±0.02	20.18±0.02
RSJ -4	3.05±0.00	2.14±0.00	3.16±0.00	4.10±0.03	8.01±0.44	21.19±0.50
RSJ -11	3.05±0.00	2.14±0.00	3.16±0.00	4.11±0.03	6.10±0.12	22.09±0.56
RSJ -13	3.05±0.00	2.14±0.00	3.16±0.00	4.08±0.00	6.00±0.00	19.15±0.00
RSJ -14	3.05±0.00	2.14±0.00	3.16±0.00	4.10±0.03	7.10±0.57	22.06±1.06
RSJ -15	3.05±0.00	2.14±0.00	3.16±0.00	4.13±0.00	7.01±0.02	21.01±0.02

Table.3: Data showing larval duration among different stages of RSJ breeds during spring rearing (2016)

Races/breeds	**Different stages (Days : h)**					**Total duration (Days : h)**
	1st instar	**2nd instar**	**3rd instar**	**4th instar**	**5th instar**	
RSJ -1	4.05±0.00	3.14±0.00	4.72±0.48	5.08±0.05	6.01±0.02	24.01±0.02
RSJ -3	4.05±0.00	3.14±0.00	4.16±0.00	5.13±0.00	7.03±0.02	25.03±0.02
RSJ -4	4.05±0.00	3.14±0.00	4.16±0.00	5.13±0.00	6.49±0.44	24.49±0.44
RSJ -11	4.05±0.00	3.14±0.00	4.16±0.00	5.13±0.00	6.10±0.12	24.10±0.12
RSJ -13	4.05±0.00	3.14±0.00	4.16±0.00	5.13±0.00	6.00±0.00	24.00±0.00
RSJ -14	4.05±0.00	3.14±0.00	4.16±0.00	5.13±0.00	6.34±0.57	24.34±0.57
RSJ -15	4.05±0.00	3.14±0.00	4.16±0.00	5.13±0.00	7.01±0.02	25.01±0.02

Table.4: Showing length of fifth instar larvae of RSJ breeds during spring (2016)

Breeds	**Days (5th instar)**					
	1	**2**	**3**	**4**	**5**	**6**
RSJ -1	4.24±0.11	5.10±0.27	6.82±0.19	7.06±0.21	7.84±0.17	8.28±0.15
RSJ -3	4.26±0.11	5.56±0.43	6.32±0.22	7.48±0.16	7.42±0.08	8.02±0.22
RSJ -4	4.28±0.15	4.60±0.34	6.06±0.21	7.06±0.21	7.44±0.11	7.96±0.13
RSJ -11	4.24±0.11	5.00±0.32	6.84±0.15	7.32±0.08	7.76±0.11	8.32±0.13
RSJ -13	4.20±0.10	5.18±0.08	6.86±0.15	7.56±0.11	7.48±0.08	7.90±0.12
RSJ -14	4.24±0.11	5.58±0.13	6.32±0.19	7.34±0.15	7.22±0.13	7.68±0.08
RSJ -15	4.24±0.11	5.20±0.10	6.36±0.11	7.02±0.16	7.34±0.18	7.74±0.11
C.D. @ 1%	-	0.35	0.23	0.21	0.16	0.18
SE. m±	0.05	0.12	0.08	0.07	0.05	0.06
C.V. (%)	2.77	5.20	2.74	2.22	1.73	1.76

http://dx.doi.org/10.22161/ijeab/2.1.18 *ISSN: 2456-1878*

Table.5: Economic parameters of RSJ breeds reared during spring (2016)

Breeds	Fecundity by No.	Hatching (%)	Larval duration (Days : h)	Larval weight (g)	Pupal weight (g)	Pupation rate (%)	Yield/ 10000 Larvae		Weight of cocoon (g)	Single cocoon weight (g)	Single shell weight (g)	Shell ratio (%)
							No.	Wt. (Kg)				
RSJ 1	493.67 (22.14)	96.54(79.27)	24.01	40.33	13.56	86.67 (68.63)	9440.00(97.15)	16.01	16.95	1.70	0.34	19.94(26.50)
RSJ 3	418.33 (20.47)	97.61(81.08)	25.03	42.09	13.37	86.67 (68.64)	9120.00(95.50)	13.44	16.23	1.24	0.26	21.12(27.33)
RSJ 4	407.00 (20.17)	91.47(73.15)	24.49	41.65	12.16	82.40 (65.20)	8746.67(93.52)	9.60	16.07	1.27	0.22	17.36(24.60)
RSJ 11	458.33 (21.42)	92.34(74.19)	24.10	42.89	11.60	85.47 (67.62)	9373.33(96.81)	12.40	16.06	1.25	0.23	18.66(25.57)
RSJ 13	384.00 (19.61)	86.88(68.92)	24.00	36.46	12.81	82.93 (65.59)	9106.67(95.43)	10.37	16.14	1.72	0.29	16.73(24.10)
RSJ 14	485.33 (22.03)	92.67(74.67)	24.34	37.96	11.36	86.13 (68.12)	9346.67(96.68)	14.27	15.18	1.65	0.35	21.09(27.32)
RSJ 15	402.00 (20.07)	92.04(74.40)	25.01	41.37	12.76	84.67 (66.95)	9100.00(95.39)	10.28	15.48	1.77	0.30	16.84(24.20)
C.D. @ 1%	-	6.88	0.48	2.44	1.11	-	1.88	1.93	-	0.17	0.02	1.83
SE. m±	0.70	2.24	0.16	0.79	0.36	1.17	0.61	0.63	0.49	0.05	0.007	0.59
C.V. (%)	5.86	5.18	1.13	3.42	5.03	3.02	1.11	8.85	5.38	6.43	4.45	4.04

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

The biological indicators studies of zooplankton in the Tigris River at the city of Baghdad

Khalid A. Rasheed[1], Hussain A. Flayyh[2], AbdulSalam T. Dawood[3]

[1]*Biotechnology Research Center/Al-Nahrain University;*
[2]*General Comp. for Elec. Indus./Ministry of Industry and Minerals;Ministry of Education and Scientific Research*

Abstract— *The study of biological indicators for zooplanktonis important factors in environmental studies to show the extent of the surrounding organisms, distribution and deployment environment affected. Zooplankton samples were collected from three stations on the Tigris River in the city of Baghdad using zooplankton net, specimens preserved and laboratory-diagnosed using internationally recognized classifications. Results show through the presence of relatively high abundance of zooplankton in the three stations and not affected by the city in addition to the species abundance is the other index gave few differences between stations, a lack of environmental pressures on these organisms in the station directory. Also, Shannon-Weiner diversity Indexpointer gave no significant differences between the study stations.*

Keywords— Tigris River, Baghdad, zooplankton, biological indicators.

I. INTRODUCTION

Life on earth depends on a balanced and accurate system of diversity, complement mutually and is losing species or group of species in an ecosystem, a reference to a defect in the function of this system (Elías- Gurtiérres *et al.*, 2001).

The aquatic monitoring, and the study of the installation of their societies and its biodiversity, gives a direct description of the state of the water body, which is the primary purpose for the management of ecosystems and the preservation of this diversity (Smith, 1999).

Zooplankton are small aquatic animals have a certain ability to swim and manipulated by the water column currents to move long distances. Moving mostly in the upper reaches of the water, it has been found in deep water also, a variety of nutrition (heterotrophic). Many of which feeds on decaying organic material (detritivorous) and play a big role in connecting the food chain by feeding on phytoplankton (Solomon, 2009).

Zooplankton consist of three groups of fresh water, (Rotifers),(Copepods) and (Cladocerans). The rotiferais great one division in fresh water, but copepod and cladocera, both are large group called the crustaceans (Smith, 2001). The Tigris River, hasmany of the studies on the prevalence and distribution of zooplankton (Nashaat 2010, Abbas and Al-Lami,2001 and Al-Lami, 2001).

The aim of the research is to study the bio-indicators of the zooplankton community as a vital proof of the water quality of the Tigris River.

II. MATERIALS AND METHODS

Study area

The study area is situated in the center of Iraq to the flat alluvial plain, which represents the western part of the continental shelf is stable to the continent of Asia, or the so-called Mesopotamian zone.

The Tigris River enters the city of Baghdad and being slow in speeding component of a number of twists river and a number of islands.The river bed consists of sand and silt and clay (Al-Aboody 1992). The water level starts to increase in October and above in April. The river view variable inside the city of Baghdad, depending on water levels between 190-500m and speed of 1.42 m/s at high discharge and 0.45 m/s at the low discharge (Iraqi Water Resources, 2011). Three stations were chosen to study, a north of the Baghdad station at Taji Bridge (station 1),station 2 in the middle of Baghdad,the station 3, lying south of Baghdad (Figure 1).

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

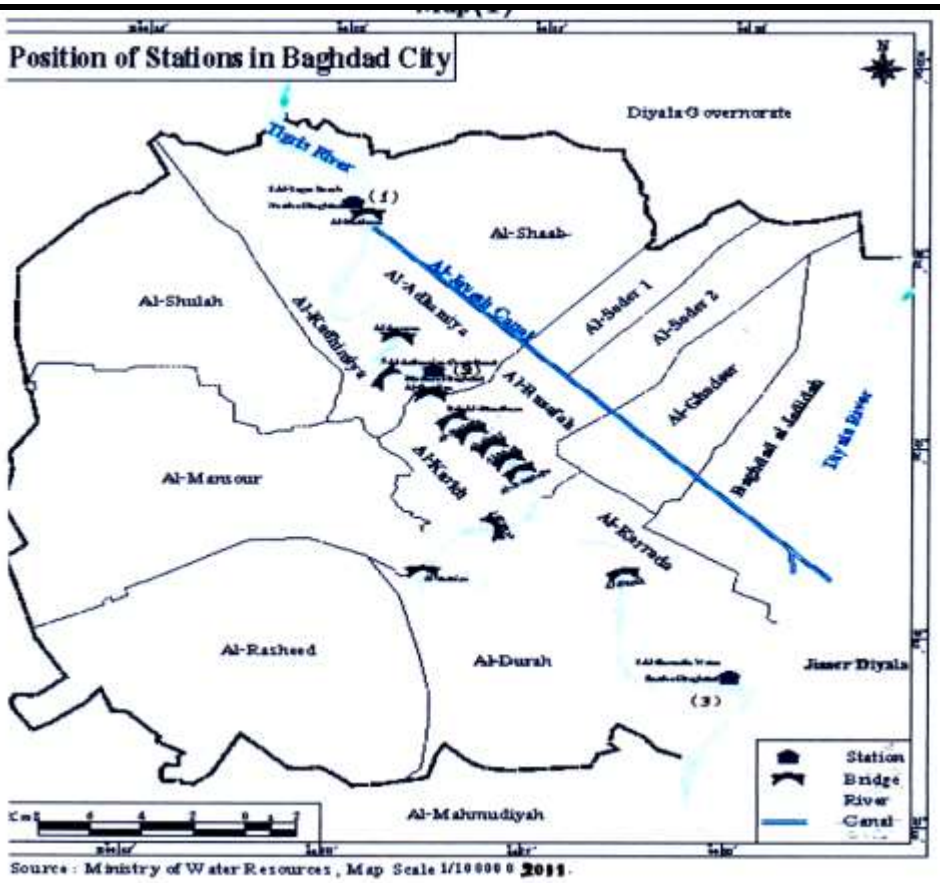


Fig.1: Map of sampling stations (Iraq Water Resources, 2011)
(Source: Ministry of water Resources, Map Scale 1/10000

Sampling collection

This study began in March 2010 until February 2011, zooplankton collected quantitative and qualitative from a depth of 30 cm by passing 60 liters of water from the river across the plankton net with mesh 55 µm in a small warehouse size of 50 ml, the sample preserved in 4% formalin solution. Diagnosed of zooplankton using a laboratory compound optical microscope using the keys (Edmondson 1959, Smith 2001, Petersen *et al*., 2010).The number of individuals calculated per cubic meter (Ind / m^3).

Biological indicators

Total Density and Relative abundance Index(Ra): This indicator was calculated using a derivative formula of Omori and Ikeda (1984) for calculating the relative abundance, as follows:

$$Ra = \frac{N}{Ns} \times 100$$

N = total number of individuals per unit taxonomic in the sample.

Ns = total number of individuals in the sample.

Since more than 70% prevalent types, 40-70% species abundant,10-40% a fewer types and less than 10% of rare species

Shannon-Weiner Diversity Index (H):

This indicator was calculated monthly using Shannon-Weiner formula as stated in (Floder and Sommer, 1999)

$$H = -\sum \frac{ni}{N} \ell n \frac{ni}{N}$$

Where ni= number of species

N= Total number of individuals

And expressed a determination unit bit/Ind. (bit=one piece of information). The values that are lower than 1 bit/Ind. hadslightly varied, while more than 3bit/Ind. was highly versatile (Porto-Neto, 2003).

The species Richness Index(D)

This index calculated from Sklar(1985) as follows"

$$D = \frac{(S-1)}{\text{Log } N}$$

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

Where s= number of species

N= Total number of species

III. RESULTS AND DISCUSSION

Total density and relative abundance index (Ra):

Station 1 recorded a less total density of zooplankton, reached about 334 individual/m^3 in July and the highest in April 2010 amounted to 3003 individual/m^3 out of 76 taxonomic units (Figure 2).

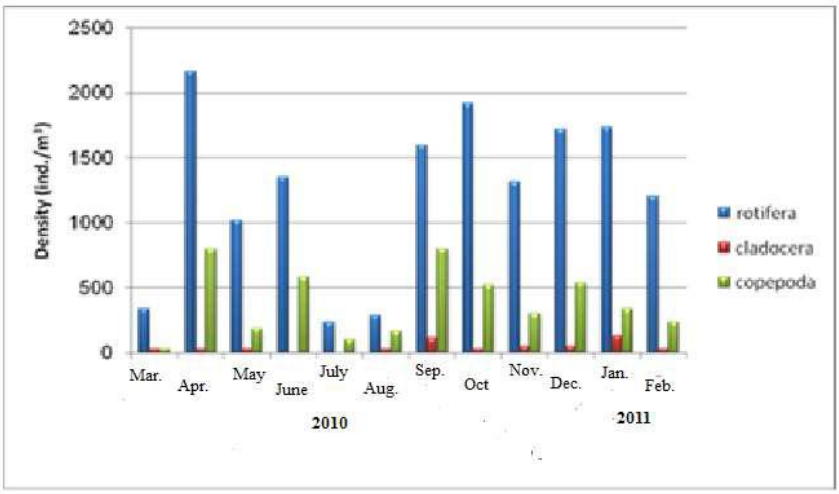


Fig.2: Total density of zooplankton in the station 1

While the total density ranged at the station 2 between 817 individual/m^3 in March 2010, and the highest density recorded in April 2010 and it was of 6018 individual/$m^3$from 64 taxonomic units (Figure 3).

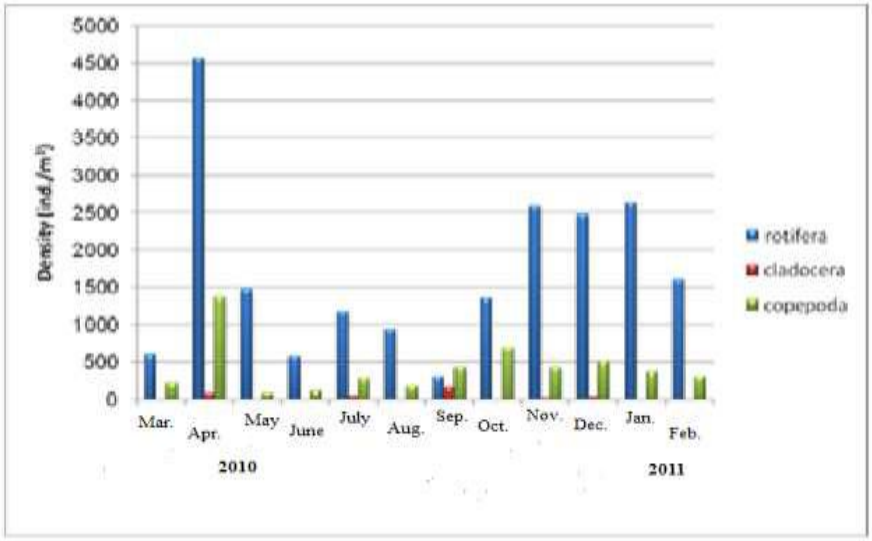


Fig.3: Total density of zooplankton in the station 2

While station 3 recorded the lowest density of zooplankton in the August 2010 reached about 235 individual/m^3 and higher density has recorded in April 2010 with 4336 individual/m^3 from 61 taxonomic units (Figure 4).

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

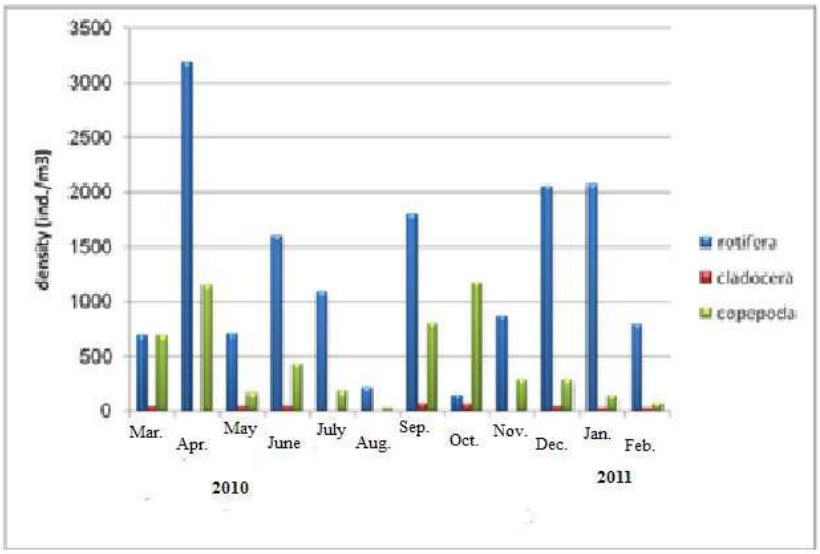


Fig.4: Total density of zooplankton in the station 3

Station 2 also recorded the highest total number of zooplankton (26.612 individual/m^3, while the lowest number in the station 1, which amounted to 20.074 individual/m^3.

The rotifera recorded the highest density compared to other groups with percentage 76.6% (Figure 5) which is most prevalent among zooplankton groups because of its ability to reproduce parthenogenesis for several generations, high fertility and their response is very rapid for environmental changes that make them are used as a guide to changing water quality (Rajashekar *et al.*, 2009). This is evident from many of the research (Shekha, 2008, Nashaat, 2010).

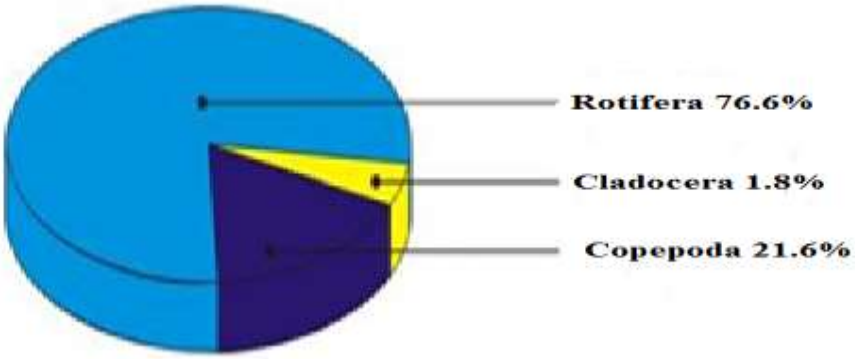


Fig.5: The percentages of zooplankton in the Tigris River at the city of Baghdad

Table 1 shows the proportions of the emergence of the species in the search for each station, where rotifera recorded the highest percentage of the species in station 1, where, the species *Keratella cochlearis* have the higher percentage(15.34%) followed by *Monostyla* sp. with 10.42%,then*Philodina roseola* by 9.39% and *Polyarthra* sp with 6.82% where the lowest percentages distributed among the rest of the species (Figure 6).

While in station 2 the relative abundance of rotifera species distributed as follows: *K. cochlearis* 14%, *Monostyla* sp. 11.42%, followed by *Philodina roseola* by 8.53%, and the lowest percentage distributed among the rest of rotifera species. In station 3,*P. roseola* recorded the highest proportion in comparison with other types of rotifera (18.92%), followed by *K.cochlearis* (15.22%) and *Monostyla* sp. (10.8%).

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

Table.1: The relative abundance of zooplankton in the three stations, and the appearance ratios, where(R) rare, less than 10%, (La) less abundant 40-10% (A), abundant species appearing 70-40% and dominant species (D) more than 70%.

	Taxa / Staion	1	2	3
	ROTIFERA			
1	*Asplanchna priodonta*	R	R	R
2	*Brachionus* sp.	R	R	R
3	*Brachionus angularis*	R	R	R
4	*Brachionus calyciforus*	R	R	R
5	*Brachionus caudate*	-	-	R
6	*Brachionus falcatus*	R	R	-
7	*Brachionus havanaenis*	R	-	-
8	*Brachionus plicatilis*	R	R	R
9	*Brachionus quadridentata*	R	R	R
10	*Cephalodella* sp.	R	R	R
11	*Cephalodella gibba*	R	R	R
12	*Colurella* sp.	R	-	R
13	*Colurella adriatica*	R	R	R
14	*Colurella obtuse*	R	R	R
15	*Colurella uncinata*	R	R	R
16	*Collotheca ornate*	R	R	R
17	*Conochilus unicornis*	-	-	R
18	*Eosphora* sp.	R	R	R
19	*Eosphora najas*	R	R	R
20	*Euchlanis deflexa*	-	-	R
21	*Euchlanis dilatata*	R	R	R
22	*Euchlanis pyriformis*	-	R	-
23	*Euchlanis triqetra*	R	-	R
24	*Filinia longuseta*	R	R	R
25	*Filinia opoliensis*	-	-	R
26	*Hexartha mira*	R	R	R
27	*Keratella* sp.	R	R	-
28	*Keratella cochlearis*	La	La	La
29	*Keratella hiemalis*	R	R	R
30	*Keratella quadrata*	R	R	R
31	*Keratella valga.*	R	R	R
32	*Lecane* sp.	R	R	-
33	*Lecane depressa*	-	-	R
34	*Lecane elasma*	R	R	R
35	*Lecane luna*	R	R	R
36	*Lecane ohioensis*	R	R	R
37	*Lepadella* sp.	R	R	-
38	*Lepadella ovalis*	R	R	R
39	*Lepadella patella*	R	R	R
40	*Macrochaetus subquadretus*	-	R	-
41	*Manfredium cadaetytotum*	-	-	R
42	*Monommata grands*	R	R	R
43	*Monostyla* sp.	La	La	La

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

44	*Monostyla bulla*	R	R	R
45	*Monostyla closterocerca*	R	R	R
46	*Monostyla lunaris*	R	R	R
47	*Mytilina mucronata*	-	-	R
48	*Mytilina ventralis*	R	-	-
49	*Notholca* sp.	R	-	-
50	*Notholca acuminate*	R	R	-
51	*Notholca striata*	-	R	-
52	*Philodina* sp.	-	R	-
53	*Philodina roseola*	R	R	La
54	*Platyias patulus*	-	R	-
55	*Platyias quadricorins*	-	R	R
56	*Polyarthera* sp.	R	R	-
57	*Polyarthera dolichoptera*	R	R	R
58	*Polyarthera vulgaris*	R	R	R
59	*Synchaeta* sp.	R	R	R
60	*Synchaeta oblonga*	R	R	R
61	*Synchaeta pectinata*	-	R	-
62	*Testudinella patina*	R	R	R
63	*Trichocerca* sp.	R	R	R
64	*Trichocerca capucina*	R	-	R
65	*Trichocerca longiseta*	R	R	R
66	*Trichocerca procellus*	R	R	R
67	*Trichocerca pusilla*	-	-	R
68	*Trichotria tetractis*	R	R	R
69	*Vanoyella globosa*	-	-	R
	CLADOCERA			
1	*Alona* sp.	La	La	La
2	*Alona guttata*	R	-	R
3	*Bosmina* sp.	-	-	R
4	*Bosmina coregoni*	R	R	La
5	*Bosmina longirostris*	La	-	R
6	*Camptocercus rectirostris*	La	La	-
7	*Ceriodaphnia* sp.	R	La	La
8	*Chydorus* sp.	R	-	La
9	*Chydorus sphaericus*	La	R	La
10	*Daphnia* sp.	R	R	-
11	*Ilyocryptus sordidus*	R	-	-
12	*Simocephalus* sp.	-	-	R
	COPEPODA			
1.	*Calanoida*	R	R	R
2.	*Cyclops*	D	D	A
3.	*Cyclopoida nauplus*	-	-	La
4.	*Diaptoms* sp.	R	-	-
5.	*Harpacticoida*	R	R	La
6.	*Macrocyclops*	R	R	R

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

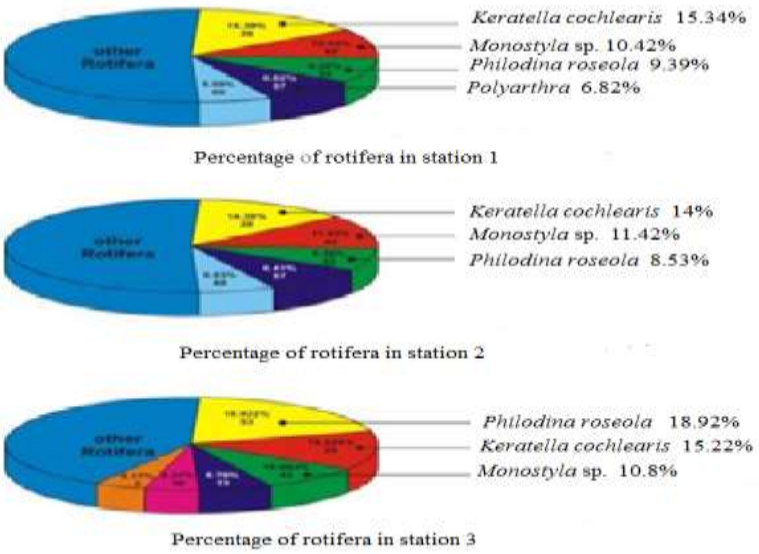


Fig.6: The percentage of rotifera in the three stations

The lack of a recording of values for the relative abundance index of rotifera gives a clear indication of the lack of environmental pressures in the river during the search, which may offer suitable conditions for the prosperity of certain types of resistance to these pressures and achieve overcome other species (Ahmad, *etal*., 2011).

The cladocera density ranged between (zero) in some months of the study to a higher intensity registered at the station 2 in September 2010 by 166 individual/m^3 (Figure 2). The relative abundance index refers to that the species*Bosmina longirostris*dominant at the station 1 by 25%, followed by *Camptocercus rectirostris* by 16.58% and *Alona* sp. by 13.9%. In the station 2 *Alona* sp. recorded the highest percentage(38%), then*Ceriodaphnia* sp. witha rate of 23.7% and then *Camptocercus rectirostris*(14.2%). *Ceriodaphnia* sp recorded the highest percentage at station 3 with 22%, then type *Bosmina coregoni*witha rate of 16.88%, followed by *Chydorus* sp. which scored about 16.5% (Figure 7).

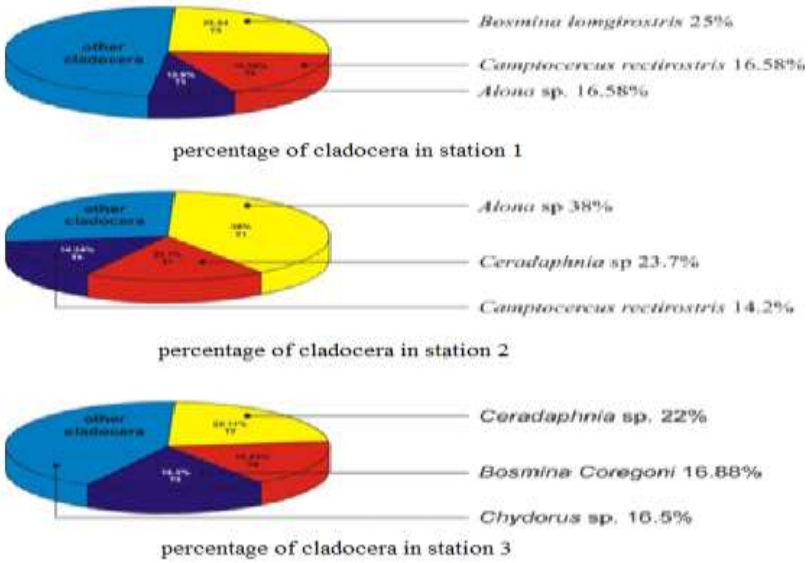


Fig.7: The percentage of cladocera in the three stations

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

The total density of cladocera in the study stations recorded as follows:station 1 ranged from 34 individual/m^3 in March 2010 to 800 individuals/m^3 in April 2010. The station 2, ranged from 184 individual/m^3 in May 2010 to 1367 individuals/m^3 in April 2010. While station 3 recorded about 17 individuals/m^3 in August 2010 to 1175 individuals/m^3 in October 2010.

The relative abundance of taxonomic units of copepoda guide to that the Cyclops is the most abundant in all studied stations compared to other taxonomic units of the same group with the rates of 84% in the station 1 and 88.58% in the station2 and 61.80% in the station 3 (Fig. 8).

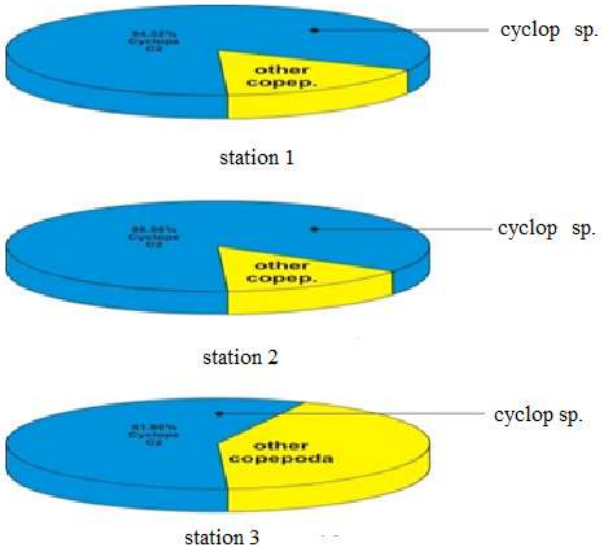


Fig.8: The percentages of copepoda in the three stations

In general, the relative density of the previous taxonomic units a few somewhat (40-10%), depending on the relative abundance index. The species that did not mention, it was rare (less than 10%) and the total stations appeared in this study was about 12 species, mostly classified as evidence of organic pollution (Ahmad *et al.*, 2011).

From the above, it illustrated the lack of taxonomic units with the increase in the relative density and this means the availability of limited types have an ability to living conditions in the river. The difference in cladocera density may be due to the increase associated with an increased appropriate food (Claps *et al.*, 2004), and that their numbers are affected by concentrations of salts and organic matter in the water, and the different larval stages of cladocera formed the highest percentage of the total density, and this is what consistent with (Al-Lami, 2001).

Species Richness Index (D)

This is an indicator expresses the fertile and rich area of study, and is described as the absolute number of taxonomic units in bio-aggregation, somewhere within the body of water,and the increase in the abundance of taxonomic units of index associatedwith the health and safety of the water ecosystem, and to measure the abundance of taxonomic units covers changes in the aquatic invertebrate community (Barbour *et al.*, 1999).

In this study rotifera group overcame 76% (out of 69 units taxonomic) for zooplankton and others, while copepoda recorded 6 taxed at a ratio of 21.6% and 1.8% for cladocera (containing 12 units taxonomic).

Station 1 recorded 2.77 for the species richness in July to 8.84 in October. At station 2 it ranged from 4.07 in May to 8.52 in September. While at the station 3 ranged from 2.53 in August to 8.17 in September (Fig. 9). It has been observed the lowest value was recorded between stations in the station 3 during August and the highest value recorded in the station1in October.

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

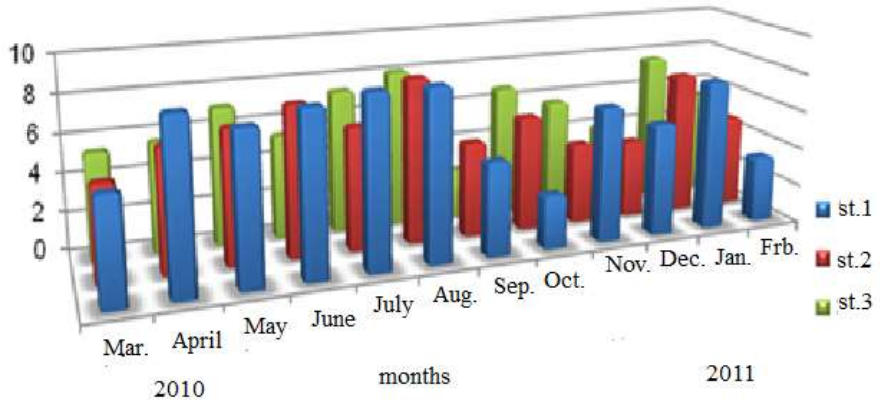


Fig.9: Species richness index for the three stations

The study stations show highlyin species richness, especially for rotifera as this group gives quantity and qualityrichness for each station, followed by copepoda, which contained abundant numerically exceeded their quantity, and less than that cladocera community, which contained few numerical and lack of quality.But in general, this indicator is based in hisaccounton the absolute number of taxonomic units, quantitative and qualitative, so it shows an envisions optimistic about the reality of the study stations in the Tigris River, which is commensurate with the availability of food productivity, as the associated change physical and chemical factors, and this means having positive relationships between the abundance of the species and the physical and chemical parameters (Al-Namrawi 2005,Nashaat 2010).

Shannon Weiner Diversity Index (H) and Species Uniformity Index(E)

The use of diversity index is important to know the developments in the eco-system changes, where the species begin to resettle themselves when appropriate environmental conditions, and decreases when the environmental condition begins changes leading to an imbalance in the stability of the whole society. Most of the contaminated water is a little diversity, so in order to assess and appropriately pollution, is favorable to have a long observation to calculate the diversity index (Goel, 2008).

Figure (10) shows the Shanon-Weiner diversity index values, where the station 1 recorded less versatile 1.90 bits/individual in July, while the highest value in November 2.86 bits/individual. Station 2 recorded the lowest versatile (1.66 bits/individual) in May, while September recorded the highest value of diversity (2.99 bits/individual). In station 3 the lowest value of diversity was 1.75 bits/individual in August and the highest in February 2.87 bits/individual.

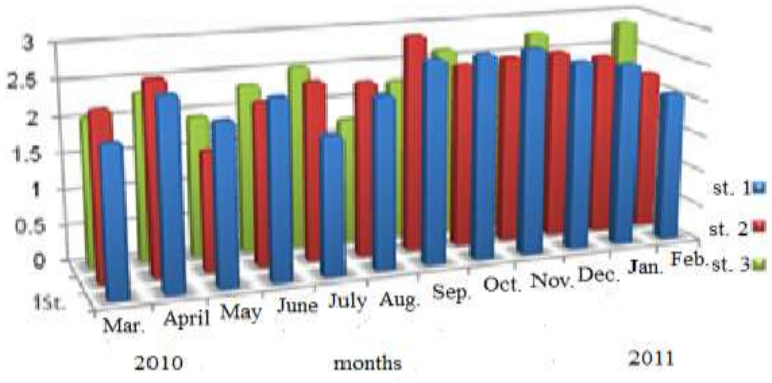


Fig.10: Shanon-Wiener diversity index values for the three stations

http://dx.doi.org/10.22161/ijeab/2.1.19 *ISSN: 2456-1878*

Generally,this indicator varied from 1.8 bits/individual and the highest value recorded was 2.99 bits/individual.Thus, according to (Goel, 2008) this indicator was depending on the number of species and the relative abundance in the body of water, which is a sign of the quality of water in the Tigris River, which can be considered as a moderate organic pollution in 2010.

The Species uniformity index (Figure 11) recorded values ranged from 0.72 to at the station 1 in February 2011 to 0z.91 in July 2010. Station 2 scored the lowest value 0.26 in May and the highest value of 0.89 in September. While the station 3 has the lowest value of 0.66 in September 2010 and the highest value of 1.01 in February 2011 and this value is the highest among all the three stations, while the minimum value of the similarity of the species between the study stations is 0.26 during May 2010 at station 2.

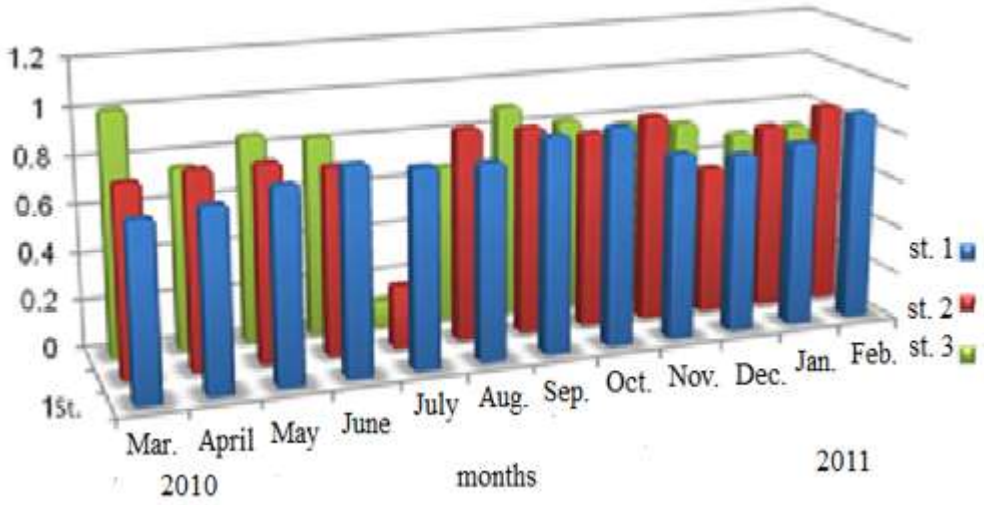


Fig.11: The species uniformity index in three stations

The highest recorded values for this indicator in these stations indicated that the environmental pressure on zooplankton species was very low, this is which referred byGreen (1993).

REFERENCES

[1] Abbas, A. K. and Al-Lami A. A. (2001).Qualitative and quantitative composition of cladocera in Tigris River, Iraq. Journal of the College of Education. 12 (4): 447-480.

[2] Ahmad, U., S. Parveen; A. A. Khan; H. A. Kabir; H. R. A. Mola and A. H. Ganai (2011). Zooplankton population in relation to physico-chemical factors of sewage fed pond to Aligarh (UP), India. Biology and Medicine. 3 (2): 333-341.

[3] Al-Aboody, Y. N. (1992). Hydro-chemical of River Tigris in Baghdad. Ms.C. Thesis, Faculty of Science, University of Baghdad. Pp. 103.

[4] Al-Lami, A. A. (2001). Zooplankton diversity in River Tigris, before and after Baghdad city. J. of Al-Fateh. 11:230-238.

[5] Al-Namrawi, A. M. (2005). Study the biodiversity of zooplankton and benthic invertebrates in the Tigris and Euphrates rivers in central Iraq. PhD thesis, Faculty of Science, University of Baghdad. P 161.

[6] Barbour, M. T., J. Gerritsen, B.D. Snyder and J.B. Stribling, (1999). Rapid Bioassessment protocols for use in stream and Wadeable rivers: periphyton, macroinvetebrates and fish, 2nd. Ed., EPA, U.S. Environmental Protection Agency, Office of water. Washington, DC.

[7] Claps M C, Gabellone N. A. and Benitez H. H. (2004) Zooplankton biomass in an eutrophic shallow lake (Buenos Aires, Argentiua): Spatio-temporal Variations. Ann. Limnol. Int. J. 4 (3): 201-210.

[8] Edmondson, W. T. (1959) Freshwater Biology 2nd Ed. John Willey & Sons, Inc., New York. 1284 pp .

[9] Elías- Gurtiérres, M.;E. Suárez- Moralesand S.S.S. Sarma (2001). Diversity neotropics: the case of Mexico. Verh. Internate. Verein. Limnol*:* 27, 2027-4031.

[10] Floder, S. and U. Sommer (1999). Diversity in plankton communities: An experimental test of the

intermediate disturbance hypothesis. Limnol. Oceanogr. 44(4): 1114-1119.

[11] Goel, P.K. (2008) Water Pollution. Causes, Effects and Control. 2nd Ed, reprint new age international (P) Limited, Publishers, New Delhi.

[12] Green, J (1993). Diversity and dominance in planktonic rotifers. Hydrobiol. 255: 345 – 352.

[13] Iraq Water Resources (2011).Discharges and the levels of the Tigris River in the city of Baghdad.The National Centre for Resource Management Department Control of Water

[14] Nashaat, M. R. (2010). Impact of Al-Durah Power plant effluents on physical, Chemical and invertebrates Biodiversity in Tigris River, Southern Baghdad, PH.D. Thesis, University of Baghdad, Iraq. 183 pp.

[15] Omori, M. and T. Ikeda (1984). Methods in marine zooplankton ecology. John Wiley and Sons, Inc. New York.

[16] Petersen F. D., Papa S. R. and Mamaril A. C. (2010) To the Philippine Freshwater Zooplankton. University of Santo Tomas, Manila, Philippines, 330 pp.

[17] Porto-Neto, V.F.(2003). Zooplankton as bioindicator of environmental quality in the Tamandane Reff System (Pernambnco- Brazil): Anthropogenic influences and interacts with mangroves. Ph. D. Thesis, Univ. Bremenm Brazil.

[18] Rajashekar, M , Vijaykumar K , Parveen Z (2009) Zooplankton diversity of three freshwater lakes in relation to trophic stats, Gulparga district, North- East Karnataka, South India. Inter. J. of systems Biology, 1(2): 32-37.

[19] Shekha, Y. A. (2008). The effect of Erbil city wastewater discharge on water quality of Greater Zab River, and the risks of irrigation. Ph.D. Thesis, College of Science- University of Baghdad. 139 pp.

[20] Sklar, F. H. (1985). Seasonality and community structure of the Back Swamp invertebrates in Alonisiana Tupelo Wetlands. Wetlands. J. 5: 69-86.

[21] Smith, P. J. (1999). Managing Biodiversity: Invertebrate by catch in sea mount Fisheries in the New Zealand exclusive economic Zone. National Institute of Water and Atmospheric Resaech. New Zealand.

[22] Smith, D. G. (2001) Pennak's Freshwater invertebrates of the united States. 4th. Ed., John Willey & Sons, Inc. New York. 538 pp.

[23] Solomon, S. G. Ataguba, G. A. & Baiyewunmi, A. S. (2009). Study of dry season Zooplankton of lower River Benue at Makurdi, Nigeria. J. of Animal & Plant Sciences, 1(3): 42-50.

http://dx.doi.org/10.22161/ijeab/2.1.20 *ISSN: 2456-1878*

Study of Al-Karamah and Sharq-Dijla drinking water purification and their byproduct effects on the Tigris River

Khalid A. Rasheed

Biotechnology Research Center, Al-Nahrain University

***Abstract*—** *Two studied stations were involved in this study included Sharq–Dijla and Al-Karamah water purification stations. Water samples collected from four sites with three replicates for each sample of each site of the river and the station: before, after, inside the stations and at the pipe. The study started in October 2012 to September 2013.*

Results showed that the minimum level of water temperature was 11ºC during (December-January) at Sharq –Dijla.While the maximum level was 30ºC during (August-September) at both AL-Karamah and Sharq –Dijla stations. pH results revealed that the highest level of pH was 8.63 during (October-November) at AL-Karamah station followed by the lowest level was 6.73 in(February-March) at Sharq –Dijla station. The highest level of EC was 1068 µS/cm during (April-May) at Sharq-Dijla station, while the lowest level was 693 µS/cm during (August- September) at the same station.

The results of DO showed that the highest level was 11.51 ppm during (December-January) at Sharq- Dijla station, while the lowest level was 4.25 ppm during (August-September) at AL- Karamah station.The BOD results recorded the highest level of BOD_5 was 4.49 ppm during (August- September), and the lowest level was 0.67 ppm during (December-January) both results at Sharq-Dijla station.

Total hardness showed that the highest level was about 404 ppm during (December-January) at AL-Karamah station, and the lowest level 162 ppm during (August-September) at the same station. Free chlorine measurements found with Iraqi limits and WHO for these two stations. The highest value of Iron concentration was 3.30ppm in (December-January) at Al-Karamah station, while the lowest value was1.63ppm in (August-September) at Sharq–Dijla station.

Keywords*— *Tigris River, Al-Karamah, Sharq-Dijla, drinking water, purification.

I. INTRODUCTION

Since the dawn of civilization several cities has been built on the banks of the Tigris River along the Baghdad city. To overcome the problem of increased population number, various hydraulic projects have been constructed along the Tigris river, according to reports of Iraqi water resources ministry, during the period of1989-1991, seven water purification units (Sharq- Dijla,Al-Karamah,Al-Kadsia ,Al-Doura, Al-Wehda, Al-Rasheed and Al-Wathba stations) were constructed on both AL-Karik and AL-Resafa with limited productionpower. All of those stations were producing potable water enough for four million people, though increasingin population in Baghdad was the most difficult problem, atthe present time population within Baghdad reached until 8-10 million people so the productivity of the potable water purification unit is not enough (Al-Ansari& Knutsson, 2011).

Enlargement of Baghdad boundaries within past thirty year, had made delivering potable drinking water to the far areas of Baghdad considered as a challenge, polluted drinking water with very low concentration of chlorine was delivered to the limited places of Baghdad , this problem resulted from deficiency in the number of water purification units ,bad situation of the water pipes with taking in consideration that 1000km of transporting pipes were changed within the period of 1984-1982(Hamza, 2007).

The aim of this project was to study the characterization of two potable drinking water in Baghdad City and their byproduct effects on the Tigris river.

II. MATERIALS AND METHODS

Study area:

Sharq -Dijla and AL-Karamah stations located in the north part of Baghdad city. The distance between these stations is about 7km (Figure 1). Three replicate samples were collected from the river representative the left side and the right side of the river and the third replicate from the middle part of the river.

http://dx.doi.org/10.22161/ijeab/2.1.20 *ISSN: 2456-1878*

Fig.1: Samplingstation (A) represented Sharq-Dijla station (S1a, S2a, S3a, S4a), (B) represented Al-Karamah station (S1b, S2b, S3b, S4b)

Sampling Procedure:

Sampling was collected bimonthly from October 2012 to September 2013, at the two sites as explained. The samples were taken about 2m from the shoreline at a depth of 45-50 cm.

Water samples for dissolved oxygen (DO) and biological oxygen demand (BOD_5) were collected in 250ml sterile dark Winkler bottles (washed and sterile by placing them in the oven for 4hr at 200^{o}C).

Parameter studies

Temperature:

The Temperature was determined by a mercury thermometer.

pH:

The pH was measured by portable pH meter type Hanna.

Electrical Conductivity (EC):

Measured by using a portable conductivity meter type Siemens. Results were recorded in µS/cm.

DO and BOD_5:

The Azide modification method described by APHA (1998) was used for measuring DO and BOD_5, BOD_5 bottles kept in the incubator at 20±1^{o}C for 5 days in the dark then measured as the following:

BOD_5= DO initial – DO after 5 days in incubator.

Total Hardness T.H.:

According to (Frohlich &Urish, 2002) the total hardness was calculated according to the following equation:

Total Hardness as $CaCO_3$ mg/l= $\frac{A\ x\ B}{ml\ of\ sample} x\ 1000$

A: ml of EDTA used in the titration

B: mg of $CaCO_3$ equivalent to 1ml of EDTA

Free Chlorine concentration:

Depending on the color of the concentration of Cl free chlorine concentration can be determined according to (Senior, 2009)

Fe concentration:

According to (Singer ,2006) using Phenanthroline method was used, the concentration of iron level was measured with mg/l according to the equation:

Fe conc. (mg/l) = $\frac{\text{conc.of standard solution}}{volume\ of\ sample}$x1000

III. RESULTS AND DISCUSSION

Temperature

Energy from the sun is the main factor influence air temperature, sunlight and air temperature both influence water temperature, as well as water flow, drought, climatic condition (Ndiongue*et al.*, 2005).

The minimum value of water temperature recorded was 11^{o}C during (December-January) at Sharq –Dijla (Figure 2). While, the maximum value of water temperature was 30^{o}C during (August-September) at both stations, (Figure 3).A significant difference between months at each station was detected, with no significant differences between the sampling location at the same station along the study period.

Temperature showed slight changes between the collection samples because they were at the same geographic location and those changes due to the time difference of collection because some samples were collected in the early morning and other collected on the moon when the sun is vertical to water surface this

phenomenon were noticed by (Ismail& Al-Saadi., 2000). The water temperature outside the normal range for a stream or river can cause harm to the aquatic organisms that live there. If the water temperature changes by even a few degrees, it could indicate a source of unnatural warming of the water or thermal pollution (John, 1989).

Inverse correlation recorded between water temperature and dissolved oxygen (r= -0.139). One important aspect of water temperature its effect on the solubility of gases, more gas can be dissolved in cold water than in warm water (Wolf.1998).

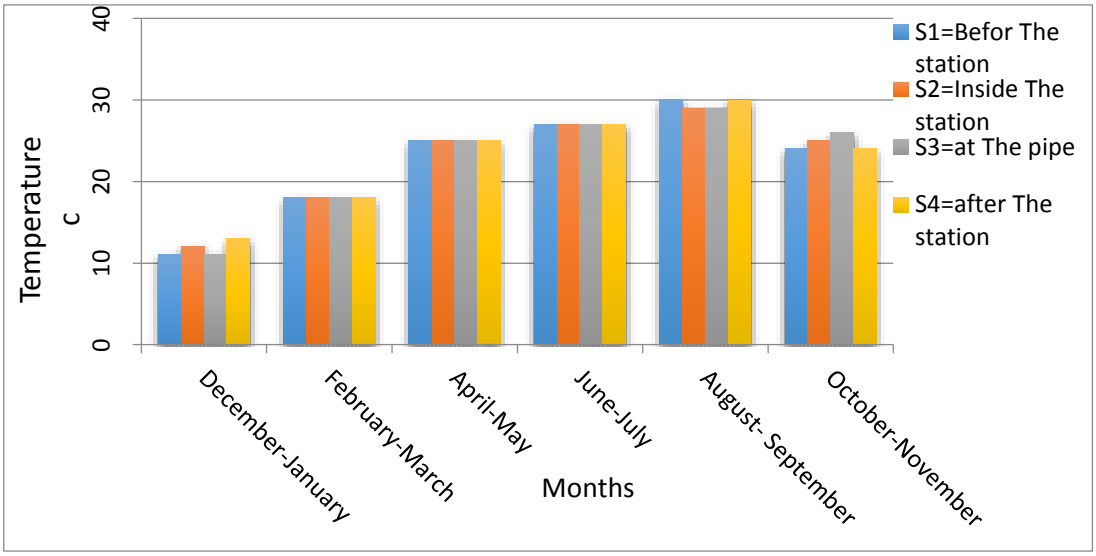


Fig.2: Monthly temperature distribution within locations in Sharq -Dijla station

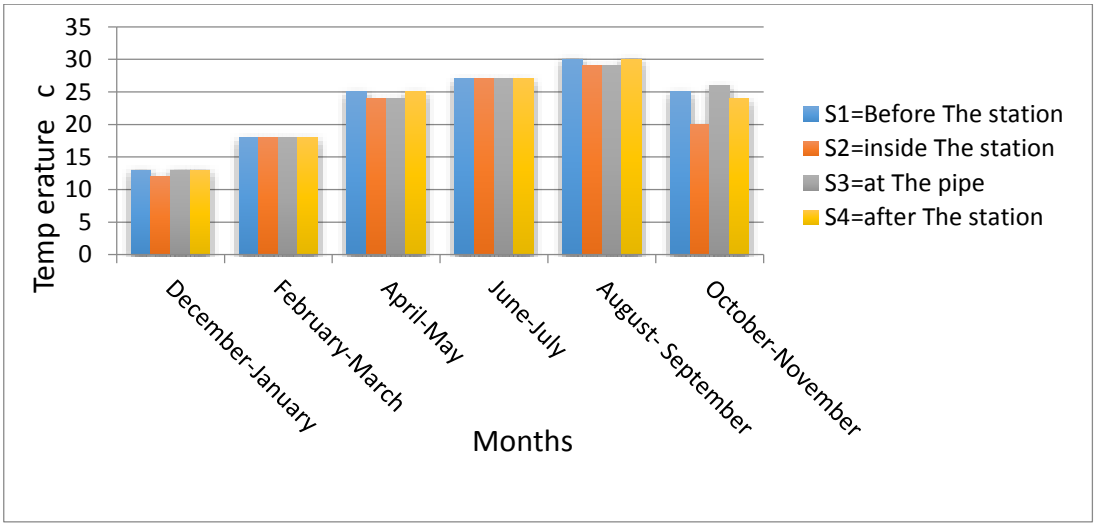


Fig.3: Monthly temperature distribution within locations in AL-Karamah station

pH values

The pH value of a water is a measurement of the activity of the hydrogen atom, because the hydrogen activity is a good representation of the acidity or alkalinity of the water (Millero, 2001).

Results showed that the highest value of pH was 8.63 during (October-November) at AL-Karamah station (Figure 4), while the lowest value was 6.73 during (February-March) at Sharq–Dijla station,(Figure5). The statistical analysis of the data revealed significant differences between months at each station, with no significant differences detected between the sampling location at the same station along the study period at ($P < 0.05$). The increase of pH values during (October-November) may be due to using additional doses of alum article to precipitate calcium carbonate ($CaCO_3$) to control pipes corrosion (Reezoqy , 2009), slight decreases of pH in (February-March) as a result of the dissolving of CO_2 in water by drop of temperature which led to form carbonic acid (HCO_3), which is weak and break down into hydrogen ion which act to when increase it concentration decrease the pH value (Chapman,1996).These results disagree with Wahab (2010) who stated that the highest value of pH was in summer. Surface water typically has a

http://dx.doi.org/10.22161/ijeab/2.1.20 *ISSN: 2456-1878*

pH value between 6.5 and 8.5. The pH of a water source can vary naturally (WHO, 1996).

While a direct correlation between pH and water temperature was found (r= 0.234), while direct correlation was noticed between pH and turbidity(r=0.92).

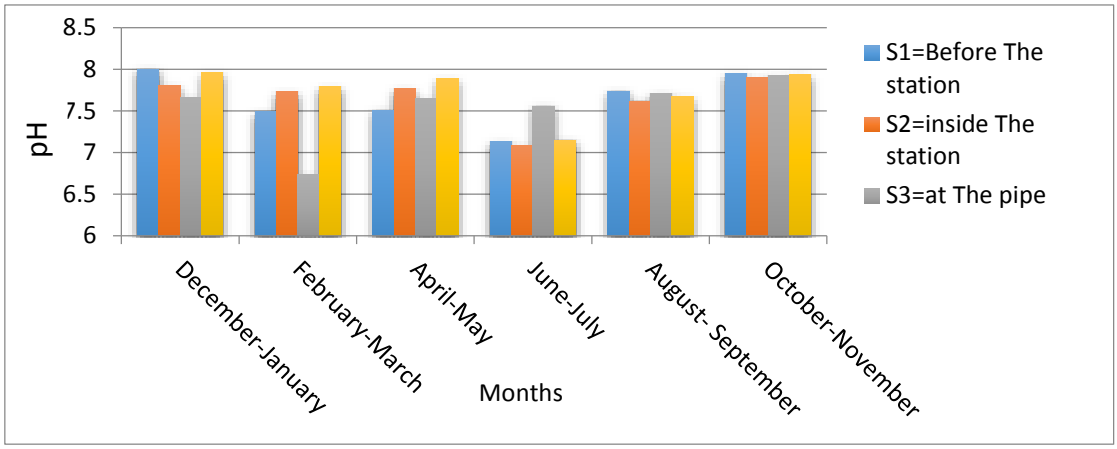


Fig.4: Monthly pH readings within locations in Sharq-Dijla station

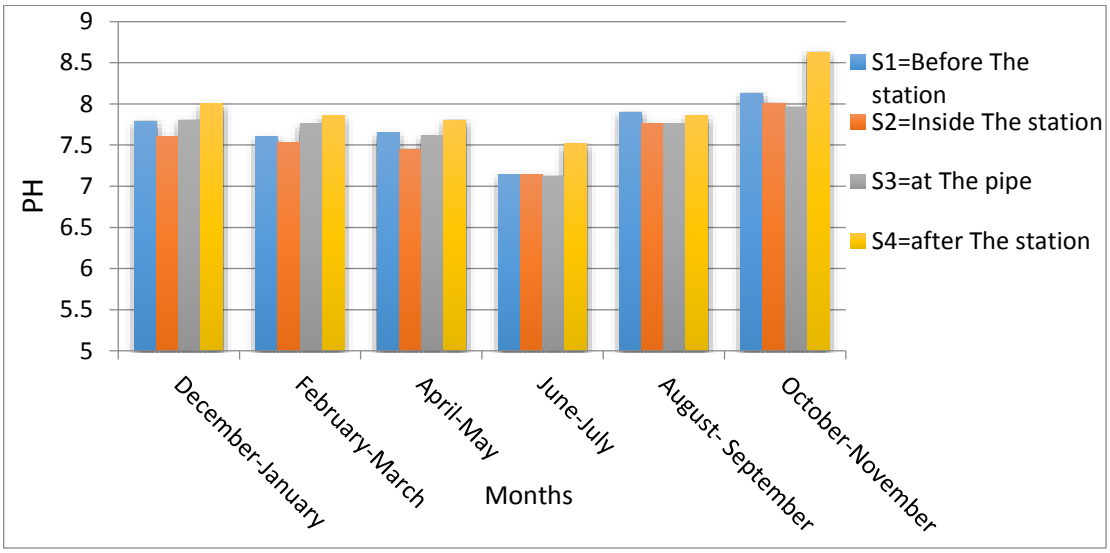


Fig.5: Monthly pH readings within locations in AL-Karamah station

Electrical conductivity:

Electrical conductivity is the ability of a substance to conduct electricity. The conductivity of water is a more-or-less linear function of the concentration of dissolved ions (Barnes, 2003).

Results showed that the highest value of EC was 1068 µS/cm during (April-May) at Sharq- Dijla station (Figure 6), while the lowest value was 693 µS/cm during (August-September) at Sharq- Dijla station (Figure 7). The statistical analysis of the data revealed significant differences between months and among sampling location of each station at the same months (P <0.05). Hashim (2010) showed the same result in his study on the Tigris river. The increase of EC during winter was due to the rains, soil wash and withdraw into the river which helped to increase the dissolved salts and decrease in summer (WHO, 1996). (Hamudat, 2009) stated that the values of EC increased during summer and decreased during winter.

The increase of EC differed with the environmental factor, including temperature and the presence of dissolved ions like Cl, K, Mn ,Na. , the increasing and the decrease in E.C. related to dissolve salts rates in water river because E.C. measurement related with total dissolved solids concentration (Rashid , 2001). An inverse correlation was found between E.C. values and water temperature (r=-0.145), and direct correlation was found between EC and TSS and TDS (r= 0.204).The maximum allowed limit of the EC under (Iraqi laws) is 2000µS/cm.

http://dx.doi.org/10.22161/ijeab/2.1.20 ISSN: 2456-1878

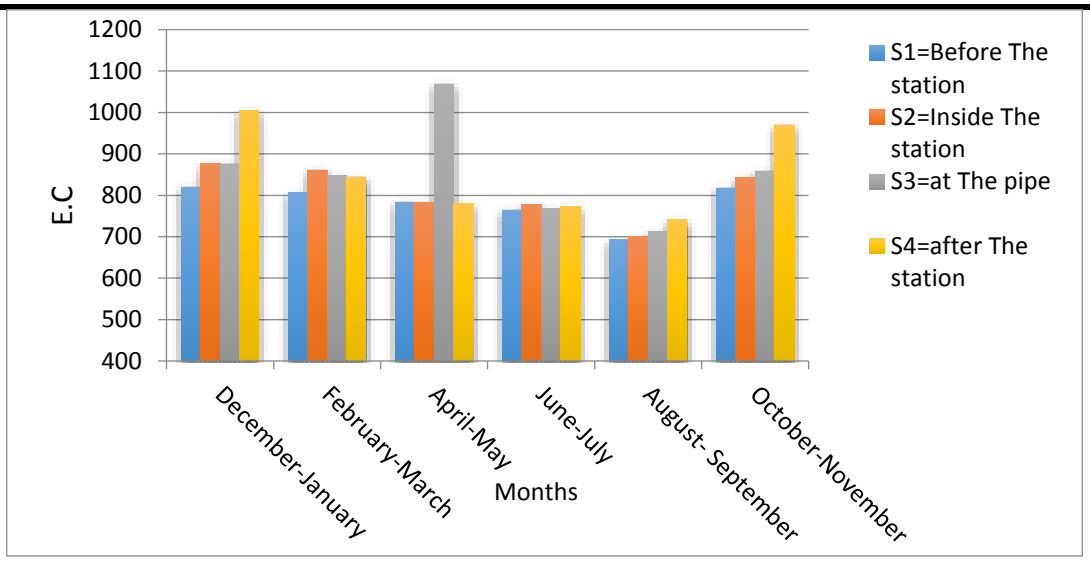


Fig.6: Monthly electrical conductivity variations(µS/cm)in Sharq-Dijla station.

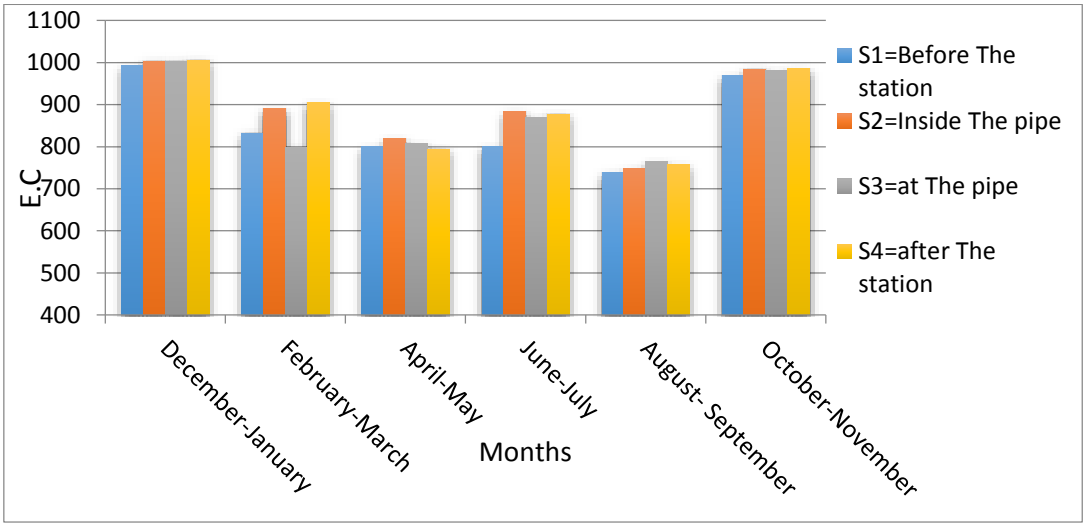


Fig.7: Monthly electrical conductivity variations (µS/cm) in Karamah station.

Dissolved Oxygen:

DO is an indicator of water quality, low levels can produce an aerobic condition leading to smelly water (Pitt, 2000). The measurement of DO can use to indicate the degree of pollution by organic matter, the destruction of organic substances and the level of self-purification of the water (Chapman, 1996).

The results of D.O showed that the highest value of DO was 11.51 ppm in (December-January) at Sharq-Dijla station, while the lowest value was 4.25 ppm in on seasonally or even over 24 hour periods in relation to temperature and biological activity such as photosynthesis and respiration. A significant difference between months and between sampling locations of each station were detected of each station at the same months along the study period. The maximum allowed limit of DO (under Iraqi) laws and WHO was>5. Increased temperature accelerates the degradation of organic matter in the overlying water and in bottom deposits which makes an increased demand on DO resources of a given system (Pitt, 2000).

http://dx.doi.org/10.22161/ijeab/2.1.20 ISSN: 2456-1878

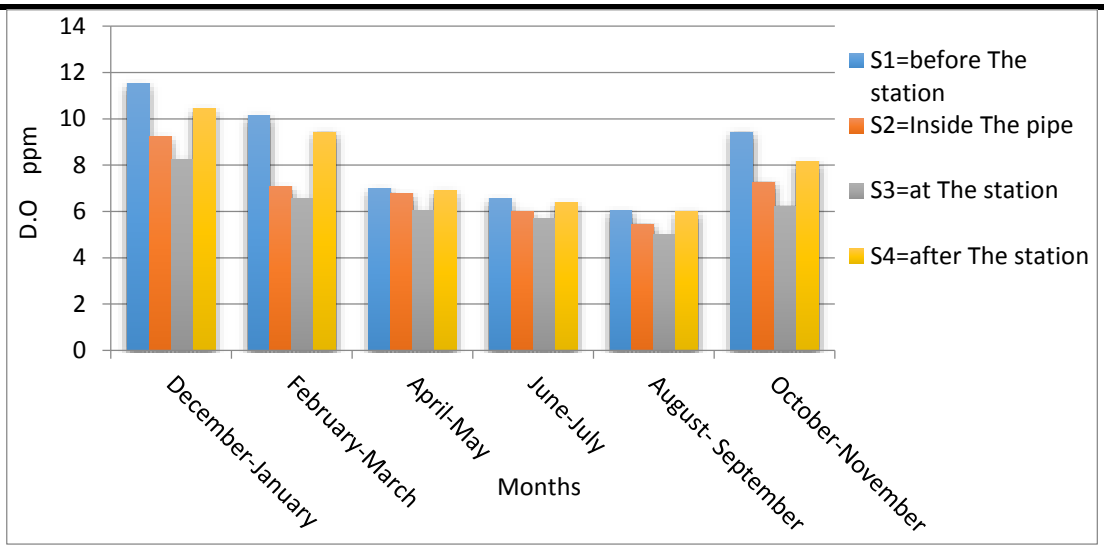


Fig.8: Monthly dissolved oxygen concentrations (ppm) in Sharq-Dijla station

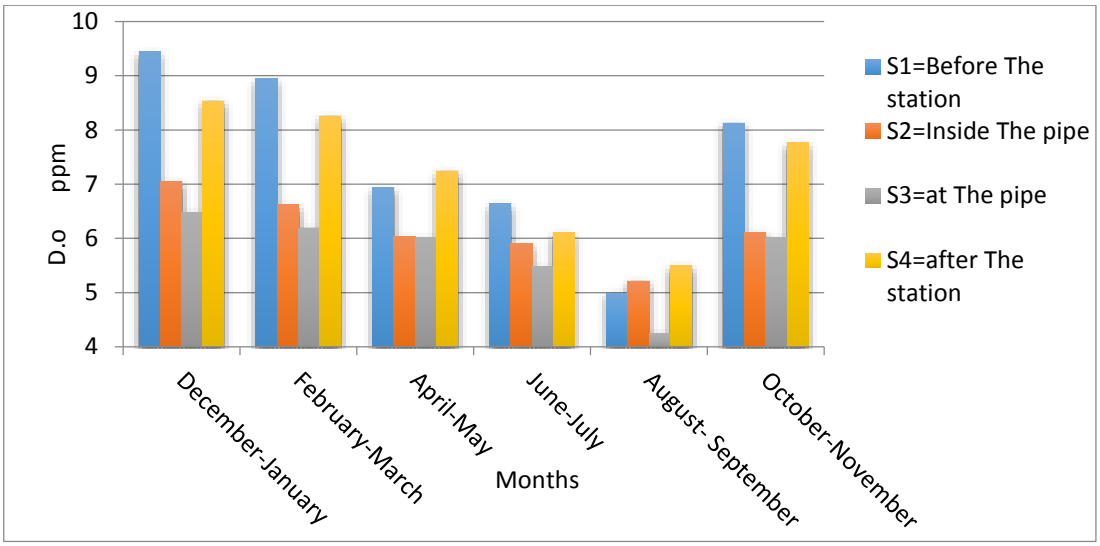


Fig.9: Monthly dissolved oxygen concentration(ppm)in AL-Karamah station

BOD:

Biological Oxygen Demand (BOD) refers to the amount of oxygen that would be consumed if all the organics in one liter of water were oxidized by bacteria and protozoa (Don, 2001).

Results showed that the highest value of the BOD was 4.49 ppm in(August- September)at Sharq-Dijla station, while the lowest value was 0.67 ppm in (December-January) at Sharq-Dijla station (Figures 10,11), the increase in BOD values due to the leaking of sewage from broken pipes or sewage treatment stations into the river or may be due to soil wash with heavy rains and domestic discharges (Sabri*et al.*, 2000). If there is a large quantity of organic waste in the water supply, there will also be a lot of bacteria present working to decompose this waste. In this case, the demand for oxygen will be high (due to all the bacteria) so the BOD level will be high. As the waste is consumed or dispersed through the water, BOD levels will begin to decline. Nitrates and phosphates in a body of water can contribute to high BOD levels (Titze& Walter, 2008).

A significant difference between months and between sampling locations of each station at the same were detected. A direct correlation between BOD and water temperature was found (r=0.913) and reverse correlation between BOD and DO (r= -0.243).The maximum allowed limits of BOD under (Iraqi laws) and WHO is < 5.

http://dx.doi.org/10.22161/ijeab/2.1.20 ISSN: 2456-1878

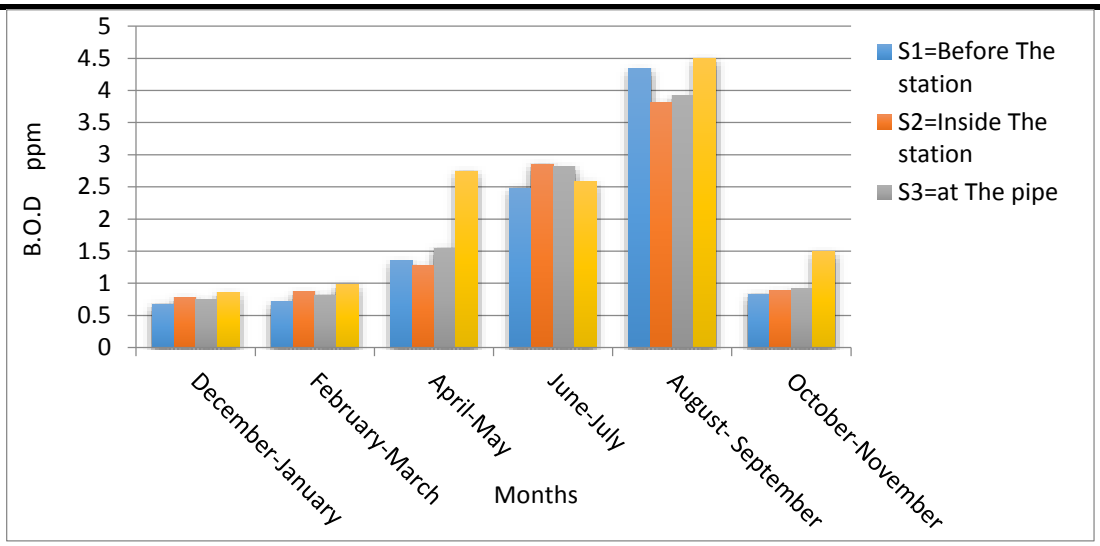


Fig.10: Monthly BOD_5 (ppm) recording within locations in Sharq-Dijla station

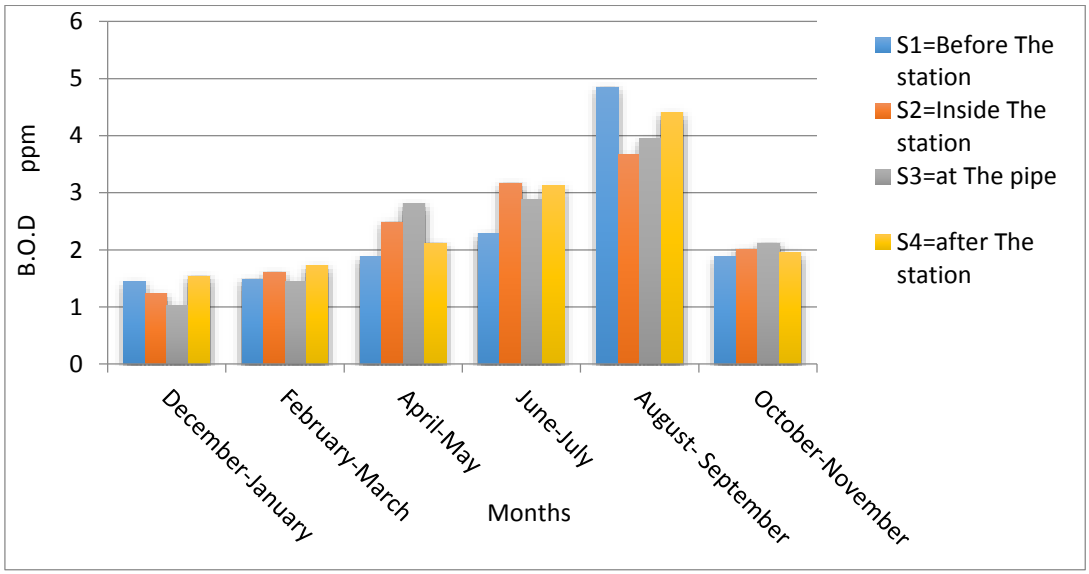


Fig.11: Monthly BOD_5 (ppm) within location in AL-Karamah station

Total hardness:

The natural sources of hardness in water are dissolved polyvalent metallic ions from sedimentary rocks, sewage and runoff from soils. A minor contribution to the total hardness of water is also made by other polyvalent ions, such as aluminum, barium, iron, manganese (Ong *et al.*,2009).

The highest value of T.H was 404 ppm during (December-January) at AL- Karamah station (Figure 12) and the lowest level 162 ppm during (August- September) at AL- Karamah station (Figure 13). The differences recorded among seasons of total hardness caused by the soil wash out, agricultural flow, or from industrial pollutants. The results showed an increase during the autumn months due to the increase in salt concentrations, especially calcium salt due to the preservation campaign mentioned earlier before to reach a high value in winter because the rainfall. Then increase of material in the water in the lands next to the water sources (Skipton *et al.*, 2004).A significant difference between months at each station and among sampling location of each station at the same months were detected.

Dissolved solids can produce hard water, which leaves deposits and films and on the insides of hot water pipes and boilers. Soaps and detergents do not produce as much lather with hard water as with soft water. As well, high amounts of dissolved solids can corrode pipes, and have a metallic taste. The same minerals that are deposited on these rocks can cause problems when they build up in pipes and(Thomas & Sach,2000). Inverse correlation between T.H and temperature (r= -0.558) were detected, and direct correlation with turbidity (r= 0.695).

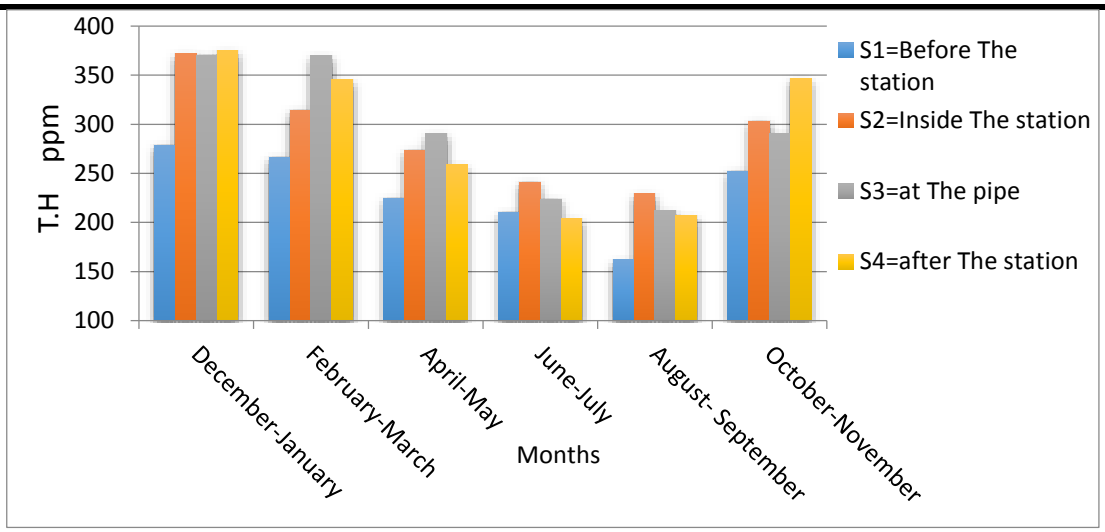


Fig.12: Monthly total hardness concentration (ppm) in Sharq-Dijla station.

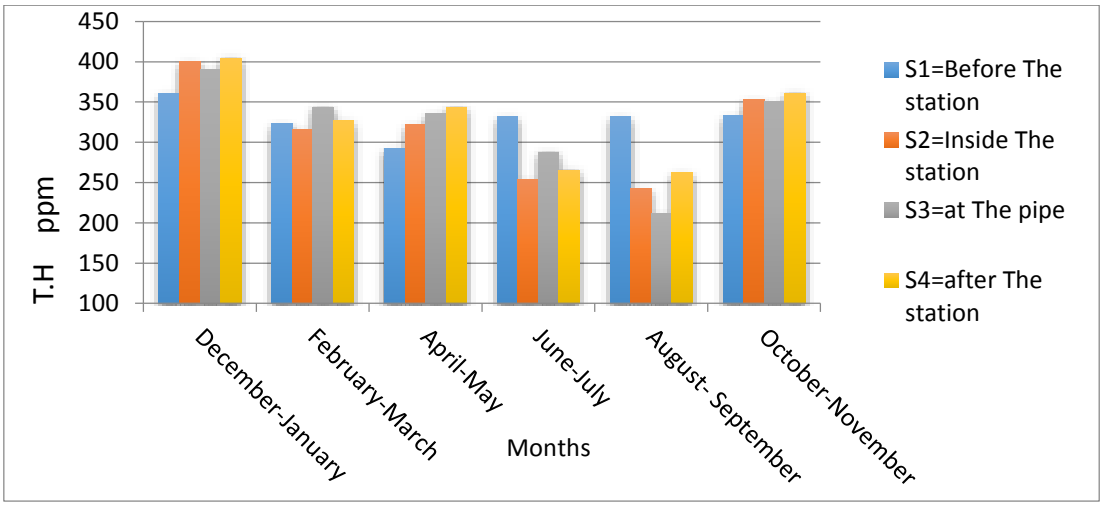


Fig.13: Monthly total Hardness (ppm) within locations in AL-Karamah station

Free chlorine:

If enough chlorine is added, some will remain in the water after all possible organisms have been destroyed. Free chlorine willremain in the water until it is used to destroy new contamination (Davis & Lambert, 2002)

The results showed that free chlorine was in the acceptable limits at the two stations and the four sampling locations of each station. The statistical analysis showed no significant difference between months of each station along the study period ($p>0.05$). These results agreed with the results of (Al-Qaisi, 2005).

The fall down in free chlorine concentration into normal values due to the decomposition of chlorine when it reacts with water into (HOCL)Hypo chloric acid, this acid decompose rapidly into Hypochlorite ion (WHO,2004). Free chlorine can associate with organic compound within water forming organic halogens this association made free chlorine concentration in water decrease (Volk *et al.*, 2002; Ndiongue *et al.*, 2005).No correlation was detected between chlorine and temperature.

The maximum allowed limit of chlorine under (Iraqi laws) and WHO is< 0.3 ppm.

Iron test:

Consequence of the growth of heavy industry has been the addition of high concentrations of heavy metals originating from anthropogenic inputs including industrial wastewater discharges, sewage wastewater, fossil fuel combustion and atmospheric deposition (Mohiuddin *et al.*, 2010).

Results showed that the highest value of Iron concentration was 3.30ppm in (December-January) at Al-Karamah station, while the lowest value was1.63ppm in (August- September) at Sharq –Dijla station (Figures 14,15). Al-Fatlawey(2007) stated that Iron concentration within river water increased in winter and decrease in summer this increase due to the industrial discharges that contain iron, rains and domestic discharges were considered as one of the main causes of the increase, but this study stated that iron concentration after the station

was approximately equal to its concentrations before the station.

A significant difference between months and between sampling location of each station at the same months.An inverse correlation was detected between Iron concentration and temperature(r=- 0.631).The maximum allowed limit of iron under (Iraqi laws) and WHO is 0.3 ppm.

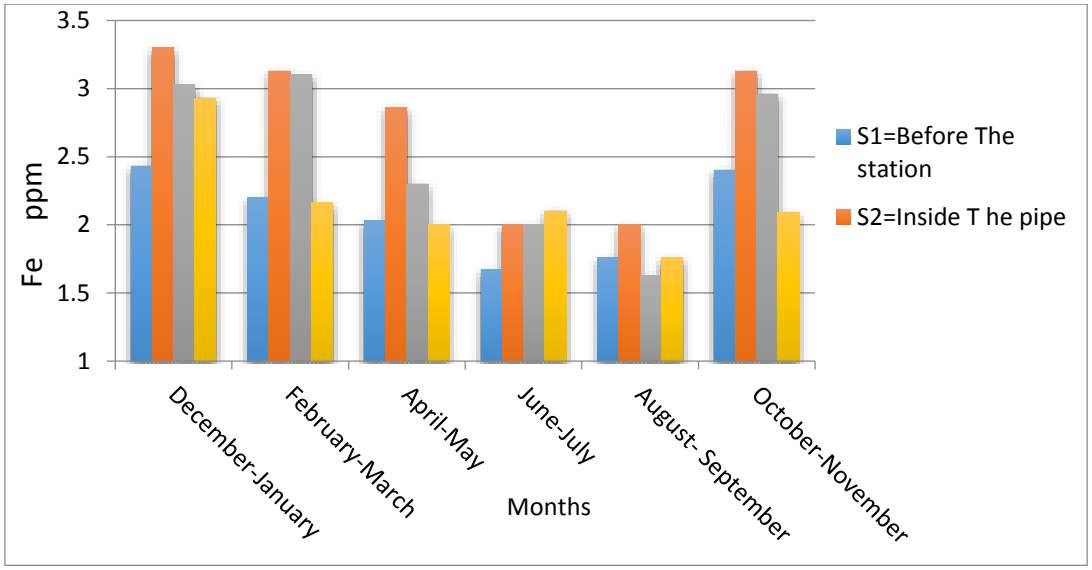


Fig.14: Monthly iron concentration (ppm)within location inSharq-Dijla station

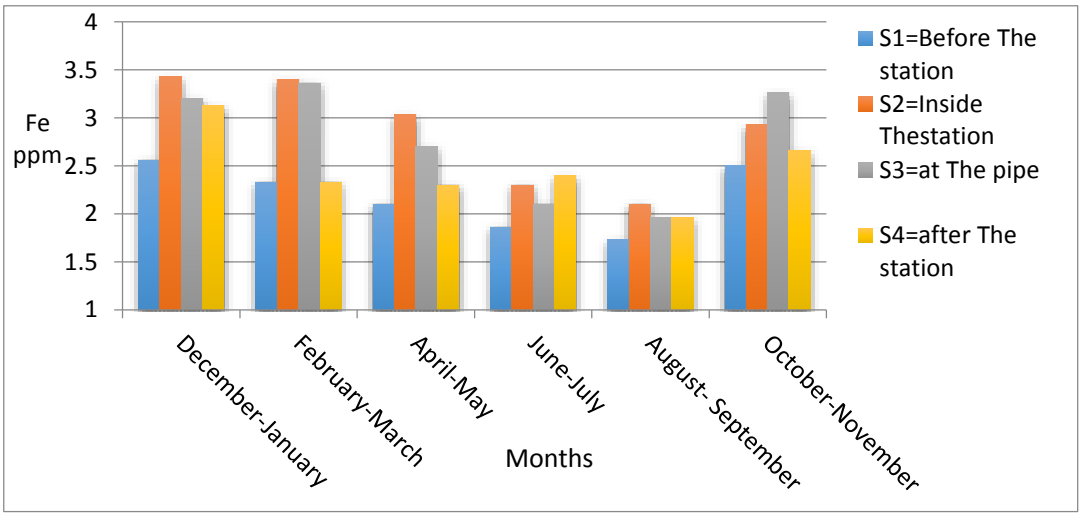


Fig.15: Monthly iron concentration (ppm) within location in AL-Karamah station

REFERENCES

[1] Al-Ansari, N. A. and Knutsson,S.2011.Toward Prudent management ofwater resources in Iraq, J. Advanced Science and EngineeringResearch, 1, 53–67.

[2] Al-Fatlawey, Y.F.K. 2007.Study the drinking water quality of some Baghdad drinking water treatment stations. Ph.D. Thesis. University of Baghdad (In Arabic).

[3] Al-Qaisi, R. K. J. 2005. Residual chlorine concentrations in Baghdad water supplies, M.Sc., Thesis Building and Constriction, Univ. of Technology

[4] APHA (American Public Health Association). 1998. Standard methods for the examination of water and waste water.20th Ed. American Public Health Association, Washington, Dc.

[5] Barnes, V. 2003. Identification of water pollutant in water and air Virginia. Virginia Cooperative Extension Fact Sheet number 427-101. Virginia State University.

[6] Chapman, D. 1996. Ed. Water Quality Assessment- A Guide to use of Biota, Sediment and Water in Environmental Monitoring. 2nded. Chapman &Hall, London.

[7] Davis J, and Lambert R. 2002. Engineering in Emergencies 2nd edition, chapter 13. (Undated) Chlorine residual testing.
[8] Don C. 2001.Drinking water treatment–understanding the processesand meeting the challenges. Water Science and Technology: Water Supply 1(1):1–7.
[9] Frohlich, R. K. and Urish, D. W.: 2002.The use of geoelectrics and test wells for the assessment ofgroundwater quality of a coastal industrial site', J. Appl. Geophys. 50, 261−278.
[10] HashimN.N.2010.Investigation of Cadmium and Mercury in water, sediments and some benthic invertebrates at section of Tigris river in Baghdad City. M.Sc. thesis, collage of science, University of Baghdad.
[11] HamudatY.R.A.2009.Study the chemical and physical characters of potable water. Msc thesis. College of Science. Baghdad University(in Arabic).
[12] Hamza, J.N. 2007.Impact of Tigris river pollution on the performance of water treatment plants efficiencies in Baghdad city," Iraqi Journal of Chemical and Petroleum Engineering, 9(3):17–24.
[13] Ismail, A. M. and Al-Saadi, H.A. 2000. Comparative limnological study of artificial lake and Tigris River, middle of Iraq. J. Coll. Educ. Women Univ.
[14] John, McPhee,1989.Atchafalaya," in The Control of Nature (New York: Farrar Strauss Girou.2nd .88-99.
[15] Millero, F. J.: 2001. The Physical Chemistry of Natural Waters, Wiley-Interscience, New York.
[16] Mohiuddin, K.M., Zakir, H.M., Otomo, K., Sharmin, S., Shikazono, N., 2010.Geochemical distribution of trace metal pollutants in water and sediments ofdownstream of anurban river. International Journal of Environmental Science and Technology 7 (1), 17-28
[17] Ndiongue, S., Huck P.M., and R.M.Slawson. 2005. Effects of temperature and biodegradable organic matter on control of biofilms by free chlorine in a model drinking water distribution system. Water Res. 39: 953 – 964.
[18] Ong CN, Grandjean AC, Heaney RP .2009. The mineral composition of water and its contribution to calcium and magnesium intake. In: Calcium and magnesium in drinking-water: Public Health Significance. Geneva, World Health Organization: 36–58.
[19] Pitt, R.E. 2000. Water quality conditions in the Cahaba River and likely pollution sources. Department of Civil and Environmental Engineering. University of Alabama.
[20] Rashid, M. J. 2001. Morphology of the sediments in Tigris River curve in Al-Jadriah area. Ms. C. Thesis. Baghdad Univ.
[21] Reezoqy.M.M.S.2009.Study the safety of potable water of a Baghdad.MSc.thesis. College of Science.Baghdad University.
[22] Sabri. A.W.; Ali.Z., Rasheed. K. A.2000. The effect of gulf war on water environment. First scientific convenience of Qatar. Baghdad. 663-672. (in Arabic)
[23] Senior, L.A.2009.Groundwater-quality Assessment.Geological Survey Scientific Investigations Report . vol(12).pp(17-28)
[24] Singer, P. C. 2006. Disinfection byproducts in drinking water:Additional Science and Policy Considerations in the Pursuit ofPublic Health Protection, National Water Research Institute, TheClarke Lecture.
[25] Skipton, S.; D. Varner; P. Jasa; B. Dvorak, and J. Kocher. 2004. Drinking water: hard water. Neb Guide, University of Nebraska Lincoln Extension,Institute of Agriculture and Natural Resources: 4 pp.
[26] Thomas KS, Sach T.H. 2000. Multicentre randomized controlled trial of ion-exchange water softeners for the treatment of eczema in children: protocol for the Softened Water Eczema Trial. British Journal of Dermatology, 159(3):561–566
[27] Titze, J., and Walter. H.2008. "Evaluation of a new optical sensor for measuring dissolved oxygen by comparison with standard analytical methods." Monatsschr. Brauwiss.(Mar./Apr.): 66-80.
[28] Volk, C.J., R.Hofmann, G.A.Chauret, G. Rangers, and R.C.Andrews. 2002. Implementation of chlorine dioxide disinfection: effects of the treatment chnge on drinking water quality in a full-scale distribution system. J. Environ. Eng. Sci. 1: 323 – 330
[29] Wahab. H.R. 2010. Investigation of lead and chromium in phytoplankton and zooplankton in section of Tigris River at Baghdad City. MS C. thesis, collage of Science, University of Baghdad.
[30] WHO .1996. Guidelines for drinking-water quality 2nd ed. Vol. 2. Health criteria and other supporting information. Geneva, World Health Organization.
[31] WHO .2004. Guidelines for drinking water quality, 3rd ed.Geneva. Switzerland.
[32] Wolf, R.E.1998. Conflict and Cooperation along International Waterways," Water Policy, Vol. 1, No. 2pp. 251-265

http://dx.doi.org/10.22161/ijeab/2.1.21 *ISSN: 2456-1878*

Analysis of the Environmental Factors Affecting the Growth Traits of Iran-Black Sheep

Reza Tohidi[1], Yunes Ismailjami[2], Arash Javanmard[3]

[1,2]Department of Animal Science, University of Torbat-e-Jam, Iran
[3]Department of Animal Science, University of Tabriz, Tabriz, Iran

Abstract*—A study was conducted to evaluate the effects of non-genetic factors on the growth behavior of Iran-Black sheep. The data of growth performances, birth weight (BW), weaning weight (W3), weight at 6, 9and 12 months of age (W6, W9 and W12, respectively), were taken from 1522 lambs belonging to data bank from Abbas Abad Sheep Breeding Station located at the North-east of Iran during a period of five years. Statistical analyses were performed using a general linear model including non-genetic factors: lamb sex, birth year and litter size as main effects, the lamb's age when weighed as covariate, and the interactions between these factors. Results showed that all traits were significantly ($P<0.001$) affected by all factors. However, no interaction between the factors was found for all traits. Environmental factors have very important roles in the development and growth of Iran-Black sheep at different ages. Therefore, a correction is necessary to increase the accuracy of direct selection on lamb weight at different growth stages.*

Keywords*—*Iran-Black sheep, Growth traits, Environmental factors.

I. INTRODUCTION

Sheep breeding is important of livestock production in Iran as there are about 50 million heads of sheep in this country (FAOSTAT, 2016). The Iran-Black is a new composite sheep that has been developed by cross breeding of Chios and Balouchi breeds in Abbas Abad sheep breeding station in Iran. This breed is more resistant to diseases and arid condition with more meat tendency and reproducibility. There are various production traits of this breed which suggest that there is a potential for improvement of economic traits. However, growth performances are preferred traits to improve due to low economic value of wool compared to meat production. In this situation, more emphasis should be placed on growth traits and carcass quality as well as reproductive traits (Snyman*et al*., 1995).Estimation of heritability indicates the potential of genetic improvement. The amount of heritability depends on both genetic and environmental variation in growth performance. Any selection program to improve growth traits should be designed based on the genetic and environmental effects on the objective traits (Yazdi*et al*., 1999). Non-genetic factors must be corrected before starting genetic analysis. Some environmental factors can be adjusted before any statistical analysis, however, there are still unknown environmental differences between animals, known as residual error. An adjustment should be made for environmental and physiological sources of variation such as age, sex, birth type or litter size, years, seasons and such other environmental variables that can be evaluated (Babar *et al*., 2004).The effect of non-genetic factors on growth performance in sheep has been investigated in several studies. These factors in different areas have their own specific effects regarding the environmental characteristics of corresponded areas (Gbangboche*et al*., 2006; Momoh*et al*., 2013). Therefore, the present study was carried out to investigate the effect of sex of lamb, year of birth and litter size on body weight of Iran-Black lambs at different ages.

II. MATERIALS AND METHODS

2.1 Animals and location of study area

The data on 1522 lambs born from 547 Iran-Black ewes sired by 60 rams kept at the Abbas Abad sheep breeding station located at a semi-arid area in the North-east of Iran during 2005-2009 were utilized to estimate the effect of environmental factors affecting BW, W3, W6, W9, and W12.The animals were raised in a closed system and fed with alfalfa, barley and straw. Sheep were supplemented in the last month of gestation and during lactation (usually barley), and births occurred mainly in April and May. Lambs were left with dams until age90 days, from this age they were kept to fatten until reaching slaughter age.

2.2 Data and analyses

The data file contained information on individuals, sire and dam identification code, sex, litter size, birth date, date of weighing and measure of body weight. The data were analyzed to estimate the effect of year of birth, litter size and sex of lamb born on the lamb growth. The mathematical model assumed for the Least-Squares Analysis was:

http://dx.doi.org/10.22161/ijeab/2.1.21 *ISSN: 2456-1878*

$Y_{ijklm} = \mu + S_i + A_j + L_k + (SA)_{ij} + (SL)_{ik} + b(Age - \overline{Age}) + \varepsilon_{ijklm}$ (1)

where Y_{ijklm} is the weight of a lamb; μ is the overall mean; S_i is the sex of lamb; A_j is the year of birth of a lamb; L_k is litter size; $(SA)_{ij}$ is the interaction between sex and year of birth; $(SL)_{ik}$is the interaction between sex and litter size; b is regression coefficient, Age is age of lamb at weighing time, ε_{ijklm} is residual error.A statistical analysisusing the univariate general linear model from the statistical package Minitab v.16 was used to analyze the effect of the fixed factors and interaction between them on the total variance of the records.

Thelamb's age at weighing time was used as covariate to correct the record of W3, W6, W9 and W12. Comparison of means was performed by Tukeytest, setting $P<0.05$ to identify significant differences between treatments.

III. RESULTS AND DISCUSSION

The data were used in the present study belonging to Abbas Abad sheep breeding station that Iran-Black breed has been created over there. As shown in Fig. 1, there was not such a big variation for all traits among different years, however, it was significant. Two reasons are supposed for this result, first, a scientific selection program has not been applied and second, environmental factors significantly influence the traits.

The effects of sex, birth year and litter size are shown in the Tables one to three, respectively. All non-genetic factors that have been investigated in this study significantly influenced on lamb weights in all ages ($P<$ 0.001). However, the interaction between these factors had non-significant effect on growth performances. Male animals were heavier than females as shown in Table 1. This fact has been reported in the other studies (McManus *et al*., 2003; Babar *et al*., 2004; Macedo and Arredondo, 2008;Baneh and Hafezian, 2009; Ulutas*et al*., 2010;Gbangboche*et al*., 2011; Momoh*et al*., 2013; Lupi*et al*., 2015). Differences in physiological functions in both sexes cause such a tendency in body weight. The nature of testosterone, a steroid hormone whose anabolic effects act as growth promoter, attributes in postnatal growth in males (Lupi*et al*., 2015).

The variation in lamb weights at different ages observed in different years (Table 2) may be due to variation in the environment, resulting primarily from differences in the amount of rainfall and the quantity and quality of herbage available. The management includes farmer manager, his ability to supervise the staff, availability of financial resources and selection strategies. Climate and environmental changes affect the quality and quantity of pasture forages, which affect the provision of food(Assan and Makuza, 2005; Momoh*et al*., 2013).Adequately fed ewes are expected to produce heavy lambs.

Litter size (single or multiple) had significant effects on living weight at different ages of lambs,single born lambs were heavier than multiple born lambs (Table 3). This result is according to the earlier studies (Dimsoski*et al*., 1999; Assan and Makuza, 2005; Hinojosa-Cuéllar*et al*., 2012; Gavojdian*et al*., 2013). The low birth weight and subsequent growth rate of twin born lambs can be attributed to competition for nutrients in utero. This could be due to uterine space and thelimited capacity of ewes to provide more nourishmentfor the development of multiples fetuses and more milkfor lambs (Gbangboche*et al*., 2006; Momoh*et al*., 2013). However, the multiple born lambs may demonstrate compensatory growth after weaning. Low birth weight was found to be leading cause of reduced lamb viability (Wilson, 1986). Therefore particular nutritional attention should be given to ewes lambing twins. Nutritional stress limits the lambs from expressing their full genetic potential (Chang and Rae, 1972) for birth weight and weaning weight.

Table 4 presents the coefficients of phenotypic correlation between body weights and corresponded Pearson correlation *P*-value. Although, all correlation coefficients are significant, the phenotypic correlations of birth weight with the body weights at subsequent ages ranged from low to intermediate and were positive. Similar results were observed in previous studies for the Tellicherry goats, Iran-Black and Lori-Bakhtiari sheep (Thiruvenkadan*et al*., 2009; Rashidi, 2013; Vatankhah, 2013, respectively). The W3 body weight had a significant, positive and moderate to high genetic correlation with the subsequent body weights (0.356 – 0.732). This indicated that selection for increased bodyweight at this age would result in genetic improvement in the subsequent ages.Phenotypic correlation between two traits includes both the genetic and environmental correlations. With appropriate design, the genetic correlation can be separated from the environmental correlation (Momoh, 2013). Therefore, in this study the environmental correlation between WW and post-weaning weights may be higher than pre-weaning weights.

IV. CONCLUSION

The results obtained in the present study revealed that environmental factors cause differences in live weight of Balouchi sheep from birth to 12 months of age.A breeding program needs to adjust records according to non-genetic effects to estimate breeding values of animals accurately. Sex of lamb, year of birth and litter size influenced body weight of Balouchi lambs. Hence, the

http://dx.doi.org/10.22161/ijeab/2.1.21 *ISSN: 2456-1878*

effect of these factors should be considered in mixed model approaches to find pure genetic values of animals.

V. ACKNOWLEDGEMENT

I would like to express my thanks to my colleagues in the Sheep Breeding Station of Abbasabad, especially Mr. Majid Jafari, for permission to use the data for this study.

REFERENCES

[1] Assan N, Makuza SM.2005. The effect of non-genetic factors on birth weight and weaning weight. Asian Australian Journal of Animal. 18, 151-157.

[2] Babar ME, Ahmad Z, Nadeem A andYaqoob M. 2004. Environmental factors affecting birth weight in Lohi sheep. Pakistan Veterinary Journal.24, 5-8.

[3] Baneh Hand Hafezian SH. 2009. Effects ofenvironmental factors on growth traits inGhezelsheep. African Journal ofBiotechnology.**8**,2903-2907.

[4] Chang TS and Rae AL. 1972. The genetic basis of growth, reproduction and maternal environment in Romney ewes. II Genetic covariation between hogget characters, fertility and environment of the ewe. Australian Journal of Agricultural Research. 23, 149-165.

[5] Dimsoski P, Tosh JJ, Clay JC and Irvin KM. 1999. Influence of management system onlitter size, lamb growth, and carcass characteristics in sheep. Journal of Animal Science.77, 1037-1043.

[6] FAOSTAT.2016. faostat3.fao.org/browse/Q/QA/E.

[7] Gavojdian D., Sauer M., Padeanu I., Pacala N., Voia S.O. 2013. Influence of production system, sex and litter size on growth rates in Turcana lambs. Animal Science and Biotechnologies. 46, 357-360.

[8] Gbangboche AB, Youssao AKI, Senou M,Adamou-Ndiaye M, Ahissou A, FarnirF,Michaux C, Abiola FA and Leroy PL. 2006. Examination of non-genetic factors affecting the growth performance of Dejallonke sheep in Soudanian zone at theOkpara breeding farm of Benin. Tropical Animal Health and Production.38,55-64.

[9] Gbangboche AB,Alkoiret TI, Salifou S, Farnir F, Leroy PL, Abiola FA. 2011. Growth pattern of purebred West African Dwarf sheep and its crosses with the West African Long Legged. Research Journal of Animal Science. 5, 6-13.

[10] Hinojosa-Cuéllar JA, Oliva-HernándezJ,Torres-Hernández G, Segura-Correa JC, Aranda-Ibáñez EM and González-Camacho JM. 2012. Factores que afectan el crecimien to predestete de corderosPelibueyen el tropic húmedo de México. Univ. Cienc. 28, 163-171.

[11] Lupi TM, Nogales S, Leon JM, Cecilio B and Delgado JV. 2015. Analysis of the non-genetic factors affecting the growth of Segureno sheep. Italian Journal of animal Science. 14, 3683-3691.

[12] Macedo R and Arredondo V. 2008. Efecto del sexo, tipo de nacimiento y lactanciasobre elcrecimiento de ovinospelibueyenmanejointensivo. Archivos deZootecnia. 57, 219-228.

[13] McManus C, Evangelista C, FernandesLAC, Miranda RM, Moreno-Bernal FE and Santos NR. 2003.Curvas de crescimento de ovinosbergamáciacriados nodistrito federal. RevistaBrasileiraZootecnia. 32, 1207-1212.

[14] Momoh OM,Rotimi EA and Dim NI. 2013. Breed effect and non-genetic factors affecting growth performance of sheep in a semi-arid region of Nigeria. Journal of Applied Biosciences. 67, 5302-5307.

[15] Rashidi A. 2013. Genetic parameters estimates of body weight traits in Iran-Black sheep. Journal of Livestock Science and Technologies. 1, 50-56.

[16] Snyman M.A., Erasmus G.J. and van Wyk J.B. 1995. Non-genetic factors influencing growth and fleece traits in Afrino sheep. South African Journal of Animal Science. 25: 70-74.

[17] Thiruvenkadan AK, Karunanithi K, Muralidharan J and NarendraBabu R. 2009. A comparative study on growth performance of crossbred and purebred Mecheri sheep raised under dry land farming condition. African Journal of Animal Science. 39, 121-125.

[18] Ulutas Z, Sezer M, Aksoy Y, Sirin E, SenU, Kuran M and Akbas Y. 2010. The effect of birth types on growth curve parameters of Karayaka lamb. Journal of Animal and Veterinary Advances. 9, 1384-1388.

[19] Vatankhah M. 2013. Genetic analysis of ewe body weight in Lori-Bakhtiari sheep using random regression models. Journal of Livestock Science and Technologies. 1, 44-49.

[20] Wilson RT. 1986. Livestock production in Central Mali: Long term studies on cattle and small ruminants in the agropastoral system Research Report 14. ILCA, Addis Ababa, Ethiopia.

[21] Yazdi MH, Johansson K, Gates P, Nasholm A, Jorjani H andLiljedahl LE.1999. Bayesian analysis of birth weight and litter size in Baluchi sheepusing Gibbs sampling. Journal of Animal Science. 77, 533–540.

http://dx.doi.org/10.22161/ijeab/2.1.21 ISSN: 2456-1878

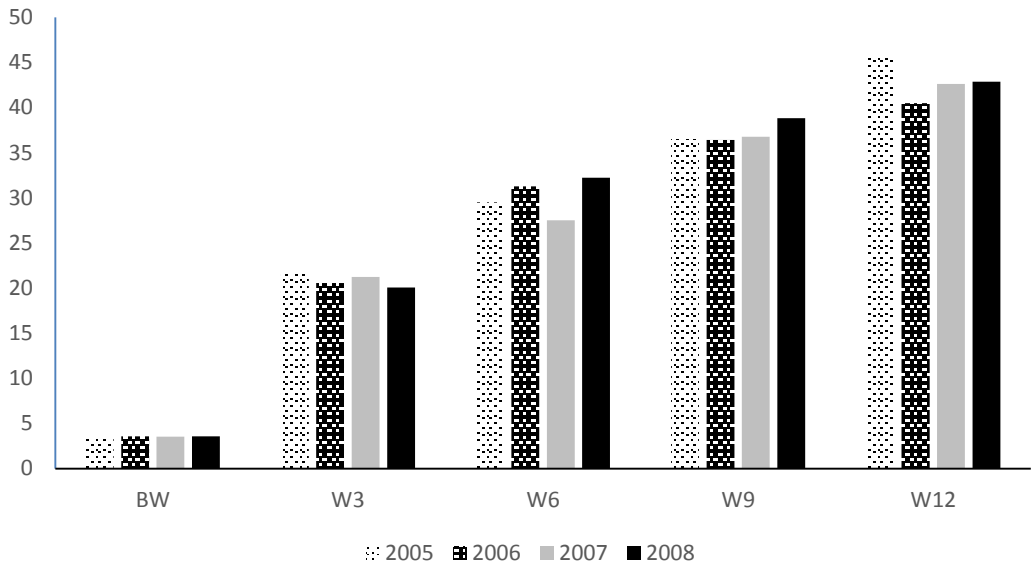


Fig.1: Least square means of growth traits according to year of birth of lambs.

Table.1: Least square means (LSM) and standard error (SE) of lambs live weights according to sex of lambs.

Trait	Sex[1]	N[2]	LSM[3]	SE
BW	M	656	3.618[a]	0.067
	F	746	3.346[b]	0.010
W3	M	423	21.970[a]	0.487
	F	531	19.750[b]	0.680
W6	M	341	32.780[a]	0.561
	F	479	27.540[b]	0.733
W9	M	266	39.250[a]	0.667
	F	316	34.520[b]	1.055
W12	M	257	45.750[a]	0.710
	F	284	40.070[b]	1.113

1 Sex of lambs; M: male, F: female

2 Number of records

3 Column with different superscripts within subclass indicate significant differences ($P< 0.001$)

Table.2: Least square means (LSM) and standard error (SE) of lambs live weights according to year of birth of lambs.

Trait	Birth year	N[1]	LSM[2]	SE
BW	2005	150	3.331[c]	0.088
	2006	334	3.550[ab]	0.065
	2007	368	3.529[abc]	0.067
	2008	205	3.580[a]	0.074
	2009	347	3.419[bc]	0.067
W3	2005	135	21.580[ab]	0.628
	2006	306	20.550[ab]	0.447
	2007	335	21.250[a]	0.461
	2008	178	20.060[b]	0.518
W6	2005	129	29.510[b]	0.715
	2006	285	31.270[a]	0.492
	2007	242	27.520[c]	0.532
	2008	164	32.340[a]	0.573
W9	2005	91	36.540[ab]	0.807
	2006	201	36.700[b]	0.645
	2007	163	35.950[ab]	0.684
	2008	127	36.090[a]	0.702
W12	2005	112	45.66[a]	0.853
	2006	153	40.490[c]	0.696
	2007	158	42.630[b]	0.720
	2008	118	42.870[b]	0.751

1 Number of records
2 Column with different superscripts within subclass indicate significant differences ($P< 0.001$)

Table.3: Least square means (LSM) and standard error (SE) of lambs live weights according to litter size.

Trait	Litter size	N[1]	LSM[2]	SE
BW	1	451	4.386[a]	0.033
	2	842	3.786[b]	0.026
	3	100	3.311[c]	0.068
	4	11	2.445[d]	0.225
W3	1	325	24.610[a]	0.264
	2	548	20.870[b]	0.219
	3	70	19.820[b]	0.556
	4	11	18.130[b]	0.514
W6	1	293	34.870[a]	0.296
	2	466	31.330[b]	0.253
	3	50	30.450[b]	0.727

http://dx.doi.org/10.22161/ijeab/2.1.21 *ISSN: 2456-1878*

	4	11	23.990[c]	1.624
W9	1	225	38.800[a]	0.286
	2	322	36.700[b]	0.250
	3	31	35.950[b]	0.741
W12	1	204	44.760[a]	0.310
	2	307	43.000[b]	0.269
	3	26	43.590[ab]	0.846

1 Number of records

2 Column with different superscripts within subclass indicate significant differences ($P< 0.001$)

Table.4: Estimates of phenotypic correlation (below diagonal) and corresponded Pearson correlationP-value (above diagonal) between lambs live weights

Trait	**BW**	**WW**	**W6**	**W9**	**W12**
BW		0.000	0.000	0.000	0.000
WW	0.486		0.000	0.000	0.000
W6	0.431	0.732		0.000	0.000
W9	0.228	0.429	0.535		0.000
W12	0.166	0.356	0.433	0.906	

http://dx.doi.org/10.22161/ijeab/2.1.22 *ISSN: 2456-1878*

Geochemical Processes and Assessment of Water Quality for Irrigation of Al-Shagaya Field-C, Kuwait

Al-Ruwaih F.M., Shafiullah G.

Department of Earth and Environmental Sciences, Kuwait University, Kuwait

***Abstract*—** *Al-Shagaya Field-C is located southwest of Kuwait City, where the brackish groundwater is produced from the Dammam aquifer. The main objectives are to recognize the major geochemical processes operating in the aquifer and controlling its quality; in addition, to evaluate the groundwater quality criteria for drinking and irrigation. The investigation was carried out by estimating pH, EC, TDS, TH, SAR, %Na, RSC, RSBC, potential salinity, magnesium ratio, chloro-alkaline index, Kelly's ratio, Permeability index, and salinity hazard respectively. The TDS ranges between 2474 and 3232 mg/l, with an average value of 2753mg/l and the water is exceeding very hard. Groundwater shows Ca-Cl and Ca-Mg-Cl genetic water types. Results revealed that the groundwater is oversaturated with respect to dolomite and calcite and under-saturated with respect to gypsum and anhydrite. The main geochemical processes controlling groundwater chemistry in the study area are due to dissolution/ precipitation process along the path flow. The major ions composition in groundwater of the study area indicated that the water is not suitable for drinking. However, the irrigation parameters revealed that the groundwater is suitable for irrigation purposes.*

***Keywords*— Dammam aquifer, saturation index, Gibb's ratio, hydro chemical facies & GIS.**

I. INTRODUCTION

Kuwait covers an area of 18,000 km^2 and lies in the north-eastern corner of the Arabian Peninsula and occupies the north-western part of the Arabian Gulf as shown in Fig (1A).The climate is extremely hot and dry in summer and mild to cold in winter . The rainfall is scarce with an annual average precipitation of 115 mm. The average evaporation is equal 17 mm/ day .The location of Kuwait within the arid gives groundwater great importance. The brackish groundwater in Kuwait is used in agriculture, gardening and domestic purposes. Moreover, it is blend with the fresh water produced by desalination plants to make potable drinking water. The groundwater is abstracted from two main aquifers, the Kuwait Group aquifer, which is leaky to water-table aquifer, and the Dammam aquifer is confined to semi-confined aquifer. Al-shagaya area is located in the southwest of Kuwait and was put in use in early 1970's .This area includes five water well fields. Fields A, B, C, D, and E supply Kuwait city with brackish groundwater produced mainly from Dammam aquifer at a peak rate of 60 MIGD, with an expected quality of 4,000 mg/l of TDS, from a total of 115 production wells distributed over the five water-well fields. The salinity of the Dammam aquifer increases from southwest to north-northeast ranging from 2,500 to, 8,000 mg/l .The major hydro chemical water types are $CaSO_4$, Na_2SO_4 and NaCl [1] . Field-C is the area under investigation, where Figs. 1B and 1C show the location and the distribution of the water wells. Al-Shagaya Field-C is located approximately 64 km to the south-west of Kuwait City, with 32 wells produced groundwater from the Dammam aquifer.

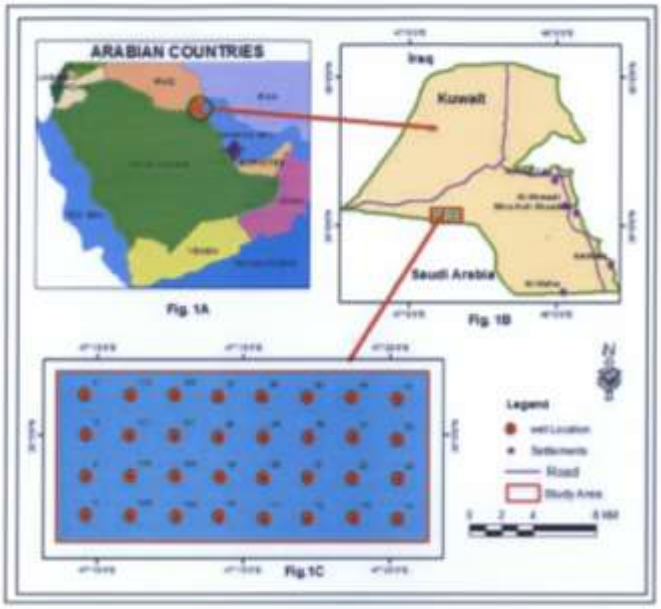


Fig. 1A: Location map of Kuwait.
Fig. 1B: Location map of the study area.
Fig. 1C: Location map of the water wells.

The objectives of this investigation are to identify the water chemical types and hydro chemical processes operating within the main aquifer, in addition to the determination of degree of saturation of groundwater with

http://dx.doi.org/10.22161/ijeab/2.1.22 *ISSN: 2456-1878*

respect to some minerals. Moreover, the suitability of groundwater for drinking and irrigation purposes will be carried out.

Several studies were conducted to date addressing water quality criteria for irrigation. In the research paper published by [2] six soil samples were collected during pre and post monsoon season from Coring mangrove region of East Godavari estuaries for physicochemical of pH, EC, TDS, TH, Cl^-, SO_4^{2-}, NO_3^-, PO_4^{3-}, Na^+, K^+, Ca^{2+}, and Mg^{2+}, and irrigation parameters such % Na, SAR, RSC, KR, and MH, were determined. The results showed that the pH ranges from 7.2 – 7.8 and 7.0 – 7.5 and indicate slight alkaline nature of the soils. Total hardness ranges from 400 – 1550 mg/l pre and post monsoon indicating the hardness of soils. The Magnesium Hazard (MH) ranges from 61.93 – 93.4 pre and post monsoon, exceeding the permissible limit of irrigation standards. Higher Magnesium level in soil causes Magnesium Hazard, so that the soil fertility will be depleted and affects the crop yields. According to [3] Salinity and Sodicity have been reported among the major problems of irrigated agriculture across the world. The methods that are commonly used as indices of salinity or sodicity in the soils include electrical conductivity, Sodium Adsorption Ratio (SAR) and Exchangeable Sodium Percentage. Also, [4] found that the effect of high SAR can be poor soil tith, and soils become sticky when wet resulting in reduced water infiltration. Aza –Gnandji et. al. [5] found that high salinity levels tend to affect soil structure and crop productivity. And chloride is an essential plant micronutrient, but, it's toxic to some crops at higher concentration. Sodium is important to some plant growth, and at high concentrations, it is toxic to many plants. The high salinity of water of C_4-S_2 class permits occasional use and then only under favorable soil and plants of high salt tolerance should be grown. Dastorani et. al. [6] reported that the groundwater resources can be available to help support development, and the limited recharge of groundwater resources is dependent on the amount duration and intensity of rainfall as well as soil properties. According to a study conducted by [7] on the groundwater quality in Abdalli area in Kuwait, it reveals that most of the groundwater samples fall within class C_3-S_4 in Wilcox salinity hazard diagram, which means poor water quality for irrigation and it can be used in well – drained soil. Moreover, with reference to [8], based on Kelly's ratio, water is classified for irrigation. Kelly's ratio of more than 1 indicates excess level of Na^+ in water. Therefore, water with Kelly's ratio of less than 1 is suitable for irrigation, while those with ratio more than 3 are unsuitable for irrigation. In addition, the authors [9] pointed out that the higher level of TDS confirms the unsuitability of water for drinking and irrigation purposes. And the presence of magnesium in water would adversely affect the soil quality rendering it unsuitable for cultivation. If MH is less than 50 the water is safe and suitable for irrigation. However, Narany et al. [10] reported that bicarbonate hazard is usually represented in term of RSC, which shows the tendency for calcium and magnesium to participation as the soil become concentrated. Therefore, the relative proportion of sodium in the water is increased in the form of sodium bicarbonate. According to [11] when electrical conductivity values exceeded the permissible of limits 4000 µ mhos/cm, the water is considered of salinity nature, and is not suitable for irrigation purposes .

II. GEOLOGY OF KUWAIT

2.1 Topography

The topography of Kuwait is generally flat, broken by occasional low hills and shallow depressions. Elevations range from sea level in the east to nearly 300 m in the southwestern corner of the country. The Jal Az-Zor escarpment form one of the main topographic features in Kuwait [12]. The major depression, Wadi Al-Batin is a valley along the western border with 8 to 11 Km and relief of 70 m. The coast lies along the east of the country and sabkha has developed along the coast. In the northeastern part of the country a few barchans dunes up to 25 m are found [13, 14].

2.2 Stratigraphy

The stratigraphical column of Kuwait was mainly influenced by the stable shelf condition of the Arabian plate, causing the deposition of shallow water sediments and evaporates. The surface of Kuwait is formed by sedimentary rocks and sediments ranging from Middle Eocene to Recent. The Dammam Formation represents the oldest exposed sedimentary rocks. The Recent deposits of fine-grained beach sands cover southern coast of Kuwait and the Neutral Zone.

Kuwait Group consists of the Dibdibba, Lower Fars, and Ghar Formations in descending order. Dibdibba Formation includes all rocks between the overlaying Holocene deposits. It consists of fluviated sequence of cross-bedded sands and gravel with subordinate intercalations or lenticular bodies of sandy clays, sandstone, conglomerate and siltstone. Lower Fars Formation consists of sands, Quartz, loosely consolidated gravels, clay and marl. The Ghar Formation consists mainly of marine to terrestrial coarse and unconsolidated sand, silt and gravel.

Hasa Group consists of three formations in descending order; Dammam, Rus and Radhuma Formations. Dammam Formation is considered the largest and the most potential productive aquifer of brackish groundwater in Kuwait. Its thickness ranges between 150 and 275 m

http://dx.doi.org/10.22161/ijeab/2.1.22 *ISSN: 2456-1878*

increases towards the northeast. Dammam Formation consists mainly of dolomitic limestone and limestone inter-bedded with shale at the base of the formation, forms the relatively impermeable lower boundary over most of the region. Rus Formation is composed of hard, dense, massive anhydrite and unfossiliferous limestone. Radhuma Formation consists mostly of anhydrite, dolomitic and marly limestone with few fossiliferous horizons [15].

2.3 Hydrology and Aquifer System

The most significant aquifer in Kuwait is the Tertiary-Quaternary system. These are the upper clastic sediments of the Kuwait Group aquifer, and the Dammam aquifer which are separated by a confining layer of cherts and/or clay [16]. Under natural hydrological conditions, the flow through the Kuwait aquifer is in SW-NE direction, from the main recharge area in Saudi Arabia to the main discharge area in the Arabian Gulf and Shaat Al-Arab. Generally, part of the natural recharge of the Kuwait Group aquifer gains by leakage from the Dammam aquifer, and also comes from infiltration through the wadies and depressions, as well as the lateral flow coming from Saudi Arabia [17]. The effect of leakage between the two main aquifers may give rise to the similarities of groundwater chemistries.

2.4 Objectives of the Study

The main objectives are to study the geochemistry of the study area in order to recognize the prevailing and the major geochemical processes that control the quality of the groundwater. Moreover, the suitability of groundwater for drinking and irrigation were evaluated by determining physiochemical and irrigation parameters.

III. METHODOLOGY

In this study, the chemical analyses of the major cations and anions such as Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, HCO_3^{-}, SO_4^{2-}, and Cl^{-} expressed in mg/l were converted to equivalent per million (e.p.m), (which is equivalent to mq/l) and %e.p.m [18]. Ion balance equation was applied to validate the accuracy of the chemical analyses where ±5% is acceptable [19]. Also, the reaction error of all groundwater samples was less than the accepted limit of ±10% [20] as in Table1 1.

To achieve these objectives a speciation model has been used to determine the degree of saturation of groundwater with respect to some minerals using WATEQ4F program [21]. Along with the application of the Gibb's ratio to assess the functional sources of dissolved chemical constituents and to recognize the main processes governing the groundwater chemistry of the study area. Hydrochemical facies interpretation is used to determine flow pattern and origin of chemical histories of groundwater by plotting of the major cations and anions on the Piper diagram [22]. The assessment of groundwater for irrigation purposes based on different irrigation indices is carried out includes SAR, RSC, %Na, residual sodium bicarbonate (RSBC), Permeability Index (P.I) Potential Salinity (P.S)), Salinity hazard, magnesium ratio (MgR), Kelly's ratio (KR), and chloro-alkaline index (CAI-1). Wilcox diagram (1955), and Doneen permeability index [23, 24] also have been utilized for classification of groundwater for irrigation. The spatial distribution of TDS, TH, RSC, SAR, gypsum and calcite parameters, were illustrated using ArcGIS10 software.

3.1 Mechanisms of Controlling Groundwater Chemistry

It is important to study the relationship between the water chemistry and the aquifer lithology. Gibb's [25] suggested a diagram that represents the ratio of dominant anions and cations plotted against the value of TDS. These ratios can be divided into two formula, the first ratio is for the cations [$(Na^{+} + K^{+}) / (Na^{+} + K^{+} + Ca^{2+})$], and the second ratio is for the anions, $Cl^{-} / (Cl^{-} + HCO^{-}_3)$ as a function of TDS. This diagram is widely used to evaluate the functional sources of dissolved constituents such as precipitation-dominance, rock-dominance, and evaporation-dominance. The chemical analyses of the study area are plotted in the Gibb's diagram as shown in Fig. 2, and showed that the predominant samples fall into the category of rock-water interaction field and few samples are located in evaporation-dominance field , which revealed that the chemical weathering of rock-forming minerals are influencing the groundwater quality by dissolution of rock through which there is circulation, while the data in the evaporation-dominance field indicate that the increasing ions of Na^{+} and Cl^{-} are in relation with the increasing of the TDS.

Table.1: Chemical analysis results of the Al-Shagaya Field - C, (mg/l)

Well No.	EC µmohs/cm	TDS	T.Hard	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}	Cl^{-}	SO_4^{-}	HCO_3^{-}
C-1	3340	2644	1308	360	12.0	338	113	476	1247	126
C-2	3460	2736	1332	365	11.5	323	128	517	1262	131
C-3	3680	2883	1379	335	10.5	330	135	535	1218	126
C-4	3700	2864	1370	470	12.0	338	128	489	1363	145

http://dx.doi.org/10.22161/ijeab/2.1.22 *ISSN: 2456-1878*

C-10	3330	2662	1355	345	12.0	345	120	439	1276	136
C-11	3720	2933	1379	355	12.0	330	135	520	1247	148
C-12	3850	3121	1460	375	12.0	338	150	535	1276	149
C-13	3430	2773	1411	305	11.5	330	143	442	1218	140
C-14	3450	2773	1411	295	11.0	330	143	451	1247	140
C-19	3410	2694	1478	345	11.5	345	150	348	1247	134
C-20	3310	2690	1209	345	12.0	323	98	467	1276	133
C-21	3430	2921	1466	345	12.3	347	146	556	1276	176
C-22	3390	2684	1478	340	12.0	345	150	429	1247	134
C-23	3430	2686	1449	345	11.5	345	143	455	1218	129
C-28	3360	2664	1337	325	12.0	338	120	439	1247	130
C-29	3340	2648	1375	340	12.0	353	120	458	1276	131
C-30	3500	2756	1288	395	13.0	330	113	467	1276	148
C-31	3360	2646	1375	330	12.5	353	120	448	1276	138
C-32	4060	3232	1549	460	16.5	398	135	505	1595	154
C-37	3510	2734	1262	395	12.0	308	120	467	1276	153
C-38	3330	2660	1370	325	12.0	338	128	420	1276	141
C-39	3370	2690	1387	330	12.0	345	128	439	1276	140
C-40	3360	2672	1355	335	12.0	345	120	429	1334	144
C-41	3880	3042	1370	445	15.0	338	128	542	1450	158
C-105	3410	2694	1478	346	11.5	345	150	438	1247	134
C-106	3380	2672	1478	340	12.0	345	150	439	1247	138
C-107	3390	2694	1370	340	12.0	338	128	429	1276	141
C-108	3370	2708	1375	335	12.0	353	120	412	1363	148
C-109	3310	2646	1346	340	12.0	353	113	458	1247	125
C-110	3320	2474	1189	350	12.0	315	98	467	1247	126
C-111	3400	2712	1339	350	12.0	323	143	467	1334	142
C-112	3360	2680	1332	330	12.0	232	128	429	1334	142
Min.	**3310**	**2474**	**1189**	**295**	**10.5**	**232**	**98**	**348**	**1218**	**125**
Max.	**4060**	**3232**	**1549**	**470**	**16.5**	**398**	**150**	**556**	**1595**	**176**
Ave.	**3467**	**2753**	**1377**	**354**	**12.13**	**336.16**	**129.50**	**462.87**	**1287.34**	**140**

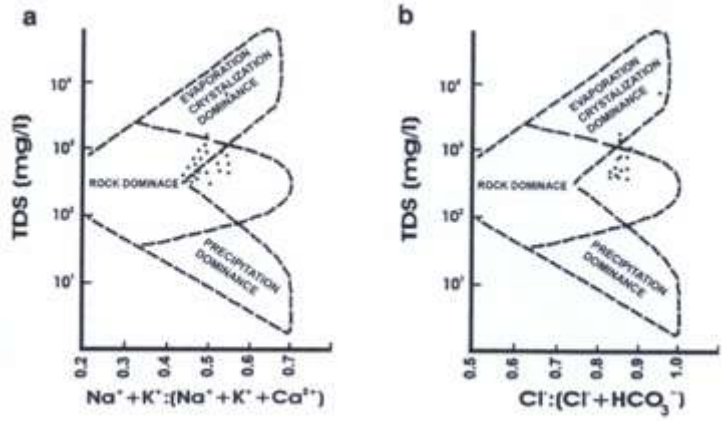


Fig.2: Gibb's diagram for controlling factor of groundwater quality in the study area

http://dx.doi.org/10.22161/ijeab/2.1.22 *ISSN: 2456-1878*

3.2 Hydrochemical Facies

Hydrochemical facies interpretation using Piper trilinear diagram is a useful tool for determining the flow pattern and origin of chemical histories of groundwater. The Piper trilinear diagram is presented in Fig.3. Two principal hydrochemical water types have been delineated. These are Ca-Cl and Ca-Mg-Cl water types respectively. The majority of the groundwater samples of the study area fall in Ca-Cl water type which suggesting an end–product water. A few of the samples show Ca-Mg-Cl water type, indicat that alkaline earth (Ca^{2+} + Mg^{2+}) exceeds the alkaline (Na^{+} + K^{+}) and strong acid (Cl^{-} and SO_4^{2-}) exceeds the weak acid (HCO^{-}_3 and CO_3^{2-}).

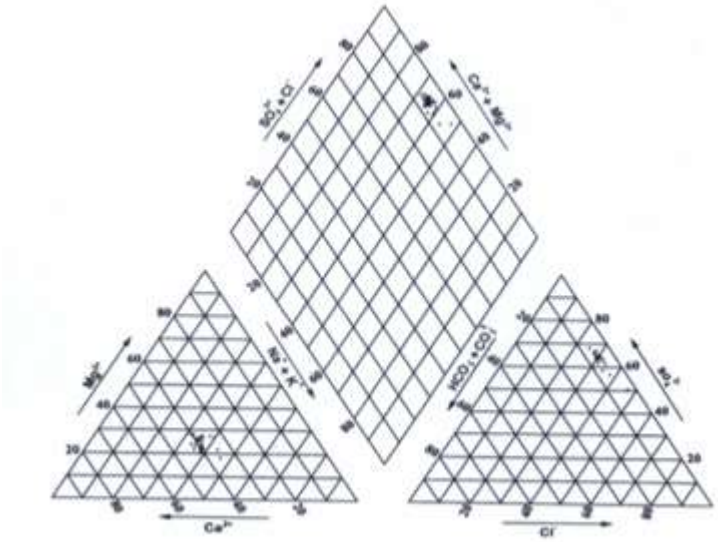


Fig.3: Piper trilinear diagram for the groundwater samples of the study area

3.3 Geochemical Modeling

Geochemical models are tools used to calculate chemical reaction in groundwater system such as dissolution and precipitation of solids, ion exchange, and sorption by clay minerals [26]. In this study, the speciation model has been applied to the groundwater samples of Al-Shagaya Field-C to determine the saturation index (SI) of minerals. The SI for a given mineral measures the degree of saturation of that mineral with respect to the surrounding system. The degree of saturation index is defined as follow [27]:

$$SI = \log \frac{K_{iap}}{K_{sp}}$$

Where "iap" is the ion activity product of the dissociated chemical species in solution, and "K_{sp}" is the solubility product of the mineral. Where SI is <0, it indicates that the groundwater is under-saturated with respect to that particular mineral. When SI > 0, it means that the groundwater is being saturated with respect to the mineral and incapable of dissolving more of the mineral. The over-saturation can also be produced by incongruent dissolution, common ion effect.

Table 2 shows the saturation indices of anhydrite, gypsum, halite, calcite and dolomite. Nearly, all groundwater samples of the study area are under saturated with respect to anhydrite, gypsum and halite and oversaturated with respect to calcite and dolomite.

Table.2: Results of thermodynamic speciation calculation of Al-Shagaya Field -C.

Well No.	Anhydrite	Gypsum	Halite	Calcite	Dolomite	P_{CO_2} Atom.
	$CaSO_4$	$CaSO_4.2H_2O$	NaCl	$CaCO_3$	$CaMg(CO_3)_2$	
C-1	-0.30	-0.33	-5.44	0.20	0.20	4.19E-03
C-2	-0.32	-0.35	-5.40	0.19	0.27	4.35E-03
C-3	-0.32	-0.36	-5.43	0.63	1.15	1.34E-03
C-4	-0.29	-0.32	-5.32	0.34	0.54	3.79E-03
C-10	-0.28	-0.32	-5.50	0.19	0.20	5.08E-03
C-11	-0.32	-0.35	-5.41	0.45	0.80	3.06E-03
C-12	-0.31	-0.35	-5.38	0.75	1.42	1.49E-03
C-13	-0.32	-0.36	-5.55	0.24	0.40	4.64E-03

C-14	-0.31	-0.35	-5.55	0.14	0.19	5.80E-03
C-19	-0.29	-0.34	-5.60	0.24	0.39	4.42E-03
C-20	-0.30	-0.34	-5.47	0.49	0.75	2.20E-03
C-21	-0.24	-0.28	-4.94	0.11	-0.01	2.91E-03
C-22	-0.29	-0.33	-5.75	0.24	0.40	4.43E-03
C-23	-0.31	-0.34	-5.48	0.32	0.53	3.37E-03
C-28	-0.30	-0.33	-5.52	0.21	0.26	4.33E-03
C-29	-0.28	-0.31	-5.49	0.62	1.05	1.69E-03
C-30	-0.30	-0.34	-5.41	0.25	0.32	4.93E-03
C-31	-0.27	-0.31	-5.51	0.25	0.32	4.58E-03
C-32	-0.18	-0.22	-5.33	0.31	0.48	5.04E-03
C-37	-0.33	-0.37	-5.41	0.33	0.35	5.10E-03
C-38	-0.29	-0.24	-5.54	0.34	0.55	3.71E-03
C-39	-0.29	-0.32	-5.52	0.35	0.55	3.67E-03
C-40	-0.27	-0.31	-5.52	0.32	0.45	3.46E-03
C-41	-0.27	-0.31	-5.31	0.22	0.30	5.78E-03
C-105	-0.30	-0.34	-5.50	0.23	0.38	4.40E-03
C-106	-0.30	-0.34	-5.51	0.25	0.41	4.55E-03
C-107	-0.29	-0.33	-5.52	0.25	0.35	4.68E-03
C-108	-0.25	-0.29	-5.54	0.28	0.36	4.92E-03
C-109	-0.28	-0.32	-5.49	0.22	0.22	4.15E-03
C-110	-0.31	-0.35	-5.46	0.37	0.51	2.64E-03
C-111	-0.30	-0.34	-5.47	0.22	0.37	4.71E-03
C-112	-0.42	-0.45	-5.53	0.37	0.77	2.30E-03
Min.	-0.42	-0.45	-5.75	0.11	-0.01	1.34E-03
Max.	-0.18	-0.22	-4.94	0.75	1.42	5.80E-03
Ave.	-0.29	-0.33	-5.46	0.31	0.48	3.93E-03

The areal distribution map of gypsum of the study area is shown in Fig. 4 and exhibits that the medium values of gypsum are concentrated in the central part, while low values are found in the southeastern corner. In addition, high values of calcite is displays in Fig. 5 and concentrated in the central part of the study area indicating that dissolution / precipitation process of these carbonate minerals along the path flow may have influenced the chemical composition of the Al-Shagaya Field-C. The partial pressure of the carbon dioxide values (Pco_2) range between 1.34×10^{-3} atm. and 5.8×10^{-3} atm., with an average value of 3.93×10^{-3} atm. This indicates that the groundwater of the Dammam aquifer become charged with CO_2 during infiltration through the soil zones. Where, according to Appelo and Postma [28] when Pco_2 values range between $10^{-2.5}$ atm. and $10^{-6.4}$ atm., it represents a closed system. Since the Dammam aquifer is acting as a confined to semi-confined aquifer, it is more likely that the groundwater represents a deep closed environment system.

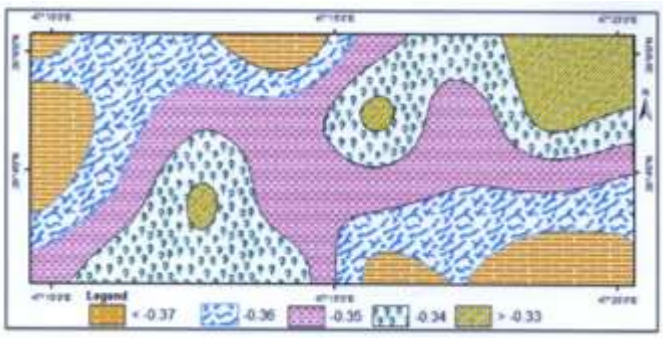


Fig.4: Spatial distribution of the saturation index of gypsum of the study area

http://dx.doi.org/10.22161/ijeab/2.1.22 *ISSN: 2456-1878*

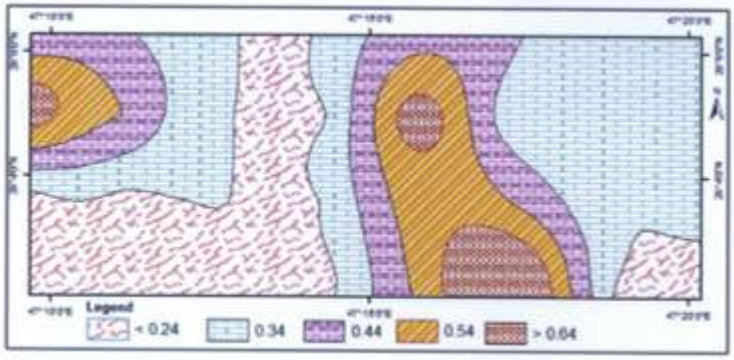


Fig.5: Spatial distribution of the saturation index of calcite of the study area

3.4 Geochemical Evolution of Groundwater

The initial composition of groundwater originates from rainfall with low concentrations of dissolved ions. During its return path to the ocean, the water composition is altered by rock weathering and evaporation causing more Ca^{2+}, Mg^{2+}, Na^{+}, SO_4^{2-}, HCO_3^{-} , Cl^{-} and SiO_2 to be added. The concentration of these ions depends on the rock mineralogy that the water encounters and its rapidity along the flow path. The abundance of the major cations in Al-Shagaya Field-C is in the order $Na^{+} > Ca^{2+} > Mg^{2+} > K^{+}$. The sequence of the anions is in order of $SO_4^{2} > Cl^{-} > HCO_3^{-}$. Calcium and magnesium present in the groundwater are mainly due to the dissolution of limestone, dolomite, gypsum and anhydrite, the most rock forming minerals of the Dammam aquifer of the study area. Calcium ions are derived also from cation exchange process [28].The concentration of calcium ions in the study area ranges from 232 mg/l to 398 mg/l and magnesium ranges from 98 mg/l to 150 mg/l, with average values of 336 mg/l and 129 mg/l respectively. This indicates that the Ca^{2+} ion concentration in the study area is relatively higher than magnesium ion. The plot of $Ca^{2+} + Mg^{2+}$ Vs. ($HCO_3^{-} + SO_4^{2-}$) as in Fig. 6A, shows that the majority of the samples fall above the equiline indicating that the carbonate weathering is the dominant processes for supply of the calcium and magnesium ions to groundwater. The plot of (Na^{+}) Vs. (Cl^{-}) of the groundwater samples of the study area presented in Fig. 6B, shows that the Na/Cl ratio is greater than (1) which is typically indicates that the sodium was released from silicate weathering. The silicate weathering is also supported by the plot of HCO_3^{-} Vs. Na^{+} as shown in Fig. 6 C, where all the samples fall below the equiline [29], this reveals that the carbonate and the silicate weathering are the dominant processes operating in the aquifer of the study area.

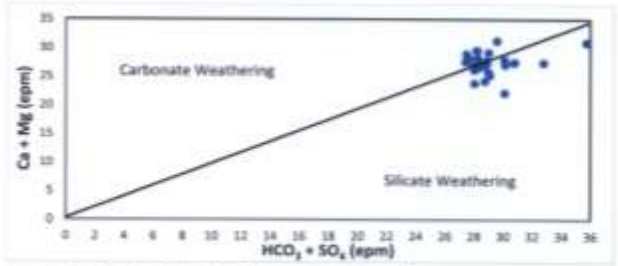


Fig. 6A: Plot of (Ca^{2+}+ Mg^{2+}) Vs (HCO_3^{-}+SO_4^{2-}) of the study area.

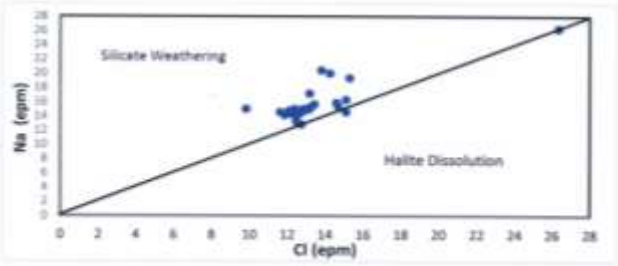


Fig. 6B: Plot of (Na^{+}) Vs (CT)) of the study area.

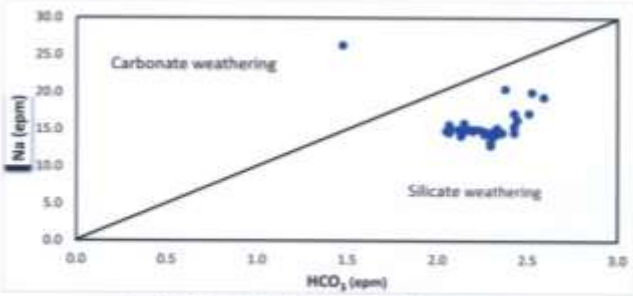


Fig. 6C: Plot of (Na^{+}) Vs (HCO_3^{-}) of the study area.

IV. DRINKING AND IRRIGATION WATER QUALITY

The assessment of the suitability of groundwater for drinking and irrigation purposes can be determined through the parameters such as pH, EC, TDS, TH, RSC, residual sodium bicarbonate (RSBC), Permeability index (P.I), Potential Salinity (P.S), SAR, salinity hazard, magnesium ratio (MgR), %Na, Kelley's ratio (KR), and chloro-alkaline index (CAI-1) as display in tables 1 and 3.

Table.3: Irrigation water quality parameters for Al-Shagaya Field – C.

Well No.	RSC	RSBC	P.I	P.S	SAR	MgR	% Na	Kelly's ratio	CAI-1
C-1	-24.10	-14.80	40.88	26.41	4.33	35.53	37.17	0.60	-0.19
C-2	-24.50	-13.97	40.78	27.72	4.35	39.51	37.08	0.60	-0.11
C-3	-25.51	-14.40	37.99	27.77	3.92	40.28	34.36	0.53	0.02
C-4	-25.02	-14.49	45.96	27.98	5.52	38.43	42.46	0.75	-0.50
C-10	-24.86	-14.99	39.20	25.67	4.08	36.44	35.39	0.55	-0.24
C-11	-25.15	-14.04	39.52	27.65	4.16	40.28	35.65	0.56	-0.07
C-12	-26.76	-14.42	39.27	28.38	4.27	42.25	35.60	0.56	-0.10
C-13	-25.94	-14.17	35.62	25.15	3.53	41.67	31.75	0.47	-0.09
C-14	-25.94	-14.17	34.94	25.70	3.42	41.67	31.04	0.45	-0.03
C-19	-27.36	-15.02	37.00	22.80	3.90	41.75	33.46	0.51	-0.56
C-20	-22.00	-13.94	42.07	26.46	4.32	33.34	38.00	0.62	-0.16
C-21	-26.44	-14.43	37.68	28.97	3.92	40.95	33.61	0.51	0.02
C-22	-27.36	-15.02	36.69	25.08	3.85	41.75	33.12	0.50	-0.25
C-23	-26.86	-15.10	37.42	25.51	3.94	40.59	33.89	0.52	-0.19
C-28	-24.61	-14.74	38.16	25.37	3.87	36.92	34.33	0.53	-0.17
C-29	-25.34	-15.47	38.45	26.20	3.99	35.91	34.73	0.54	-0.17
C-30	-23.34	-14.04	43.64	26.46	4.79	36.08	39.70	0.67	-0.33
C-31	-25.22	-15.35	37.90	25.92	3.87	35.91	34.05	0.52	-0.16
C-32	-28.44	-17.34	42.37	30.85	5.09	35.86	38.93	0.65	-0.43
C-37	-22.73	-12.86	44.24	26.46	4.84	39.11	40.21	0.68	-0.33
C-38	-25.08	-14.56	37.70	25.13	3.82	38.43	33.79	0.52	-0.22
C-39	-25.45	-14.92	37.70	25.67	3.85	37.95	33.85	0.52	-0.18
C-40	-24.73	-14.86	38.67	25.99	3.96	36.44	34.72	0.54	-0.23
C-41	-24.81	-14.28	44.85	30.38	5.23	38.43	41.07	0.71	-0.29
C-105	-27.36	-15.02	37.06	25.34	3.92	41.75	33.52	0.51	-0.24
C-106	-27.29	-14.95	36.74	25.37	3.85	41.75	33.12	0.50	-0.22
C-107	-25.08	-14.56	38.66	25.39	4.00	38.43	34.81	0.54	-0.25
C-108	-25.06	-15.19	38.35	25.81	3.93	35.91	34.40	0.53	-0.28
C-109	-24.86	-15.57	38.90	25.90	4.03	34.54	35.21	0.55	-0.17
C-110	-21.71	-13.65	42.72	26.16	4.42	33.90	38.73	0.64	-0.18
C-111	-25.55	-13.79	38.86	27.06	4.08	42.19	35.07	0.55	-0.18
C-112	-19.78	-9.25	43.55	25.99	4.32	47.63	39.04	0.65	-0.21
Min.	-28.44	-17.34	34.94	22.80	3.42	33.34	31.04	0.45	-0.56
Max.	-19.78	-9.25	45.96	30.85	5.52	47.63	42.46	0.75	0.02
Ave.	-25.13	-14.48	39.49	26.46	4.17	38.80	35.68	0.56	-0.21

4.1 Drinking Water Quality

The suitability of groundwater in the study area is evaluated for drinking by comparing with the standard guide line values [30]. According to WHO specifications, TDS up to 500 mg/l is the highest desirable and up to 1500 mg/l is the maximum permissible level. Based on this classification, the TDS of the groundwater of the study area ranges between 2474 and 3232 mg/l with an average value of 2753 mg/l which exceed the recommended limit. The areal distribution map of the TDS is plotted in Fig. 7, and showed that the minimum values are located in the southwestern corner of the study area. However, the major cations and anions composition of the study area are all above the standard guideline of the WHO for drinking purposes. Moreover, the total hardness of the study area is varying from 1189 to 1549 mg/l as $CaCO_3$, with an average value of 1377 mg/l as shown in Table 1. The areal distribution map of TH is shown in Fig. 8, where, the lower values of TH are found in the southwestern part, which seems to be the best quality zone in the study area. The analytical result of TH indicates that the groundwater of the study area is

exceeding very hard water type according to [31] and as shown in Table 4. Therefore, according to TDS and TH standards the groundwater is not suitable for drinking purposes.

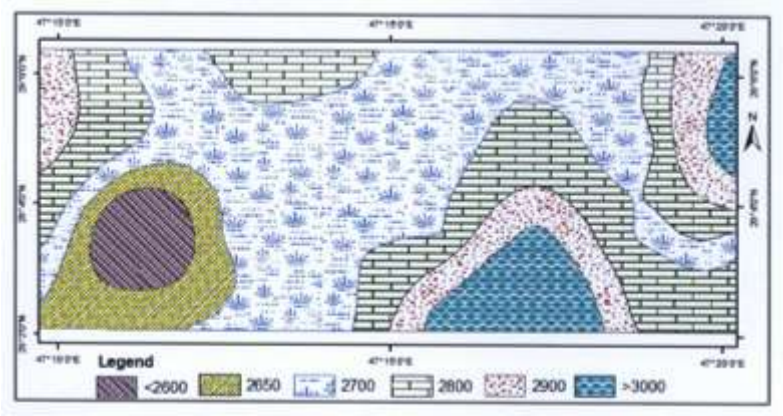


Fig.7: Spatial distribution of TDS of the study

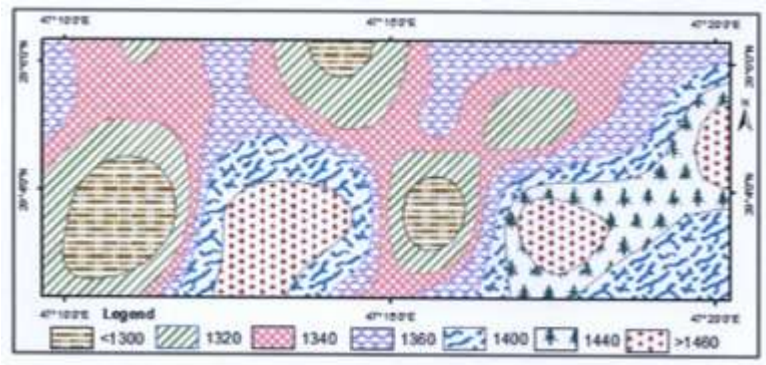


Fig.8: Spatial distribution of total hardness of the study area

Table.4: Water Classes (After Sawyer and McCarthy, 1967).

Total Hardness as $CaCO_3$ (mg/l)	Water Class
<75	soft
75-150	moderately hard
150-300	hard
>300	very hard

Water hardness causes more consumption of detergents at the time of cleaning, and some evidences indicate its role in heart disease [32]. The total hardness (TH) was determining by the following equation according to [33, 21 and 34].

$$TH = 2.5\ Ca^{2+} + 4.1\ Mg^{2+}$$

Where Ca^{2+} and Mg^{2+} concentration are expressed in mg/l as $CaCO_3$. Hardness of water is by inhabitation of soap action in water due to precipitation of Ca^{2+} and Mg^{2+} salts like carbonate, sulphates and chlorides. Hardness of water causes scaling of pots, boilers and irrigation pipes. In order to examine the degree of correlation between the different chemical parameters affecting groundwater quality of the study area, the correlation matrix was determined between the different parameters as display in Table 5. It is found that there is a good correlation between TH and Ca^{2+}, Mg^{2+}, Cl^- respectively , which indicates that the hardness of groundwater is mainly due to $CaCl_2$ and $MgCl_2$.

Table.5: Correlation matrix for different water quality parameters in the study area.

	EC	**TDS**	**Na**	**K**	**Ca**	**Mg**	**Cl**	**SO_4**	**HCO_3**	**T.Hard**
EC	**0.000**	0.985	0.889	0.656	0.541	0.148	0.908	0.478	-0.316	0.493
TDS	0.985	**0.000**	0.842	0.650	0.549	0.200	0.880	0.491	-0.274	0.543

Na	0.889	0.842	**0.000**	0.726	0.507	-0.067	0.821	0.569	-0.273	0.304
K	0.656	0.650	0.726	**0.000**	0.503	-0.115	0.486	0.847	0.089	0.316
Ca	0.541	0.549	0.507	0.503	**0.000**	0.102	0.467	0.305	-0.340	0.583
Mg	0.148	0.200	-0.067	-0.115	0.102	**0.000**	-0.052	-0.057	0.138	0.778
Cl	0.908	0.880	0.821	0.486	0.467	-0.052	**0.000**	0.242	-0.565	0.273
SO_4	0.478	0.491	0.569	0.847	0.305	-0.057	0.242	**0.000**	0.354	0.255
HCO_3	-0.316	-0.274	-0.273	0.089	-0.340	0.138	-0.565	0.354	**0.000**	-0.129
T.Hard	0.493	0.543	0.304	0.316	0.583	0.778	0.273	0.255	-0.129	**0.000**

4.2 Irrigation Water Quality

The suitability of groundwater for irrigation is depending on the effect of mineral composition of water on the soil and plants. The effect of the salt on soils causes change in soil structure, permeability, and hence, it effects on plant growth.

4.2.1 Residual Sodium Carbonate

Residual sodium carbonate (RSC) has been calculated to determine the hazards effects of carbonate and bicarbonate on quality of water for irrigation and is expressed by the equation:

$$RSC = (HCO^-_3 + CO_3^{2-}) – (Ca^{2+} + Mg^{2+})$$

Whereas, all ionic concentrations are expressed in meq/l. The classification of irrigation water according to the RSC presents in Table 6 after [35] , where water containing more than 2.5 meq/l of RSC are not suitable for irrigation, while those having < 1.25 meq/l is good for irrigation [36].

Table.6: Water classes based on RSC (after Richards, 1954).

RSC value	Water quality
<1.25	suitable
1.25-2.5	marginal
>2.5	not suitable

Eaton (1950) indicated that if waters which are used for irrigation contain excess of HCO_3^- + CO_3^{2-} than its equivalent Ca^{2+} + Mg^{2+}, there will be a residue of Na^+ + HCO_3^- when evaporation takes place and the pH of the soil increase up to 3 [37]. When total carbonate levels exceed the total amount of calcium and magnesium the water quality diminished [38]. The calculated RSC values of the groundwater samples of the study area are ranged from -28.44 to -19.78 meq/l with an average value of -25.2 meq/l. Negative RSC indicates that sodium buildup is unlikely, since sufficient calcium and magnesium are in excess of what can be precipitated as carbonates [39]. Hence, the groundwater of the study area is safe for irrigation, and the minimum values of RSC are distributed in the southeastern as well as central part of the study area as displayed in Fig. 9.

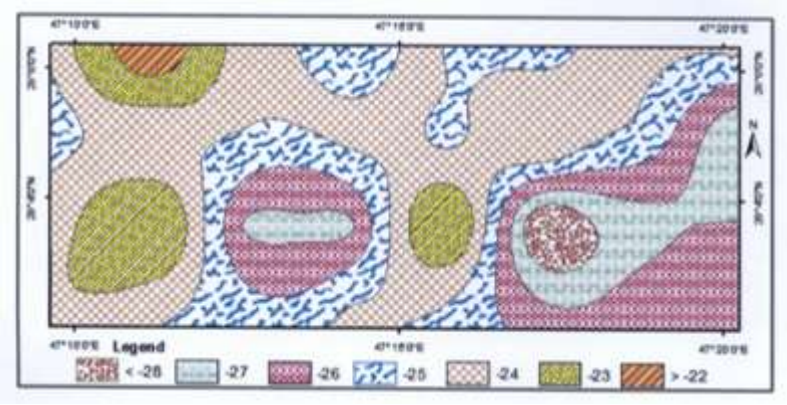


Fig.9: Spatial distribution of RSC of the study area

http://dx.doi.org/10.22161/ijeab/2.1.22 *ISSN: 2456-1878*

4.2.2 Residual Sodium Bicarbonate (RSBC)

Residual sodium bicarbonate (RSBC) is calculated by the following formulae according to [40]:

$$RSBC = HCO_3^- - Ca^{2+}$$

It was found that the groundwater is considered satisfactory with <5 meq/l for irrigation, according to the criteria set by [40] and [41]. In the study area, the values of the RSBC ranges between -17.34 and -9.25 meq/l with an average value of -14.48 meq/l, which indicate that groundwater is good for irrigation.

4.2.3 Permeability Index (P.I)

The permeability of soil is affected by long-term use of irrigation water and is influenced by sodium, calcium, magnesium and bicarbonate contents in soil. Doneen (1964) set a criteria for assessing the suitability of water for irrigation based on permeability index (P.I), accordingly, waters can be classified as Class I, Class II and Class III. The Class I and Class II waters are categories as good for irrigation with 50-75% or more of maximum permeability. Whereas, Class III water is unsuitable with of 25% maximum permeability. Therefore, soil permeability is affected by consistent use of irrigation water which increases the presence of sodium, calcium, magnesium and bicarbonate in the soil [42].

The permeability index is used to measure the suitability of water for irrigation purpose when compared with the total ions in meq/l, it's expressed as follow:

$$PI = \frac{Na^+ + \sqrt{HCO_3^-}}{Ca^{2+} + Mg^{2+} + Na^+} * 100$$

In the present study, the P.I of the groundwater samples ranged from 34.94% to 45.96 % with a mean value of 39.49 %, and it's observed that all the groundwater samples fall in class II category of Doneen Chart (Fig.10). Therefore, the groundwater of the study area is good for use in irrigation.

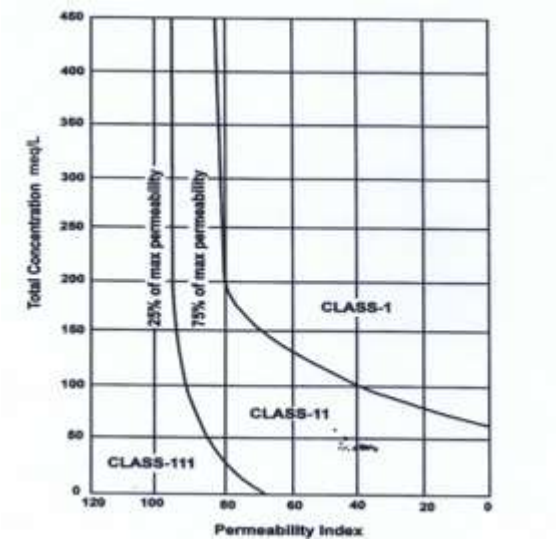


Fig.10: Showing Doneen's Chart of Permeability Index (after Doneen, 1964)

4.2.4 Potential Salinity (P.S)

Doneen, 1961 introduced an important parameter "Potential Salinity" for assessing the suitability of water for irrigation uses, which defined as chloride concentration plus half of the sulphate concentration expressed in meq/l.

$$\text{Potential Salinity} = Cl^- + \tfrac{1}{2}\, SO_4^{2-}$$

On the basis of the potential salinity Doneen (1961) subdivided the irrigation water into three classes as presented in Table 7. The potential salinity of the majority of the analyzed groundwater samples of the study area ranges between 22.8 meq/l and 30.85 meq/l with an average value of 26.46 meq/l indicates high values of potential salinity. However, it is found that the classification of the groundwater of the study area for irrigation purposes fall in Class III, therefore, the groundwater should be used in case of a soil of high permeability [43].

Table.7: Classification of irrigation water based on potential salinity.

Class of water / Soil Characteristics	Class I	Class II	Class III
Soil of low Permeability	<3	3-5	>5
Soil of medium Permeability	<5	5-10	>10
Soil of high Permeability	<7	7-15	>15

http://dx.doi.org/10.22161/ijeab/2.1.22 *ISSN: 2456-1878*

4.2.5 Sodium Adsorption Ratio (SAR)

Sodium concentration is considered an important factor to express reaction with the soil and reduction in its permeability. Therefore, sodium adsorption ratio is considered as a better measure of sodium (alkali) hazard in irrigation water as it is directly related to the adsorption of Na^+ on soil, and is an important critera for estimating the suitability of the water for irrigation. SAR can be computed as follow:

$$SAR = \frac{Na^+}{\sqrt{\frac{Ca^{2+} + Mg^{2+}}{2}}}$$

Where all ionic concentrations are expressed in meq/l. The SAR of the study area ranges between 3.42 and 5.52, with an average value of 4.17. According to the classifications of water based on SAR values [33, 35], the SAR values of all the study area are found to be <10, and are classified as being excellent for irrigation i.e S_1 category. The areal distribution map of the SAR values of the study area is presented in Fig.11, and it is exhibited that the lower values of SAR are concentrated in the southeastern and central part of the study field, which means that the groundwater of this part is suitable for irrigation.

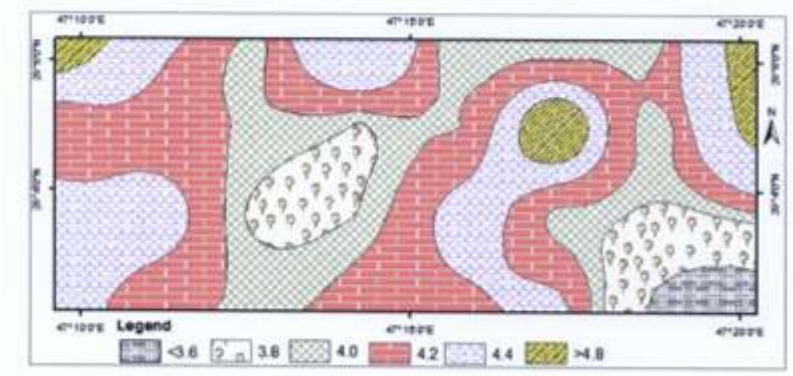


Fig.11: Spatial distribution of SAR of the study area

4.2.6 Salinity Hazard

The most important criteria regarding salinity and water availability to the plant is the total salt concentration. Since there exist a straight line correlation between electrical conductivity (EC) and total salt concentration of waters, the most expedient procedure to evaluate salinity hazard is to measure its electrical conductivity measured in (µmohs/cm) [44]. On the basis of salt concentration, US Salinity Laboratory Staff [35] divided the irrigation water into four classes as displayed in Table 8. It is found that the values of EC of the study area range from 3310 to 4060 µmohs/cm with an average value of 3467 µmohs/cm which is considered of C4 high salinity hazard class. For rating irrigation waters, the US salinity diagram was used, in which the SAR is plotted against EC as shown in Fig.12, where, all the samples of the study area fall in the category of the C_4-S_2, indicating high salinity/ medium sodium type. Therefore, the groundwater of C_4-S_2 class can be used with tolerant crops of clayey, sandy loam and loamy sand soil texture [45]. Based on these specifications, the groundwater of the study area is considered safe for irrigation.

Table.8: Salinity hazards of irrigation waters based on EC values (Richards, 1954).

EC of irrigation water (µmohs/cm)	Salinity Class	Salinity Hazards
100 – 250	C1	very low
250 – 750	C2	low
750 - 2250	C3	medium
2250 - 4000	C4	high salinity

http://dx.doi.org/10.22161/ijeab/2.1.22 *ISSN: 2456-1878*

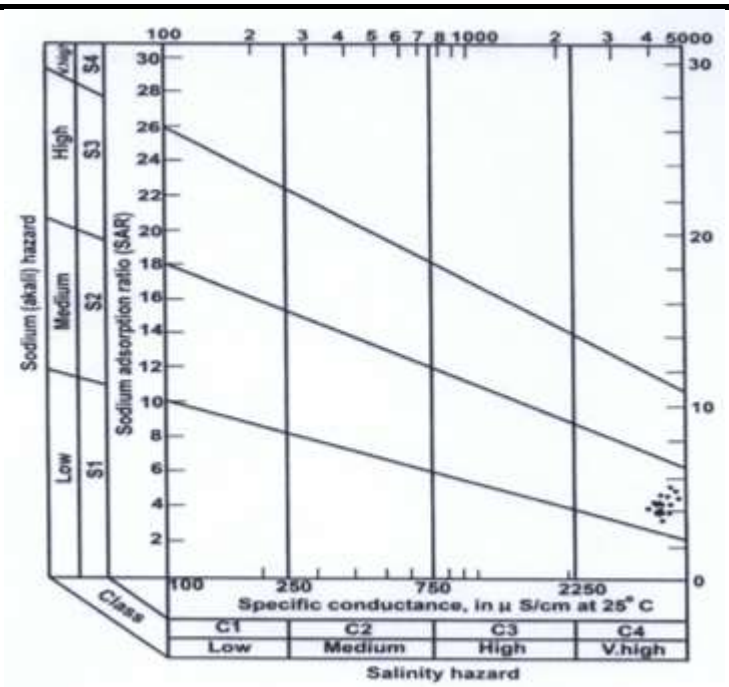


Fig.12: Showing USSL salinity hazard diagram of the study area

4.2.7 Magnesium Ratio

In most waters calcium and magnesium maintain a state of equilibrium. A ratio namely index of magnesium hazard was developed by [46]. According to this, high magnesium hazard value >50% has an adverse affect on the crop yield as the soil becomes more alkaline, and effect on the agricultural yield.

$$\text{Mg ratio} = \frac{Mg^{2+}}{(Ca^{2+}+Mg^{2+})} \times 100$$

Where all ionic concentration are expressed in meq/l.

In the study area, the magnesium hazard values falls in the range value of 33.34% to 47.63% with an average value of 38.8 %, i.e. magnesium hazard ratio < 50%, which is recognized as suitable for irrigation.

4.2.8 Sodium Percentage (%Na)

Sodium is an important ion used for the classification of irrigation water due to its reaction with soil, reduces its permeability. The %Na is computed as:

$$\%Na^{+} = \left(\frac{(Na+K)^{+}}{Ca^{2+}+Mg^{2+}+K^{+}+Na^{+}}\right) \times 100$$

Where, all ionic concentrations are expressed in meq/l. According to [47] in all natural waters %Na is a common parameter to assess its suitability for irrigation purpose as shown in Table 9. If the concentration of Na^{+} is high in irrigation water, Na^{+} gets absorbed by clay particles, displacing Mg^{2+} and Ca^{2+} ions. This exchange process of Na^{+} in water for Ca^{2+} and Mg^{2+} in soil reduces the permeability of soil and eventually results in poor internal drainage of the soil, and such soils are usually hard when dry [48, 49]. The values of %Na of the study area varies from 31% to 42.46% with an average value of 35.68% which fall in good to permissible category, showing that the groundwater of the study area is suitable for irrigation.

Table.9: Classification of groundwater based on %Na (Wilcox, 1955).

Water quality	Sodium %
Excellent	<20
Good	20-40
Permissible	40-60
Doubtful	60-80
Unsuitable	>80

4.2.9 Kelly's Ratio

Kelly's ratio is used for the classification of water for irrigation purposes. A Kelly's index (>1) indicates an excess level of sodium in waters [50]. Therefore, water with a KR (<1) is suitable for irrigation. KR is calculated by using the formulae; where all the ions are expressed in meq/l.

$$\text{Kelly's Ratio} = \frac{Na^{+}}{(Ca^{2+}+Mg^{2+})}$$

The values of the KR in the present study varied between 0.45 and 0.75 with an average value of 0.56 which is <1. Accordingly, the groundwater of the study area is suitable for irrigation.

4.2.10 Ion-Exchange Processes

It is essential to identify the various changes in chemical composition occur in groundwater during its travel in subsurface [51]. This can be done by the computation of

the chloro-alkaline index -1 which is suggested by [52] to indicate ion exchange between the groundwater and its host environment during residence or travel. The value of the index CAI-1, can be positive or negative. If the value is positive then it explains that the exchange of Na^+ and K^+ ions are from water with Mg^{2+} and Ca^{2+} ions of the rocks. And if the index is negative, then it means that there is an exchange Mg^{2+} and Ca^{2+} of water with Na^+ and K^+ ions from rocks, so the exchange is in indirect base indicating chloro-alkaline disequilibrium. The chloro-alkaline index-1 is calculated using the following formulae:

$$\text{Chloro-alkaline index} = \frac{Cl^- - (Na^+ + K^+)}{Cl^-}$$

Whereas, all ionic concentrations are expressed in meq/l.

The chloro-alkaline index -1 is calculated for the groundwater samples of the study area and it has been found that CAI-1 values all are negative, and range from -0.56 to -0.21,with an average value of -0.21 indicating that all the groundwater samples have indirect base-exchange reaction.

V. CONCLUSION

The interpretation of the hydrochemical analysis of Field-C reveals that the groundwater is brackish and exceeding very hard. The sequence of the major ions is in the following order: $Na^+ > Ca^{2+} > Mg^{2+} > K^+$ and $SO_4^{2-} > Cl^- > HCO^-_3$. Alkali earth exceeds alkalis and strong acids exceed weak acids. The dominated hydrochemical facies of groundwater is Ca-Cl and Ca-Mg-Cl genetic water types. The determination of the saturated index indicated that all groundwater samples of the study area were under-saturated with respect to the sulphate minerals, and oversaturated with respect to carbonate minerals. Gibb's plot revealed that the chemical weathering of rock-forming minerals is the dominant process, where there is an interaction between rock chemistry and percolating waters in the subsurface. The irrigation parameters reveal that the groundwater is good and suitable for irrigation and concentrated along the southwestern and central parts of the study area. Meanwhile, the major ions compositions in groundwater indicate that the water is not suitable for drinking purpose.

ACKNOWLEDGMENTS

The authors would like to thank the Ministry of Electricity and Water for providing the chemical analyses of the Al-Shagaya Field-C. In addition, authors are highly appreciated to the distinguished reviewers for their insightful suggestions and comments.

REFERENCES

[1] Al-Ruwaih, F.M. (2001). Hydrochemical investigation on the clastic and carbonate aquifers, Kuwait Bulletin of Engineering Geology and the Environment. vol. 60, 4, 310 –314.

[2] Kumari, B. V., Murthy, Y. L. N., & Machiraju, P. V. S, (2015). Quality Evaluation of soil in Coringa Mangroves region in East Godarari District for Assessement of its Quality for Application. International Journal.of Engineering and Technical Research. ISSN:2321-0869, vol. 3, Issue–5, 461-468.

[3] Chemura, A., Kutywayo, D., Chagwesha, T.M., & Chidoko, P. (2014). An assessment of irrigation water quality and selected parameters at Mutema irrigation scheme, Zimbabwe. Journal of water Resources and protection. vol. 6, 130 – 140.

[4] Yidana, M.S., Sakyi, P.A., & Starmp, G. (2011). Analysis of the salinity suitability of surface water for irrigation purposes: The Southwest and Coastal River System in Ghana. Journal of Water Resources and Protection. vol.3, no. 1, 695 – 710.

[5] Aza-Gnandji, CD R., Xu, Y., Raitt, L., & Levy, J. (2013). Salinity of irrigation water in Phillippi farming area of the Cape Flats, Cape Town, South Africa. Water of South Africa, Vol. 39, no. 2, 199 – 209.

[6] Dastorani, M.T., Heshmati, M., & Sadeghzadeh, M.A. (2010). Evaluation of the efficiency of surface and subsurface irrigation in dryland environments. Irrigation and Drainage. 559, 129 – 137.

[7] Al-Enezi, A. H. (2015). Groundwater quality and its effect on soil in Abdalli area in the state of Kuwait. Kingdom of Bahrain. Arabian Gulf University. College of Graduate Studies M.sc. Thesis, 75pp.

[8] Ramesh, k., & Elango, L. (2012). Groundwater quality and suitability for domestic and agriculture use in Tondiar river basin, Tamil Nadu, India. Environmental Monitoring Assessement. 184, 3887 – 3899.

[9] Lakshmi, J. K., Machiraju, P.V.S., & UdayaBhaskar, P. (2014). Physicochemical and microbial characterization of subsurface waters near a non point source for assessment of quality for end use applications. International Journal of Current Chemical sciences. vol. 3, Issue 1, 4 – 11.

[10] Narany, T. S., Ramli, M. F., Aris, A. Z., Sulaiman, W. N. Z., & Fakharian, K. (2012). Assessment of groundwater quality for irrigation purpose in Amol Plaine, Iran. 6th Internatioal symposium on Advances in Science and Technology, Malaysia, Kuala Lumpur, 21 – 25 March.

[11] Rama Rao, D., Satyanarayana, T., & Machiraju, P. V. S. (2012). Assessment of groundwater quality for application in KaKinada coast. Pelagia Research Library. Der Chemica Sinica. vol. 3, 1, 287-291.

[12] Fuchs, W., Gattinger, T.E., & Holzer, H.F. (1968). Explanatory text to the synoptic geologic map of Kuwait. A surface geology of Kuwait and the Neutral zone. Geological Survey of Austria, Vienn, 87 pp.
[13] Omar, S.S., Al-Yaqubi, A., & Senay, Y. (1981). Stratigraphy of the Kuwait-Basrah area. In: Hibitat of Oil. American Association of Petroleum Geology Bulletin. 22,1252-1278.
[14] Al-Sulaimi, J.S., & Al-Ruwaih, F.M. (2005). Geology and natural resources of Kuwait. Academic Publication Council, Kuwait University, 501 pp.
[15] Al-Ruwaih, F.M., & Ben-Essa, S.A. (2004). Hygrogeological and hydrochemical study of the Al-Shagaya Field-F, Kuwait. Bulletin of Engineering Geology and Environment. 63,57-70.
[16] Al-Ruwaih, F.M., Talebi, L., & Hadi, K.M. (2005). Major geological processes in the evolution of Eocene carbonate aquifer, Kuwait. Kuwait Journal of Science and Engineering. 32, 1,119-144.
[17] Al-Ruwaih, F.M., Al-Hmoud, J. M., & Al-Dhaferri, Z.M. (2012). Characterization and evaluation of the confined limestone aquifer in Kuwait. Journal of the Gulf and Arabian Peninsula Studies. vol.3, no.146, 17-49.
[18] Fetter, W.C. (2001). Applied hydrogeology. 4th ed. Prentice-Hall, Inc. 598pp.
[19] Saether, O.M., & De-Caritat, P. (1997). Geochemical processes, weathering and groundwater recharge in catchments. Balkema, Rotterdam. The Netherlands, 400pp.
[20] Hem, J.D. (1991). Study and interpretation of chemical characteristics of natural Water. (3rd ed). Book 2254: Scientific publ. Jodhpur, India.
[21] Ball, J.W., & Nordstrom, D.K. (1992). Geochemical model to calculate speciation of major, trace and redox elements in natural waters. Report, U.S. Geological Survey, International Groundwater Modeling Center, U.S.A.
[22] Piper, A.M. (1953). A graphical procedure in geochemical interpretation of water analyses. Trans. Am. Geophys. Union . 25, 914-923.
[23] Doneen, L.D. (1961). The influence of crop and soil on percolating water. Proceedings of Groundwater Recharge Conference, California, U.S.A.
[24] Doneen, L.D. (1964). Notes on water quality in agriculture. In Davis, C.A., Water Science and Engineering, University of California.
[25] Gibbs, R.T. (1970). Mechanisms controlling world's water chemistry. Science, vol., 170, 1088-1090.
[26] Plummer, L.N. (1984). Geochemical modeling: A comparison of forward and inverse method. In: Hitchon, B., & Wallick, E.I. (Eds.). Proceeding of First Canadian Conference of Hydrogeology: Partial application of groundwater geochemistry. National Water Well Association, Canada, Worthington, OH, U.S.A.
[27] Deutsch, W.J. (1997). Groundwater geochemistry: fundamentals and applications to contamination. Lewis Publishers, New York, , U.S.A, 221pp.
[28] Appelo, C.A.J., & Postma, D. (2005). Geochemistry, groundwater and pollution. Balkema, Rotterdam, The Netherlands, 649pp.
[29] Datta, P.S., & Tyagi, S.K. (1996). Major ion chemistry of groundwater in Delhi area: chemical weathering processes and groundwater flow regime. J. Geol. Soc. India. 47,179-188.
[30] WHO. (2008). Guidelines for drinking-water quality [Electronic Source]: incorporations, 3rd ed. WHO, Geneva, 515pp.
[31] Sawyer, G.N., & McCarthy, D.L. (1967). Chemistry of sanitary engineers, 2nd ed., Mc Grow Hill, New York, 518pp.
[32] Schroeder, H.A. (1960). Relations between hardness of water and death rate from certain chronic and degenerative diseases in the United States, J. Chron. Disease. 12,586-591.
[33] Todd, D.K., & Mays, L.W. (2005). Groundwater hydrology. John wiley & sons, Inc, 3rd ed., 636 pp.
[34] Raghunath, H.M. (2007). Groundwater. 3rd ed., New age International Publishers, 504pp.
[35] Richards, L.A. (1954). Diagnosis and improvement of saline and alkaline soils. US Department of Agriculture Hand Book, 60pp.
[36] Taqveem, A.K., & Abbasi, A.M. (2013). Synthesis of parameters used to check the suitability of water for irrigation purposes. International J. of Environmental Science, vol.3, no.6 , 2031-2038.
[37] Eaton, E.M. (1950). Significance of carbonate in irrigation water. Soil Sci. 69, 12-133.
[38] Sundaray, S.K., Nayak, B.B. , & Bhatta, D. (2009). Environmental studies on river waters quality with reference to suitability for agricultural purposes: Mahanadi river esturarine system, India- a case study, Environmental Monitoring and Assessment, 155, 227-243.
[39] Satyanarayanan, M., Balaram, V., Al Hussin, M. S., Al Jemaili, M. A., Rao, T.G., Mathur, R., Dasaram, B., & Ramesh, S.L. (2007). Assessment of groundwater quality in structurally deformed granitic terrain in Hyderabad, India.. Environmental Monitoring and Assessment, 113, 117-127.
[40] Gupta, S.K. , & Gupta, I.C. (1987). Management of saline soils and water. Oxford and IBH Publication Coy, New Delhi, India, 399pp.

[41] Oladeji, O.S., Adewoye, A.O., & Adegbola, A.A. (2012). Suitability assessment of groundwater resources for irrigation around Otte Village, Kwara State, Nigeria. Int. J. of Applied Sciences and Engineering Research, Vol. 1, Issue 3, 434-445.
[42] Chandu, S. N., Subbarao, N.V., & Prakash, S. R. (1995). Suitability of groundwater for domestic and irrigation purposes in some part of Jhansi District, U. P. Bhujal Newa, 10, 1, 12-17.
[43] El-Nakhal, H.A., & Ashour, M.M. (1986). Water quality of the area west of Karak City, Jordan. Qatar Univ. Sci. Bull, 6, 273-285.
[44] Chhabra, R. (1996). Soil salinity and water quality. A.A. Balkema Publishers, U.S.A. 284pp.
[45] Kanwar, J.S., & Kanwar, B.S. (1969). Quality of irrigation waters. Trans. 9th Intern. Congr. Soil Sci.1, 391-403.
[46] Paliwal, K.V. (1972). Irrigation with saline water. Monogram No.2 (new series). I A R I, New Delhi, 198pp.
[47] Wilcox, L.V. (1955). Classification and use of irrigation waters. US Department of Agriculture, Washington. D.C, 19pp.
[48] Collins, R., & Jenkins, A. (1996). The impact of agricultural land use on stream chemistry in the middle hills of the Himalayas, Nepal. J. Hydrology, 185,71-86.
[49] Saleh, A., Al-Ruwaih, F. M., & Shehata, M. (1999). Hydrogeochemical processes operating within the main aquifers of Kuwait. J. Arid Environments, 42,195-209.
[50] Kelly, W.P. (1951). Alkali soils- their formation properties and reclamation. 3rd ed. Reinhold publication, New York, U.S.A, 92pp.
[51] Al-Ahmdi, M. E. (2013). Hydrochemical characterization of groundwater in wadi Sayyah, Western Saudi Arabia . Applied Water Science. 3, 721-732.
[52] Schoeller, H .(1977). Geochemistry of groundwater. In groundwater Studies- An international guide for research and practice (ch. 15, p.p. 1-18) .Paris : UNESCO.

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

The Suitability of Groundwater for Domestic and Irrigation Purposes: A Case Study of Ikere-Ekiti, SW-Nigeria

Talabi A.O.

Department of Geology, Ekiti State University, P. M. B. 5363, Ado-Ekiti, Nigeria.

Abstract**—** *Shallow groundwater in Ikere-Ekiti was assessed for potability and irrigation employing chemical and bacterial analyses. Twenty two groundwater samples were collected and analyzed using Atomic absorption spectrometer for cations and ion chromatographic method for anions determinations (˚C), pH and electrical conductivity (EC) (µS/cm) were measured in the field using pH Testr meter. The bacteriological analysis was carried out using nutrient agar medium to obtain plate count of living bacteria. Results of the analysis revealed that all EC values were less than 1000µS/cm indicating fresh water. The pH with average values of 9.48, 7.82 and 7.44 in migmatite, granite and charnockitic terrains respectively exceeded the approved standard (6.5 – 8.5) for drinking water in two samples from migmatite, one sample from granitic terrain and none from the charnockites. Sodium was the dominant cation with average concentrations (mg/L) of 95.65, 38.33 and 6.61 in migmatite, granite and charnockite respectively while* K^+ *ions in the same order of rock units have average concentrations (mg/L) of 60.49, 32.33 and 15.77. The average concentrations (mg/L) of* Ca^{2+} *ions in groundwater located on migmatite, granite and charnockitic terrains were 36.67, 24.63 and 10.98 respectively while those for* Mg^{2+} *were 9.94, 7.48 and 4.57. The order of cation abundance was* $Na^+ > K^+ > Ca^{2+} > Mg^{2+}$*. In respect of the major anions,* Cl^- *was dominant with average concentrations (mg/L) in charnockites (187.20) within approved standard of 250mg/L while the average values (mg/L) in migmatite (475.2) and granite (340.62) exceeded the standard value. Following the same sequence of rock units,* HCO_3^- *average concentrations (mg/L) were 34.6mg/L, 27.07mg/L and 25.7. Sulphate and nitrate were less dominant ions and the order of anions abundance in the groundwater was* $Cl^- > HCO_3^- > SO_4^{2-} > NO_3^-$*. Bacteria evaluation revealed that all sampled groundwater tested positive to bacteria with TBC values (CFU/100ml) ranging from* $1.76X10^8$ *to* $1.78X10^9$ *in migmatite,* $5.3x10^5$ *to* $8.9x10^8$ *in granite and* $2.55x10^7$ *to* $8.2x10^8$ *in charnockite. Gibb's diagram revealed that chemical weathering of rock-forming minerals has contributed to solute source in the groundwater of the area. Water type on migmatite was mainly NaCl while granite and charnockite had NaCl and CaCl types revealing lithologic effects. Irrigation water quality assessment employing Sodium absorption ratio (SAR), Soluble sodium percent (SSP), Residual sodium bicarbonate (RSBC) and Permeability index (PI) revealed that the groundwater is suitable for irrigation purpose. Groundwater in the study area is low mineralized, chemically potable, suitable for irrigation but infected by bacteria pollutants. Differences in rock types affected the chemistry of the groundwater as reflected in their physico-chemical compositions, water facies and irrigation quality.*

Keywords**— Rock units, groundwater, potable, bacteria, irrigation.**

I. INTRODUCTION

All over the world, population surge, industrialization and rising standards of living have put water demand on the rise; though without corresponding increase in the required quantity of the resource (Ali 2012). Records of population in Nigeria revealed that the population of the study area (Ikere-Ekiti) was 59,257 in 1963, 114,780 in 1991 and 147,355 in 2006 (NPC 2006). The population of the area will continue to increase considering the fact that the study area is the gateway to Ekiti-State and its nearness to Ado-Ekiti, the state capital has resulted into human migration into the town. With increasing population and reduction in surface water supply during the dry season and contamination by floods during the rainy season, the increase in demand for domestic water can only be met through digging of localized shallow wells that tap the small discrete bodies of groundwater present in the weathered zone of basement terrains of the area. In addition to the above crucial factors, the rock types in an area, particularly the thickness of their weathered products/fracture characteristics and rainfall contribute greatly to the chemistry of its groundwater. This in essence determines groundwater suitability for domestic, agricultural and industrial application. As groundwater migrates, it reacts with the minerals that

make up the host-rocks. These mineral may be soluble and percolate into the groundwater system thereby altering the exisisting geochemical characteristics of the groundwater. It is obvious that groundwater can be contaminated through natural processes such as chemical weathering and dissolution (Abimbola et al. 2002; Amadi et al. 2015). Thus, water- rock interactions alter greatly the chemistry of groundwater apart from contributions from anthropogenic contaminants. Groundwater is generally preferred to surface water due to natural protection from pathogenic contamination and buffer against climatic variability. At Ikere-Ekiti, access to groundwater provides the only realistic option for a sustainable safe drinking water supply. The town has sizable numbers of boreholes and wells which if properly managed will serve as recipe to safe drinking water.

Specific publications on the study area are few. Odeyemi et al. (2011) worked on Bacteriological, Physicochemical and Mineral Studies of Water Samples from Artesian bore-hole, spring and Hand dug well located at Oke-Osun of the study area and concluded that the groundwater was contaminated by bacteria. Aturamu (2012) also concluded that the groundwater at Ikere-Ekiti was contaminated bacteriologically. Similar researches in other parts of Ekiti-State (Omotoyinbo 2007; Ayodele and Aturamu 2011) as well as the work of Talabi and Ogundana (2014) covering the whole state also revealed bacterial contamination of groundwater.

Groundwater regime is dynamic and possible amelioration of bacterial contamination of groundwater in the area cannot be ruled out especially with recent health education in the state. However, according to World Health Organization (2004) about 85% of communicable diseases are water borne or water related. The quality of groundwater in an area is a function of its chemistry and the nature of the aquifer characteristics (Amadi et al. 2015). Groundwater quality appraisal is gaining importance, due to intense urbanization, industrialization and agricultural activities putting the soil and groundwater to greater risk of contamination (Sayyed and Wagh, 2011; Tiwari 2011). Water pollution also threats human health, economic development and social prosperity (Milovanovic 2007).

This study was tailored towards assessing the suitability of groundwater at Ikere-Ekiti for domestic and irrigation purposes. The research attempted deciphering the effects of rock units on the chemistry of the groundwater and discussed potential adverse chemical/health effects of the groundwater on domestic uses and irrigation.

II. LOCATION, GEOLOGY AND HYDROGEOLOGY OF THE STUDY AREA

Ikere-Ekiti is situated in the southern part of Ekiti-State, southwestern Nigeria between latitudes 7° 29′ and 7° 31′N and longitudes 5° 12′ and 5° 14′E covering a total area of 346.5 km^2. It is a town endowed with magnificent hills, including Orole and Olosunta. The town is the gateway to Ekiti State, located between Ado-Ekiti (the capital of Ekiti State) and Akure (the capital of Ondo State) (Fig. 1). The town is situated in the humid tropical region and rugged basement terrain that is generally 250m above sea level. The mean annual rainfall is 1500mm while the annual temperature ranged from 23 - 28°C with mean annual relative humidity of 75%.

Geologically, Ikere-Ekiti is underlain by crystalline igneous and metamorphic rocks of the Precambrian basement complex. The area is made up of migmatite-gneiss quartzite complex, charnockites and Older granites. The Precambrian basement complex was affected by the Pan-African orogeny (600Ma±150Ma) thereby occupying the re-activated region which resulted from the plate collision between the passive continental margin of the West African Craton and the active Pheurasian continental margin (Burke and Dewey 1972; Dada 2006).

The migmatite-gneiss complex is the most widespread and abundant rock type in the Basement Complex into which other successions of rocks have been emplaced (McCurr 1973; Rahaman 1988).

The Older Granites and charnockites occur as intrusive bodies of various dimensions in the pre-existing basement rocks, that is, the migmatite-gneiss units and the schist belts. One striking feature of the older granites is their occurrence as picturesque inselbergs and such prominent hills rising sharply above their surrounding plains in the study area include Olsunta and Orole hills. The charnockitic rocks outcropped as oval or semi-circular hills of between five and ten meters (10m) high with a lot of boulders at some outcrops. Most of the charnockitic rocks in the study area occurr along the margins of Older Granites bodies especially the porphyritic granites. Differential weathering occurs on each rock unit due to difference in mineralogical and chemical composition and consequently, groundwater occurrence is localized and these variations may result into differences in groundwater chemistry based on rock units.

The major surface water in the study area is river Osun rising from the hills at the western end of the area with highest topographical point of 598m above main sea level. River Owururu is a major tributary which along with other tributaries/streams meander through intersecting valleys. The volume of water in the streams depends on the response to wet and dry seasons. During the rainy season, there is a great increase in water volume in the major rivers while there is hardly water in some of the streams during the dry season. Rainfall is the dominant factor that determines the occurrences of groundwater. Rainy season, in the area is characterized

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

with high amount of uniform rainfall with the pick in August and the lowest in November. Differential weathering occurs on each rock unit due to difference in mineralogical and chemical composition and consequently, groundwater occurrence is localized which may result into differences in groundwater chemistry based on rock units.

III. METHODOLOGY

The sampling of groundwater in this research was based on the three major rock units (migmatite, granite and charnockite) in the study area. Prior to groundwater sampling, reconnaissance survey of the study area was carried out to decide on the number of samples per rock unit and the number was based on the spread of a specific rock outcrop in the area. Granite predominates with 13 water samples, followed by charnockites with 5 samples while migmatite has the least with only 4 samples. Three set of groundwater samples put in one liter pre-washed polyethylene bottles) were obtained per location following standard sampling procedure (Stednick 1991).

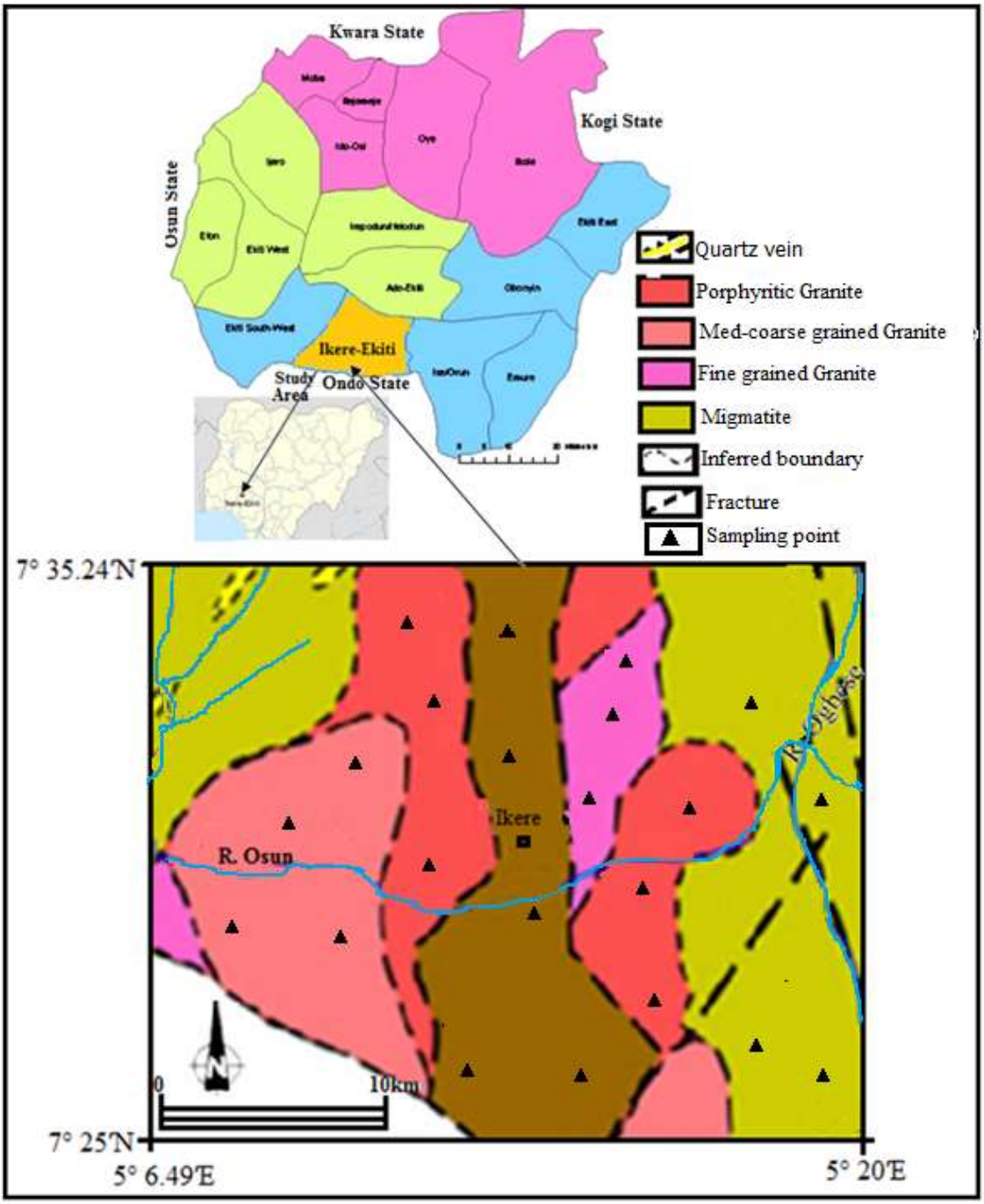


Fig.1: Location and Geology of the of Study Area

The three set of samples were for cations, anions and bacterial analyses respectively. Water samples for cations

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

determination were acidified to a pH<4 using concentrated Nitric acid and all samples were preserved (refrigerated) prior to analyses.

Temperature, pH and EC of water samples were measured in-situ employing portable pH Testr meter. In addition, water level and depth of sampled wells were measured using dip-meter. TDS was estimated in this research employing the relationship that:

$$TDS = EC * 0.75. \quad (1)$$

While total hardness (TH) was calculated using the relation:

$$TH = 2.5Ca^{2+} + 4.1Mg^{2+} \text{ (Fournier, 1981)} \quad (2)$$

In this research all laboratory analyses were carried out at Fatlab Nigeria Company limited, Ibadan Nigeria. Ions analyses were carried out using Atomic absorption spectrometer for cations and ion chromatographic method for anions determinations.

The basic criterion by which the sanitary quality of water may be judged is the kind and number of bacteria present in it. The presence of the coliform group of bacteria in water is accepted traditionally as an indication of pathogenic content particularly Escheria coli which are normal inhabitants of the large intestine of human begins and other animals and are consequently present in faeces. The samples were analyzed for bacteria count employing nutrient organ medium to obtain plate count of living bacteria.

Furthermore, the data from the hydrochemical analysis were subjected to evaluation for irrigation purpose employing sodium adsorption ratio (SAR) (Richard 1954), soluble sodium percentage (SSP) (Todd 1980), residual sodium bicarbonate (RSBC) (Gupta 1983), Kelly's ratio (KR) (Kelly 1963), permeability index (PI) (Doneen 1964) and magnesium adsorption ratio (MR) (Raghunath 1987). The irrigation parameters in this study were estimated employing:

$$SAR = \frac{Na^+}{\sqrt{(Ca^{2+} + Mg^{2+})/2}} \quad (3)$$

$$SSP = \frac{Na^+ + K^+}{Ca^{2+} + Mg^{2+} + Na^+ + K^+} \quad (4)$$

$$RSBC = HCO_3^- - Ca^{2+} \quad (5)$$

$$KR = \frac{Na^+}{Ca^{2+} + Mg^{2+}} \quad (6)$$

$$PI = \frac{Na^+ + \sqrt{HCO_3^-} \times 100}{Ca^{2+} + Mg^{2+} + Na^+} \quad (7)$$

$$MAR = \frac{Mg^{2+} \times 100}{Ca^{2+} + Mg^{2+}}$$

Furthermore, the sodium in irrigation waters denoted as per cent sodium was determined using the following formula (Wilcox 1995);

$$\% Na = (Na^+) \times 100 / (Ca^{2+} + Mg^{2+} + Na^+ + K^+) \quad (8)$$

where the quantities of Ca^{2+}, Mg^{2+}, Na^+ and K^+ are expressed in milliequivalents per litre (epm). Data obtained from the analysis were subjected to statistical evaluation employing Microsoft excel software.

IV. RESULTS AND DISCUSSION

The results of the physical parameters of sampled groundwater from the study area are presented in Table 1 while those for the chemical concentrations are in Table 2. Wells depths revealed average values of 6.03m, 7.35m and 5.5m in wells located on migmatite, granite and charnockite respectively. The depth values showed that all the wells are shallow and the depth is a reflection of the degree of weathering in the study area. The physical parameters (EC, TDS and TH) have low values that are within WHO (2004) approved standard for drinking water. The pH values signified alkaline water. The pH concentrations were greater than 7 in all the groundwater samples and exceeded the approved WHO standard of 6.5 – 8.5 in two samples from migmatite. Only one sample from granitic terrain exceeded the standard value of WHO while all samples from charnockite fell within the value. In similar trends, all measured chemical parameters have concentrations within WHO (2004) approved standard. Water in the area is chemically potable. EC (µS/cm) on migmatite gneiss, granite and charnockite ranged from 598 – 650, 83 – 998 and 76 – 347 while TH (mg/L) on the same rock units was from 111 – 256, 33 – 268 and 16 – 87 respectively. These trends clearly revealed that rock units affected the chemistry of groundwater in the study area. Migmatite gneiss appeared to have more dissolved constituents with an average EC value of 629.5 (µS/cm) while this was followed by granite (av. 383.62 µS/cm) and charnockite (195.20 µS/cm) respectively (Fig.2A). The relatively high value of dissolved substances in migmatite gneiss reflects the mixed nature of the rock. All EC and TDS values irrespective of rock units were less than 1000 µS/cm and 500 mg/L. Water can be classified into fresh (TDS <1,000 mg/ L), brackish (TDS>1,000 mg/ L), saline (TDS>10,000 mg/ L) and brine (TDS>100,000 mg /L) categories on the basis of TDS concentrations (Freeze and Cherry, 1979). Based on this classification, the groundwater of the study area belongs to fresh water.

The total hardness (TH) represents the properties of water that prevents the lather formation with soap and causes increase in the boiling point of water. Water hardness is caused primarily by the presence of cations such as

calcium and magnesium and anions such as carbonate, bicarbonate, chloride and sulfate in water. Hard water is not suitable for domestic purpose. Water hardness has no known adverse effects; however, some evidence indicates its role in heart disease (Schroeder 1960). McGowan (2000) indicated that water containing calcium carbonate at concentrations below 60 mg/L is generally considered as soft; moderately hard (60–120 mg/L), hard (120–180 mg/L) and very hard (>180 mg/L). Thus groundwater on migmatite gneiss was in the moderately hard to vey hard category while the groundwater from granite and charnockite fell into soft to very hard and soft to moderately hard classes respectively. Sixty percent (60%) of groundwater from charnockitic terrain fell into the soft water category while the remaining 40% were in moderately hard class.

Table.1: Physical parameters of groundwater from the study area.

Code	**Rock type**	**EC (µS/cm)**	**TDS (mg/L)**	**Temp (°C)**	**pH**	**TH (mg/L)**	**Water**	**Depth (m)**
						$CaCO_3$	level (m)	
ID 1	Mig. gneiss	640	480	25.7	9.7	154	2.75	6.2
ID 2	Mig. gneiss	598	448.5	26.4	9.6	111	3.3	5.1
ID3	Mig. gneiss	630	473	25.8	9.2	256	4.2	5
ID4	Mig. gneiss	650	488	26.2	9.4	219	2.6	7.8
	Min	598	448.5	25.7	9.2	111	2.6	5
	Max	650	488	26.4	9.7	256	4.2	7.8
	Mean	629.5	472.38	26.03	9.48	185	3.21	6.03
	Stdev	22.53	17.06	0.33	0.22	65.03	0.72	1.3
1D5	Granite	84	63	27.6	8.2	154	6.3	9.9
ID 6	Granite	315	236.25	25.9	7.8	111	10.95	11.3
ID 7	Granite	106	79.5	27.1	7.3	256	6.45	7
ID 8	Granite	180	135	26.4	7.3	219	10.1	14.2
ID 9	Granite	83	62.25	26.1	7.3	111	5.2	6.2
ID 10	Granite	261	195.75	28.4	7.2	154	4.5	10.9
ID 11	Granite	998	748.5	27.8	8.2	268	2.9	5.9
ID 12	Granite	817	612.75	27.4	7.3	172	7.7	8.3
ID 13	Granite	876	657	27.4	8.6	155	1.45	2.65
ID 14	Granite	187	140.25	28.1	8.3	33	4.3	5.25
ID 15	Granite	285	213.75	28.6	7.9	101	4.2	4.7
ID 16	Granite	621	465.75	28.7	7.8	147	2.7	6.6
ID 17	Granite	174	130.5	27.7	8.5	56	2.1	2.6
	Min	83	62.25	25.9	7.2	33	1.45	2.6
	Max	998	748.5	28.7	8.6	268	10.95	14.2
	Mean	383.62	287.71	27.48	7.82	149	5.3	7.35
	Stdev	325.94	244.45	0.91	0.51	69.69	2.94	3.45
ID 18	Charnockite	283	212.25	26.6	8.1	74	3.2	4
ID 19	Charnockite	347	260.25	26.7	7.5	87	6.9	7
ID 20	Charnockite	134	100.5	26.9	7	18	6	6.5
ID 21	Charnockite	136	102	26.6	7.6	37	2.5	5.3
ID 22	Charnockite	76	57	27.4	7	16	3.58	4.7
	Min	76	57	26.6	7	16	2.5	4
	Max	347	260.25	27.4	8.1	87	6.9	7
	Mean	195.2	146.4	26.84	7.44	46	4.44	5.5
	Stdev	114.25	85.69	0.34	0.46	32.57	1.91	1.24
	WHO (2004)	1500	1000	-	6.5 – 8.5	500	-	-

http://dx.doi.org/10.22161/ijeab/2.1.23 ISSN: 2456-1878

Table.2: Chemical parameters of groundwater from the study area.

Code	Ca2+ (mg/L)	Mg2+ (mg/L)	K+ (mg/L)	Na+ (mg/L)	Cu (mg/L)	NO_3 (mg/L)	SO_4^{2-} (mg/L)	HCO_3^- (mg/L)	Cl^- (mg/L)	TBC (CFU/100ml)
Migmatite										
ID1	43.21	11.23	62.53	97.84	0.1	1.86	2.8	36.6	475.2	$1.78X10^9$
ID2	30.12	8.65	58.45	93.45	0.02	0.71	2	32.6	475.2	$1.4X10^9$
ID3	67.6	21.2	64.4	98.8	0.06	1.6	3.2	34.6	485.2	$1.62X10^9$
ID4	58.6	17.8	60.4	94.8	0.01	2.8	2.8	36.2	464.5	$1.76X10^8$
Min	30.12	8.65	58.45	93.45	0.02	0.71	2	32.6	475.2	$1.76X10^8$
Max	43.21	11.23	62.53	97.84	0.1	1.86	2.8	36.6	475.2	$1.78X10^9$
Mean	36.67	9.94	60.49	95.65	0.06	1.28	2.4	34.6	475.2	$1.03x10^9$
Stdev	9.26	1.82	2.88	3.1	0.05	0.81	0.57	2.83	0	$7.92x10^8$
Granite										
ID5	6.54	1.13	2.2	3.01	0.01	0.05	0.25	28.5	122.4	$1.56X10^8$
ID6	37.65	1.57	31.12	7.42	0.02	1.77	0.17	24.4	302.4	$1.46X10^8$
ID7	2.3	3.11	10.01	2.34	0.05	0.59	0.68	18.3	100.8	$1.08X10^8$
ID8	6.78	1.78	23.65	9.7	0.07	0.13	0.03	20.3	158.4	$2.56X10^8$
ID9	2.97	0.86	5.04	2.31	0.06	0.15	0.06	19.4	122.4	$2.56X10^8$
ID10	23.14	8.12	16.35	4.22	0.05	1.94	0.05	21.2	216	$2.77X10^8$
ID11	79.84	16.63	61.12	172.53	0.12	5.37	5.33	42.7	734.4	$8.9X10^8$
ID12	40.4	17.36	56.57	137.06	0.02	10.76	1.54	12.2	626.4	$8.8X10^8$
ID13	37.43	14.97	82.35	100.57	0.03	1.53	1.82	61	655.2	$5.4X10^6$
ID14	5.56	4.78	15.43	8.01	0.04	2.1	1.38	24.4	180	$6.2X10^7$
ID15	24.56	9.64	38.65	14.96	0.06	0.52	0.94	27.7	374.4	$2.27X10^8$
ID16	39.78	11.66	68.42	31.23	0.13	5.12	1.43	26.4	619.2	$5.3X10^5$
ID17	13.25	5.68	9.43	4.98	0.06	0.26	0.48	25.4	216	$6.9X10^7$
Min	2.3	0.86	2.2	2.31	0.02	0.05	0.03	12.2	100.8	$5.3x10^5$
Max	79.84	17.36	82.35	172.53	0.13	10.76	5.33	61	734.4	$8.9x10^8$
Mean	24.63	7.48	32.33	38.33	0.06	2.33	1.09	27.07	340.62	$2.56x10^8$
Stdev	22.3	6.06	26.7	58.48	0.36	3.09	1.42	12.43	234.29	$2.94x10^8$
Charnockite										
ID18	16.57	7.83	28.25	3.63	0.23	1.364	2.35	30.2	295.2	$8.2X10^8$
ID19	21.33	8.22	24.63	23.64	0	1.855	3.79	31.8	216	$8.09X10^7$
ID20	4.66	1.45	14.32	2.92	0.03	2.263	0.08	24.4	194.4	$1.40X10^8$
ID21	8.35	3.96	7.92	1.75	0.03	0.05	1.36	18.3	122.4	$3.6X10^7$
ID22	3.97	1.41	3.75	1.1	0.04	0.241	0.04	23.8	108	$2.55X10^7$
Min	3.97	1.41	3.75	1.1	0	0.05	0.04	18.3	108	$2.55x10^7$
Max	21.33	8.22	28.25	23.64	0.23	2.263	3.79	31.8	295.2	$8.2x10^8$
Mean	10.98	4.57	15.77	6.61	0.07	1.15	1.52	25.7	187.2	$2.2x10^8$
Stdev	7.65	3.32	10.52	9.57	0.09	0.98	1.59	5.42	75.86	$3.38x10^8$
WHO 2004	200	-	200	200	1.00	50.00	250.00	240.00	250.00	0.00

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

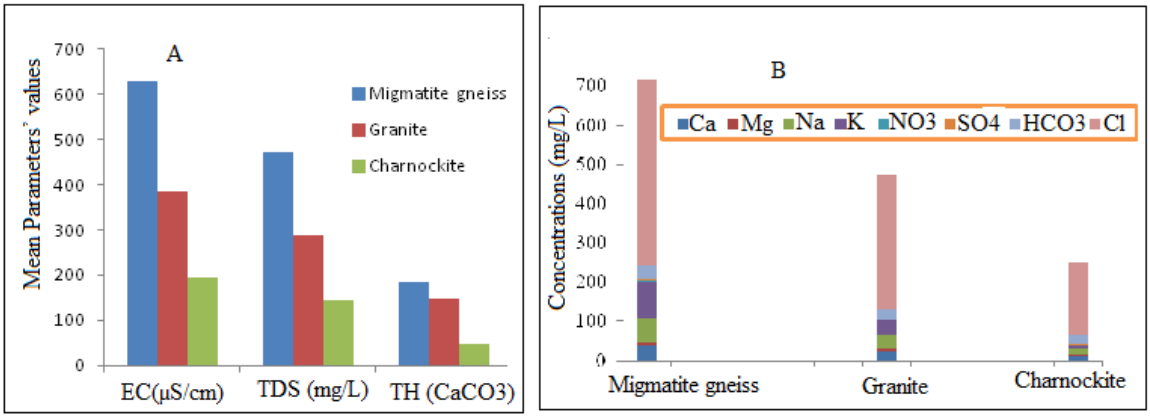


Fig.2: Variations in the concentrations of physical parameters from the study area

Effects of rock units on the chemistry of groundwater in the area were further exemplified with 50% of groundwater from migmatite gneiss terrain in the very hard category while moderately hard and hard categories each had 25% representation (Table 1). The chemical parameters (Table 2) revealed generally low chemical values that fell within approved WHO (2004) standard. Among major cations, Na^+ was the dominant ions with an average values (mg/L) of 95.65, 38.33 and 6.61 in migmatite gneiss, granite and charnockite respectively. Following the same order of rock units, this was closely followed by K^+ ions having average concentrations (mg/L) of 60.49, 32.33 and 15.77. Ca^{2+} ions have appreciable concentrations compared with Mg^{2+} ions.

The average concentrations (mg/L) of Ca^{2+} ions in groundwater located on migmatite, granite and charnockitic terrains were 36.67, 24.63 and 10.98 respectively while those for Mg^{2+} were 9.94, 7.48 and 4.57. The order of cations abundance was $Na^+ > K^+ > Ca^{2+} > Mg^{2+}$ (Fig.2B). Among the major anions, Cl^- was generally dominant with average concentration of 475.2mg/L in migmatite, 340.62mg/L in granite and 187.20mg/L charnockite. The second dominant anion was HCO_3^-. Its concentrations (migmatite (av. 34.6mg/L), granite (av.27.07mg/L) and charnockite (av. 25.7mg/L)) clearly showed that rock units have significant influence on the chemistry of groundwater. Sulphate and nitrate were less dominant ions and the order of anions abundance in the groundwater was $Cl^- > HCO_3^- > SO_4^{2-} > NO_3^-$. The chemical concentrations of ions in the groundwater of the study area indicated soft mineralized water that is chemically potable except in few locations where Cl^- exceeded the approved WHO (2004) standard. However, the results (Table 2) revealed that the groundwater was contaminated by bacterial as all sampled groundwater tested positive to bacteria with e-coli values (CFU/100ml) ranging from $1.76X10^8$ to $1.78X10^9$ in migmatite, $5.3x10^5$ to $8.9x10^8$ in granite and $2.55x10^7$ to $8.2x10^8$ in charnockite.

4.1 Characterization of groundwater from the study area

Variations in the concentrations of the different hydrogeochemical constituents dissolved in groundwater determine its usefulness for domestic, industrial and agricultural purposes (Obiefuna and Sheriff 2011). In order to gain better insight into hydrochemical processes of groundwater chemistry in the study area, Gibbs's diagrams representing the ratios of $Na^+ + K^+ / (Na^+ + Ca^{2+})$ and $Cl^- / (Cl^- + HCO_3^-)$ as a function of TDS was employed (Sivasubramanian et al. 2013). Gibbs's diagrams are widely used to assess the functional sources of dissolved chemical constituents, such as precipitation-dominance, rock-dominance and evaporation-dominance (Gibbs 1970). The chemical data of groundwater in this study were plotted in Gibbs's diagrams (Fig. 3). The distribution of sample points revealed that the chemical weathering of rock-forming minerals have influenced the groundwater quality. Furthermore, rock units have no significant influence on the Gibb's Diagrams as virtually all groundwater samples irrespective of rock type plotted in the rock dominance portion of the diagrams. Furthermore, to buttress the assertion that ions in the groundwater of the study area were derived from rock weathering, few bivariate plots of (a) $Ca^{2+} + Mg^{2+}$ vs HCO_3^-, (b) $Ca^{2+} + Mg^{2+}$ vs $HCO_3^- + SO_4^{2-}$, (c) $Ca^{2+} + Mg^{2+}$ vs total cation, (d) $Na^+ + K^+$ vs. Cl^-, (e) Na^+ vs Cl^- and (f) $Na^+ + K^+$ vs total cation were made as presented in Fig. 4. From Fig. 4, it is clear that rock units have effects on the groundwater chemistry of the study area. For example, in Figs 4a and 4b, the data points fell mostly away from the equiline. However, Fig. 4b has a pecularity in which all samples from the granitic terrain fell below the 1:1 line. In addition, all samples from migmatite were above the 1:1 line in Figs.4b and 4d. Fig. 4a signified that the data point irrespective of rock units fell away from equiline 1:1 to 2:1 and 1:2.

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

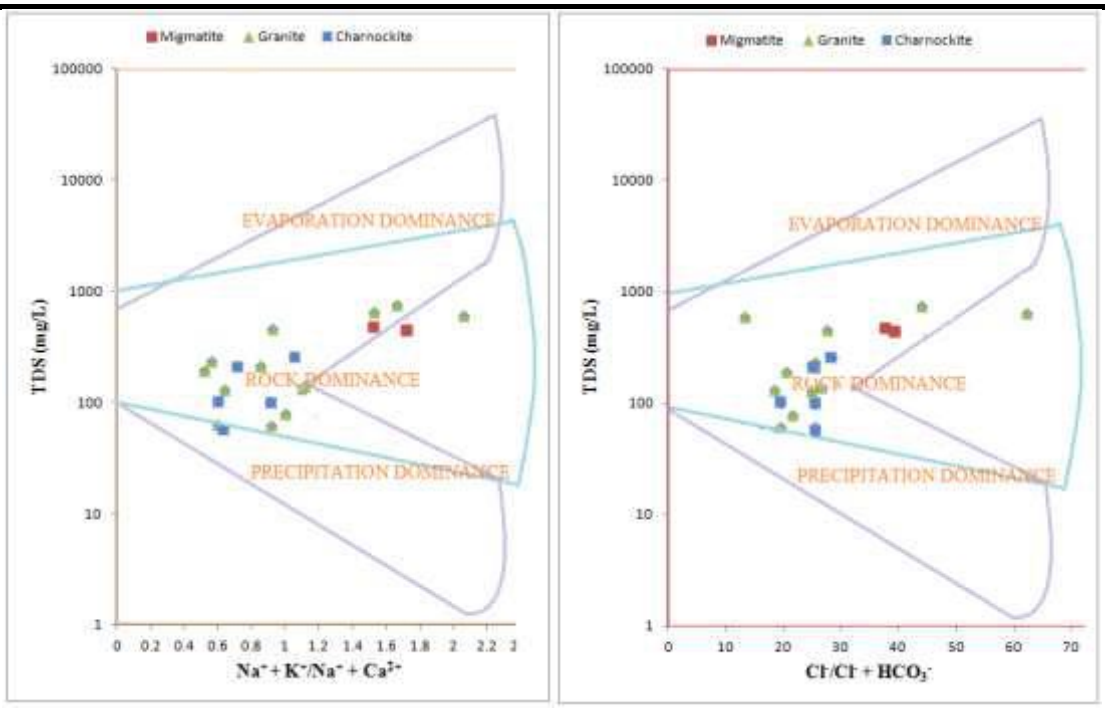


Fig.3: Gibb,s Diagrams of groundwater samples from the study area.

Sixty four percent (64%) of the groundwater samples fell below the equiline indicating predominance of bicarbonate zone due to the reaction of the feldspar minerals with carbonic acid in the presence of water, which releases HCO_3^-. The remaining 36% of the groundwater samples fell above the equiline indicating silicate weathering by alkali earth (Elango et al. 2003). In figs. 4d and 4e most data fell above the equiline indicating weathering process of both alkali and alkali earth from feldspars (Jeelani and Shah 2006). In addition contribution of ions to groundwater of the study area could be from alkali/saline soil and reaction process (cation exchange) irrespective of rock units as exemplified in Fig. 4f.

Piper-Hill diagram is used to infer hydro geochemical facies (Piper 1953). Chemical data of samples from the study area were plotted on a Piper-Tri-linear diagram (Fig.5). The diagram revealed the analogous, dissimilarities and different types of waters in the study area which include NaCl water type (dominant, 72%), CaCl water type (23.5%) and mixed CaMgCl type (4.5%). Water could be categorized into distinct zones depending on the dominant ions. This concept of hydrochemical facies came up in order to understand and identify the water composition in different classes (Back 1966). Facies represent recognizable parts of different characters belonging to any genetically related system. Hydrochemical facies are zones with distinct ions concentrations. Hydrochemical properties of groundwater vary with lithology, modalities and time tracking in the different aquifers. Effects of rock units were manifested as indicated in the Piper diagram (Fig. 5) as all water samples from migmatite terrain fell into the NaCl water type whereas those samples from granitic and charnockitic rocks fell mainly in the two major water facies (NaCl and CaCl) of the area.

Main ionic constituents of groundwater (SO_4, HCO_3, Cl, Mg, Ca, Na and K) in the study area in milli equivalents per liter of solution (meq/L) were plotted on a Schoeller diagram (Schoeller, 1965). The Schoeller diagram (Fig. 6) represents a semi-logarithmic diagram of the concentrations of the groundwater samples of the study area. Concentrations of each ion in each sample are represented by points on six equally spaced lines and points are connected by a line. The diagram in this study supporting the Piper diagram revealed Na and Cl as dominant cation and anion respectively (Fig. 6).

4.2. Bacteriological Evaluation of groundwater of the study area

Pollution of groundwater occurs when contaminants are discharged to, deposited on, or leached from the land surface above the groundwater. Ground water contaminated with bacteria, chemicals, pesticides, gasoline or oil can result in various human health problems, ecological imbalance etc. Specifically total bacteria counts of all groundwater samples from the study area were carried out to unveil the presence or otherwise of bacteria pollutants in the water. The results of the bacteriological analysis (Table 1) suggested that all the groundwater samples have been contaminated due to human activities and closeness to pit latrines/soak away and other domestic refuse dumps.

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

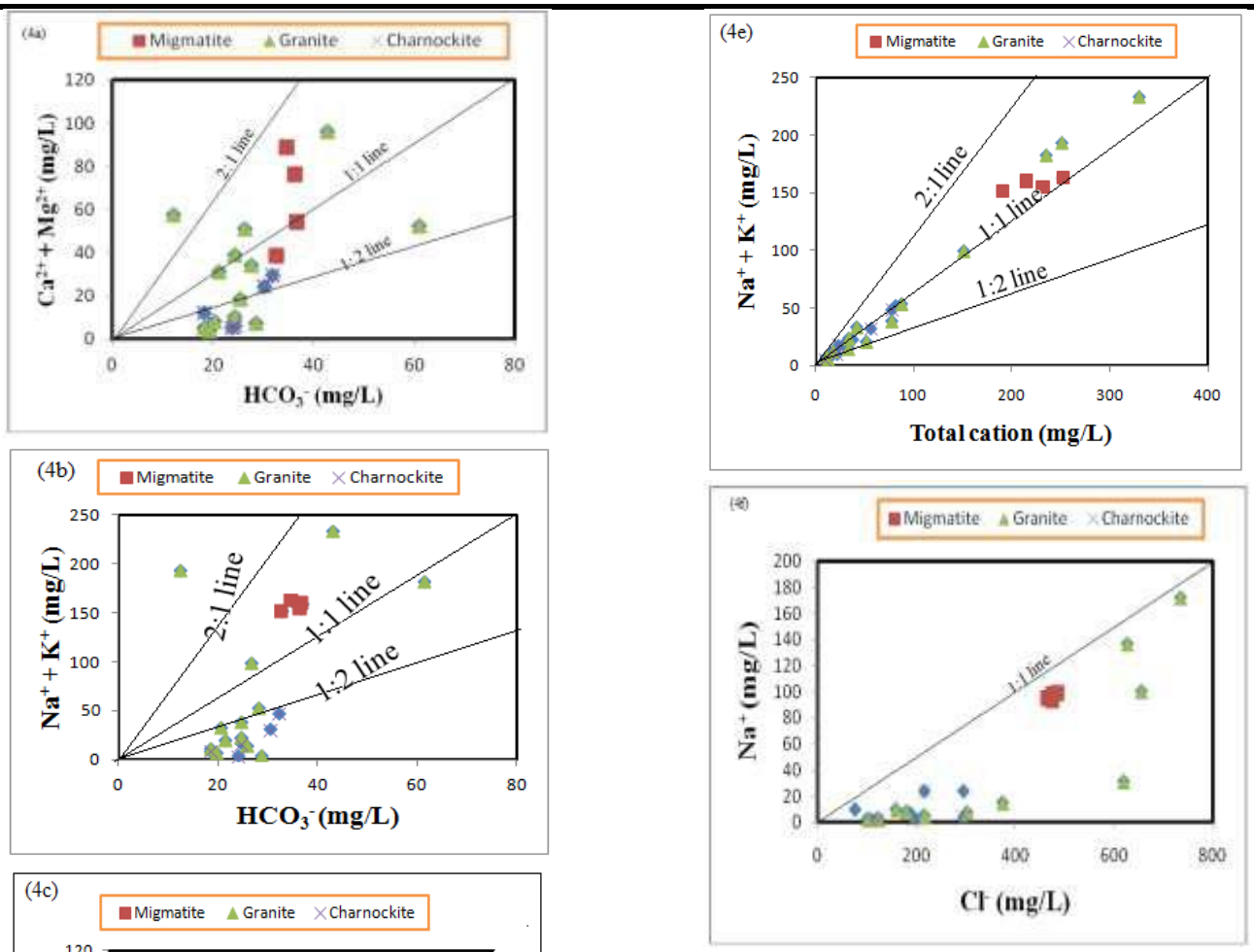


Fig.4: Bivariate plots of chemical data of groundwater from the study area

Generally, the surfaces of most rocks (migmatite, granite and charnockite) except the inselbergs were littered with human and animal faeces and dungs respectively. Obviously wash off from the faeces and dungs have been leached into the groundwater system of the area thereby contaminating it. In addition pit latrines are common in the study area and leakages through septic tanks constituted part of the sources of pollutants to the groundwater. Both the NO_3^- and Cl^- concentrations have link with the surface materials (animals' dungs, human faeces and waste dumps) as indicated in the bivariate plots in Fig. 7A with positive correlation (r = 0.45) of TBC vs Cl^- while the low positive correlation (r = 0.22) was recorded in TBC vs NO_3-. Both the correlation values of TBC vs Cl^- and TBC vs NO_3^- are low but yet signified that the TBC and NO_3^- as well as Cl^- have some common source.

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

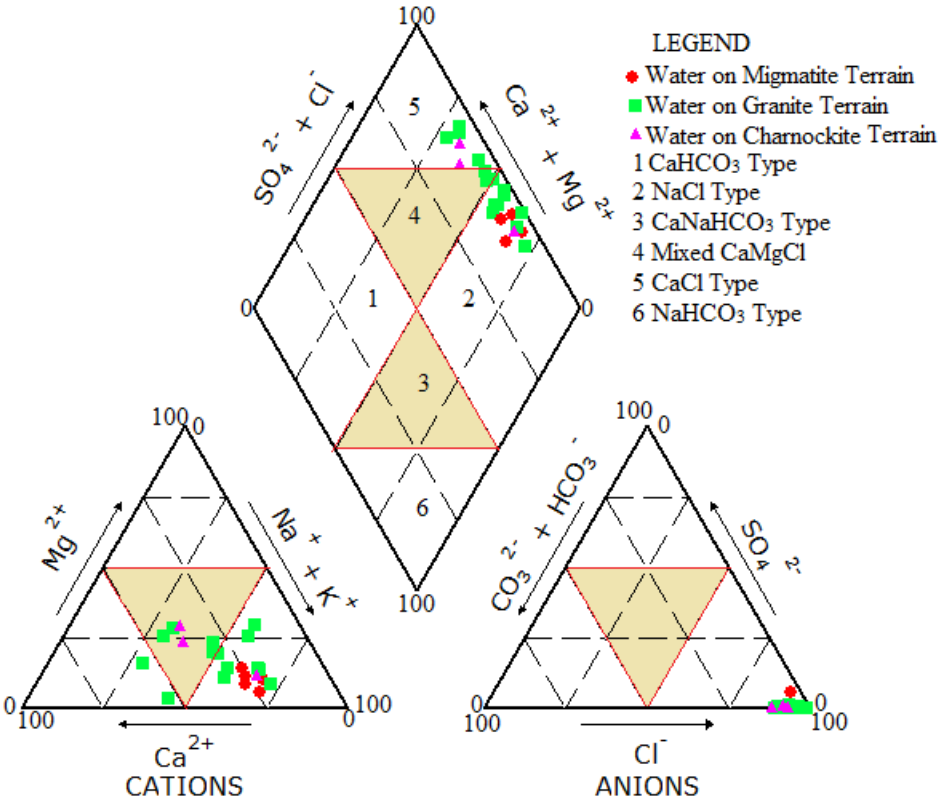


Fig.5: Piper Trilinear diagram of groundwater samples from the study area.

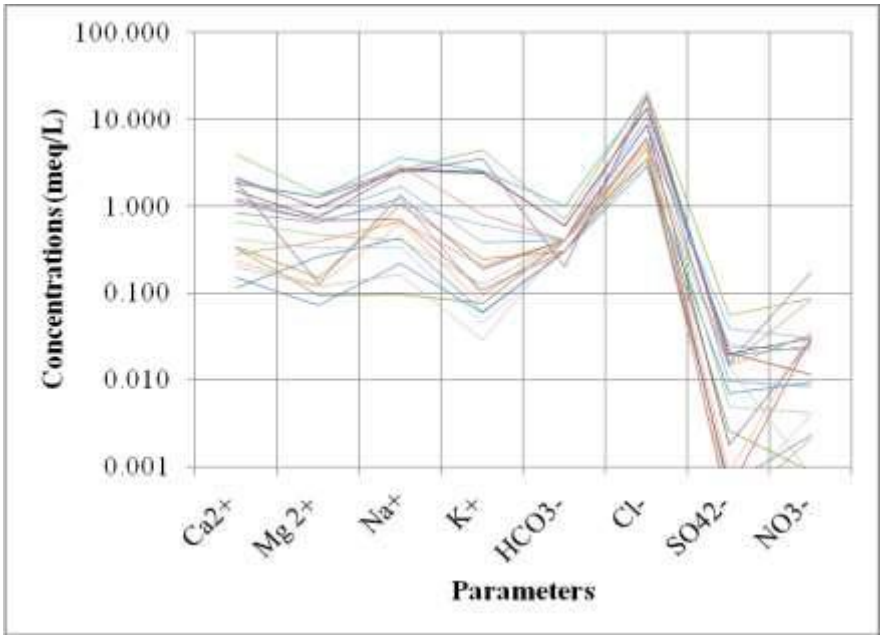


Fig.6: Schoeller Diagram of Groundwater samples from the study area.

4.3 Irrigation Quality Assessment of Groundwater of the study area.

Water is considered as an important resource which is required for the plant growth in agricultural production (Tiwari et al., 2011). The suitability of groundwater for irrigation depends on how its mineral constituents affect both the plant and the soil. High salts contents in groundwater can be highly harmful. Growth of plants can be physically affected as taking up of water is reduced through modification of osmotic processes. Also, plant growth may be damaged chemically by the effect of toxic substance arisen from metabolic processes. Use of poor water quality can create four types of problems such as toxicity, reduction in water infiltration rate, salinity and miscellaneous (Ayers and Westcot, 1985). Assessment of water quality for irrigation could be carried out

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

employing EC, sodium adsorption ratio (SAR), chemical concentration of elements like Na^+, Cl^- and/or B^- and residual sodium carbonate (RSC) (Raghunath 1987; Raju 2006). In the present study, irrigation water quality assessment were carried out employing the individual chemical parameters, SAR, SSP, RSBC, KR, PI and MR. The results of some of the essential irrigation parameters are presented in Table 3 while the USSL (1954) classification of irrigation quality assessment based on electrical resistivity of groundwater is in Table 4.

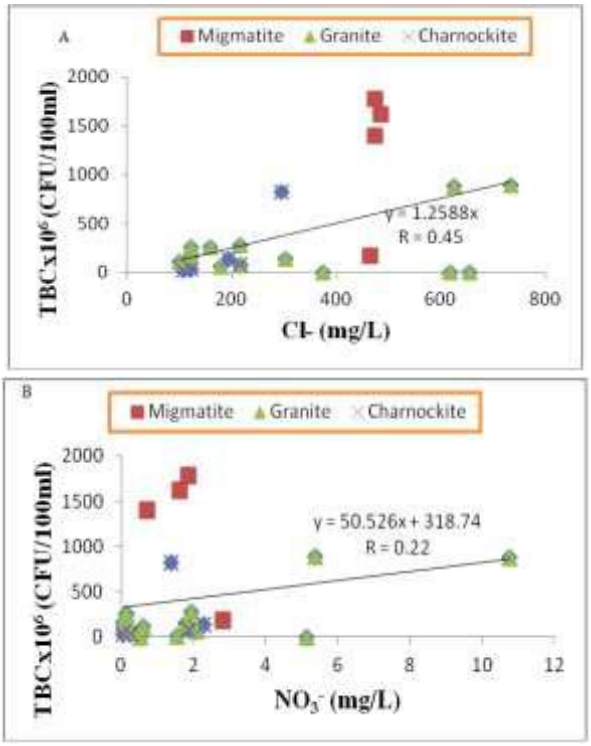


Fig.7: Bivariate Plots of TBC vs Cl^- and NO_3^-

The results in Table 3 showed SAR<10 for all groundwater samples from the study area indicating water of low sodium hazard. Sodium absorption ratio (SAR) is an important parameter for determining the suitability of groundwater for irrigation because it is a measure of alkali/sodium hazard to crops (Subramani et al. 2005). The SAR values ranged from 0.04 – 1.62 and all samples are in the excellent irrigation water category (Richards 1954). However, classification based on electrical conductivity revealed that eight (8samples) (5 from granite and 3 from charnockite) out of the 22samples had EC< 250µS/cm. Thus, only 36% of the groundwater fell into excellent irrigation class (Table 4). Fifty percent of the samples (11 samples) are in the good irrigation quality category. Three (3) samples (14%), all from granitic terrain fell into the doubtful irrigation class. Based on the USSL (1954) classification, the groundwater from the study area is suitable for irrigation and the effects of rock units on irrigation is equally justified as only granite has samples in the doubtful class (Table 4).

Table.3: Summary of Irrigation parameters of groundwater from the study area

Parameters	Min	Max	Mean	Stdev
SAR	0.04	1.62	0.51	0.56
SSP	6.68	54.79	26.48	16.21
RSBC	-3.29	0.19	-0.84	1.04
KR	0.30	4.40	1.72	1.24
PI	29.62	68.15	51.29	11.32
MAR	7.08	225.62	66.17	43.73

Table.4: Classification of groundwater for irrigation based on EC, SAR

Quality of water	**Electrical conductivity (S/cm)**	**Sodium adsorption Ratio (SAR)**
Excellent	<250	<10
Good	250–750	10–18
Doubtful	750–2250	18–26
Unsuitable	>2250	>26

Replacement of adsorbed Ca^{2+} and Mg^{2+} by Na^+ through cations exchange process can be dangerous to plants and such constitute hazard as soil structures are damaged and the soil may be compacted and becomes impervious. The analytical data plotted on the US salinity diagram (Richards, 1954) illustrates that 86% of the groundwater samples fall in the field of C1S1 and C2S1, indicating low to medium salinity and low sodium water, which can be used for irrigation on all types of soil without danger of exchangeable sodium (Fig. 8). Residual sodium bicarbonate (RSBC) calculated to determine the hazardous effect of carbonate and bicarbonate on the quality of water for agricultural purpose revealed that RSBC values ranged from -3.29 to 0.19. According to the US Department of Agriculture, water having RSBC<1.25 is good for irrigation, those with RSBC between 1.25 and 2.5 are in the doubtful category while any water with RSBC >2.5 is unsuitable for irrigation purpose. Based on this classification, all the groundwater samples in the area are in the good irrigation quality category.

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

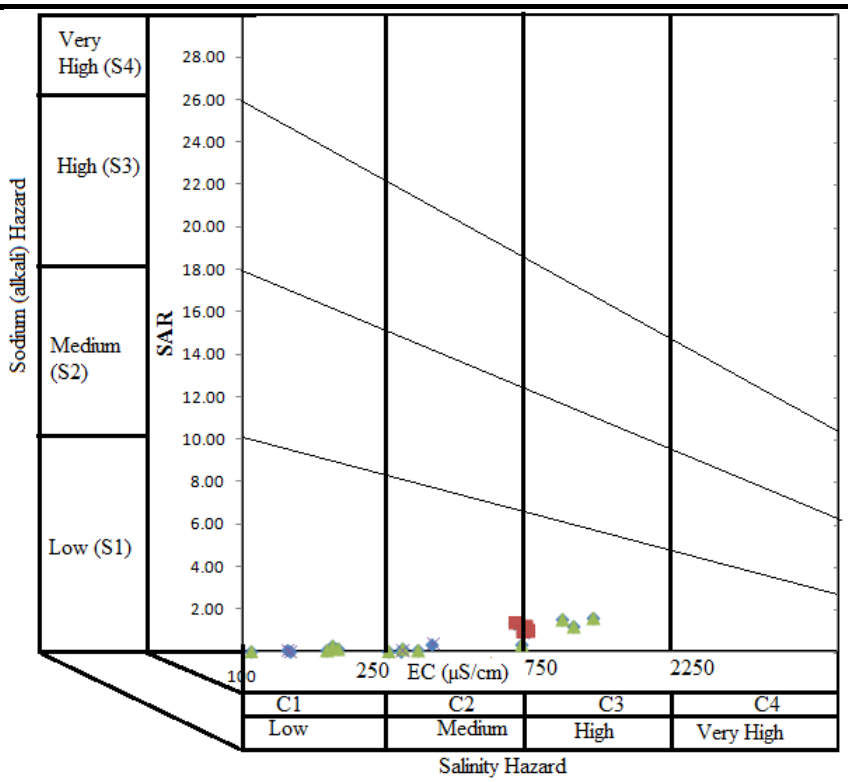


Fig.8: USSL Classification of Groundwater from the Study area

According to Paliwal (1972), MAR>50 is unsuitable for irrigation. MAR values of groundwater samples in this study varied from 7.08 – 225.62 with an average of 66.17 (Table 3). Only 7samples (31.81%) of the groundwater have MAR<50 and as such suitable for irrigation. Higher levels of TDS, Na+, HCO3-, Cl- etc in irrigation water can affect the permeability of soil. Doneen (1964) developed a criterion to assess the suitability of water for irrigation based on permeability index. PI values for groundwater samples in the area ranged from 29.62 - 68.15 %. According to Doneen's (1964) chart (Fig. 9) all the well waters fell under Class-I & II (Good Water). Furthermore all samples from migmatite terrain fell under Class-1 while samples from the other rocks (granite and charnockite) cut across Class-I and II, signifying the effects of rock units on the chemistry of the water and inadvertently on the irrigation quality of the water. Further assessments of irrigation quality of groundwater in the study area were carried out using KR and SSP. The KR for groundwater samples from the study area ranged from 0.3 – 4.4 (av. 1.72) while the SSP varied between 6.68% and 54.79% (av. 26.48%) (Table 3). Kelly (1963) suggested that the ratio for irrigation water should not exceed 1.0meq/L. The estimated mean value of KR for groundwater samples from the study area exceeded 1.0meq/L. However, nine (9) samples (6 from granite, 3 from charnockite) have KR<1.0meq/L. Thus KR values clearly indicate that the groundwater is moderately suitable for irrigation. The effects of rock units are again demonstrated as all samples from migmatite terrain have KR>1.0meq/L. As for the Soluble Sodium Percentage **(SSP), i**rrigation water with an SSP greater than 60% may result in Na^+ accumulation and possibly a deterioration of soil structure, infiltration, and aeration (Scianna et al., 2007). All groundwater samples from the study area are suitable for irrigation based on SSP values.

http://dx.doi.org/10.22161/ijeab/2.1.23 *ISSN: 2456-1878*

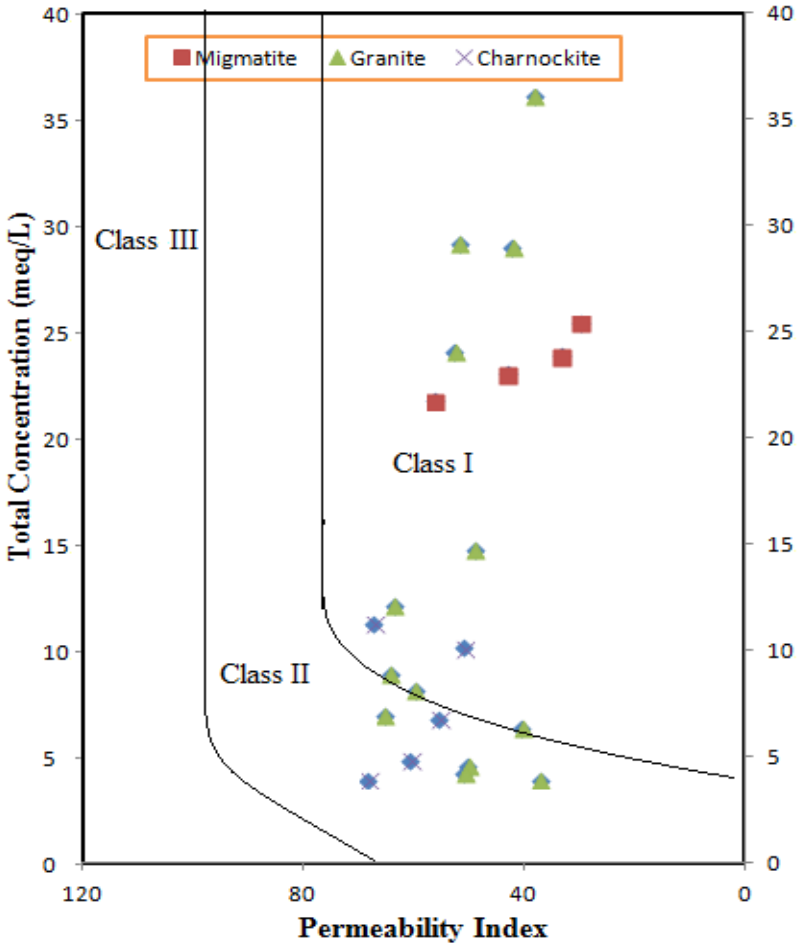


Fig. 9: Classification of irrigation water based on toatal concentration and permeability index (Doneen, 1964)

V. CONCLUSIONS

This study assessed the impacts of lithology on the chemistry of groundwater in shallow wells at Ikere – Ekiti. The study area is characterized by three major rocks; migmatite, granite and charnockite. The physico-chemical parameters of groundwater in the area have low values that are within WHO (2004) approved standard for drinking water except for pH that exceeded the standard (6.5 – 8.5) in two samples from migmatite and one sample from granite. All EC (µS/cm) values of groundwater samples irrespective of rock units were less than 1000 µS/cm indicating fresh water. All groundwater samples were polluted by bacteria. Groundwater in the area is chemically potable but bacteriologically infected. Total hardness of groundwater from migmatite gneiss was in the moderately hard to vey hard category while the groundwater from granite and charnockite fell into soft to very hard and soft to moderately hard classes respectively. In general, the order of cation abundance was Na^{+}> K^{+} > Ca^{2+}> Mg^{2+} while that of the anion was Cl^{-} > HCO_3^{-}> SO_4^{2-}> NO_3^{-} though this order varied in the individual rock units of the area. Gibb's diagram revealed that chemical weathering of rock-forming minerals has contributed to solute source in the groundwater of the area. Two water facies (Nacl and CaCl) were identified in the study area. All water samples from migmatite terrain fell into the NaCl water type whereas those samples from granitic and charnockitic rocks cut across the two facies (NaCl and CaCl) revealing lithologic effects. Irrigation water quality assessment employing the individual chemical parameters, SAR, SSP, RSBC, PI and MR revealed that the groundwater is suitable for irrigation purpose except for the KR and MR that indicated 41% and 31.5% suitability respectively. Classification of irrigation water based on toatal concentration and permeability index showed that all samples from migmatite terrain fell under Class-1 while samples from the other rocks (granite and charnockite) cut across Class-I and II, signifying the effects of rock units on the chemistry of the water and inadvertently on the irrigation quality of the water.

Groundwater in the study area is low mineralized, chemically potable but infected by bacteria pollutants. The water is suitable for irrigation purpose. Differences in rock types affected the chemistry of the groundwater as reflected in their physico-chemical composition, water facies and irrigation quality.

REFERENCES

[1] Abimbola AF, Odukoya AM, Adesanya OK (2002) The environmental impact assessment of waste disposal site on ground water in Oke Ado and Lagos, southwestern Nigeria. Proceedings 15th Annual conference Nigerian Association of Hydrogeologists Kaduna, Nigeria, 42pp.

[2] Ali KA (2012) Development of Water Supply Infrastructure in Nigeria: Challenges and Prospects. Being 2012 Nigerian Society of Engineers October Lecture.

[3] Amadi AN1, Tukur A, Okunlola, IA, Olasehinde, PI1, Jimoh MO (2015) Lithologic Influence on the Hydrogeochemical Characteristics of Groundwater in Zango, North-west Nigeria

[4] Aturamu AO (2012) Physical, Chemical and Bacterial Analyses of Groundwater in Ikere Township, South western Nigeria. International Journal of Science and Technology. Vol.2, No.**5**, pp.301-308.

[5] Ayers RS, Westcot DW (1985) Water quality for agriculture, irrigation and drainage (Paper No. 29). FAO, Rome.

[6] Ayodele O S, Aturamu AO (2011) Portability Status of Some Hand Dug Wells in Ekiti State, Southwestern Nigeria. International Journal of Science and Technology. Volume 1 No. 2, pp.102 – 109

[7] Back W (1966) Hydrochemical facies and groundwater flow patterns in Northern part of Atlantic Coastal Plain. USGS Professional Paper 498-A. Washington D. C.; USGS.

[8] Burke KC, Dewey FJ (1972) Orogeny in Africa. In: Dessauvagie, T.F.J. and Whiteman, A.J., Ed., African Geology, Ibadan University Press, Ibadan, 583-608.

[9] Dada SS (2006) Proterozoic Evolution of Nigeria. In: Oshi, O., Ed., The Basement Complex of Nigeria and Its Mineral Resources (A Tribute to Prof. M. A. O. Rahaman), Akin Jinad & Co., Ibadan, 29-44.

[10] Doneen LD (1964) Notes on water quality in agriculture. Davis: Water Science and Engineering, University of California.

[11] Elango L, Kannan R, Senthilkumar M (2003) Major ion chemistry and identification of hydrogeochemical processes of ground water in a part of Kancheepuram district, Tamil Nadu, India, Environmental Geosciences, 10(4), pp 157–166.

[12] Fournier RO (1981) Application of water Geochemistry to geothermal exploration and reservoir engineering in L. Rybach and L.J.P. Muffler, eds., Geothermal Systems, Principles and Case histories, John Wiley & Sons, New York. Pp. 109 – 143.

[13] Freeze RA, Cherry JA (1979) Groundwater. Prentice-Hall, Englewood Cliffs, NJ USA.

[14] Gibbs RJ (1970) Mechanisms controlling world water chemistry. Science 17:1088–1090

[15] Gupta, S. K. and Gupta, I. C. (1987). Management of Saline Soil and Water. *Oxford and IBH Publication. Co.* New Delhi, India, pp. 399.

[16] Jeelani G and Shah A Q 2006 Geochemical characteristics of water and sediment from the Dal Lake, Kashmir Himalaya, India: Constraints on weathering and anthropogenic activity; *Env. Geol.* **50** 12–23.

[17] Kelly W P (1963) Use of Saline Irrigation Water. *Soil Sci.* Vol. 95(4):pp. 355-391.

[18] McCurry P (1973) Geology of Degree Sheet 21, Zaria, Nigeria. Overseas Geol. Mineral Res., 10 (45): 1-45.

[19] Milovanovic Mimoza (2007). Water quality assessment and determination of pollution sources along the Axios/Vardar River, Southeastern Europe. Science Direct, Desalination 213,l 159–173

[20] McGowan W (2000) Water processing: residential, commercial, light-industrial, 3rd ed. Lisle, IL, Water Quality Association.

[21] National Population Commision. (2006) 1963, 1991, and 2006 Cencus of Each Local Government Area of Ekiti State. Ado-Ekiti: National Population Commision, Ekiti State Chapter.

[22] Obiefuna GI, Sheriff A (2011) Assessment of shallow ground water quality of Pindiga Gombe Area, Yola Area, NE, Nigeria for irrigation and domestic purposes. Res J Environ Earth Sci 3(2):131–141

[23] Odeyemi AT, Akinjogunla OJ, Ojo MA (2011) Bacteriological, Physicochemical and Mineral Studies of Water Samples from Artesian bore-hole, Spring and Hand dug Well located at Oke-Osun, Ikere-Ekiti, Nigeria. Scholars Research Library Archives of Applied Science Research, 3 (3):94-108

[24] Omotoyinbo OS (2007) Determination of the Level of Contamination of Underground Water (hand- dug well) by Organic Waste: A case Study of Ado-Ekiti, Nigeria. Ethiopia Journ of Education and Sc, vol3 no1, P43-50

[25] Paliwal, K.V. (1972). Irrigation with saline water, Monogram no. 2 (New series). New Delhi, IAR1, p198.

[26] Piper AM (1953) A Graphic Procedure in the Geochemical Interpretation of Water Analysis, Washington D.C., United States Geological Survey. ISBN ASIN: B0007HRZ36.

[27] Rahaman MA (1988) Recent Advances in the Study of the Basement Complex of Nigeria. In: Precambrian Geology of Nigeria, Geological Survey of Nigeria Publication, 11-43.
[28] Raghunath IIM (1987) *Groundwater*. Second edition; Wiley Eastern Ltd, New Delhi, India.
[29] Raju NJ (2006) "Hydrogeochemical parameters for assessment of groundwater quality in the upper Gunjanaeru River basin, Cuddapah District, Andhara Pradesh, South India," Environmental Geology.
[30] Richards LA (1954) Diagnosis and improvement of Saline and Alkali Soils. Agricultural Handbook 60, USDA and IBH Publishing Co. Ltd. New Delhi, India: pp 98-99.
[31] Sawyer CN, McCarty PL (1967) Chemistry for sanitary engineers. McGraw-Hill, New York
[32] Sayyed MRG, Wagh GS (2011) An assessment of groundwater quality for agricultural use: a case study from solid waste disposal site SE of Pune, India. Proceedings of the International Academy of Ecology and Environmental Sciences, 1(3-4): 195-201
[33] Schroeder HA (1960) Relations between hardness of water and death rates from certain chronic and degenerative diseases in the United States, *J. Chron disease*, *12*:586-591
[34] Schoeller H (1965) Qualitative evaluation of groundwater resources. In: Methods and techniques of groundwater investigations and development, UNESCO, pp 54–83.
[35] Sivasubramanian P, Balasubramanian N, Soundranayagam JP, Chandrasekar N (2013) Hydrochemical characteristics of coastal aquifers of Kadaladi, Ramanathapuram District, Tamilnadu, India. Appl Water Sci 3:603–612
[36] Stednick JD (1991) "Wildland water quality sampling and analysis," Academic Press Inc., San Deigo, 216p.
[37] Subramani T, Elango L, Damodarasamy SR (2005) "Groundwater quality and its suitability for drinking and agricultural use in Chithar River Basin, Tamil Nadu, India," Environmental Geology, Vol. 47, pp. 1099–1110.
[38] Talabi AO, Ogundana AK (2014) Bacteriological Evaluation of Groundwater In Ekiti-State, Southwestern Nigeria. International Journal of Scientific & Technology research volume 3, issue 9, pp. 288 – 293.
[39] Todd KD (1980) *Ground water hydrology*. 2nd ed. New York: John Wiley and Sons.
[40] Tiwari RN (2011). Assessment of groundwater quality and pollution potential of Jawa Block Rewa District, Madhya Pradesh, India. Proceedings of the International Academy of Ecology and Environmental Sciences, 2011, 1(3-4):202-212
[41] US Salinity Laboratory (1954) "Diagnosis and improvement of saline and alkali soils," Agricultural Handbook, USDA, No. 60, pp. 160.
[42] World Health Organization (WHO) (2004) Guidelines for Drinking-Water Quality. Third Edition Incorporating the First and Second Addenda. Vol. 1. Recommendations.

http://dx.doi.org/10.22161/ijeab/2.1.24 *ISSN: 2456-1878*

Study the concentrations of Ni, Zn, Cd and Pb in the Tigris River in the city of Baghdad

Khalid A. Rasheed[1], Hussain A. Flayyh[2], AbdulSalam T. Dawood[3]

[1]Biotechnology Research Center/Al-Nahrain University;
[2]General Comp.for Elec. Indus./Ministryof Industry and Minerals, Ministry of Education and Scientific Research

Abstract— *Four heavy metals were selected to estimate their concentrations on the Tigris River in the Baghdad area, the water samples collected from three stations on the river represented the northern, central and southern Baghdad, using apolyethylene bottles of 2-liter for the period from March 2010 until February 2011 and then on a monthly basis.*

It observed from the results, that all of the concentrations of heavy metals under the study, were within the permissible limits for the three stations depending on the values of Iraqi Rivers Maintenance Regulation No. 25 of 1967.In many of the recoding data it was within intangible readings because of the low concentration of the heavy metals in the sample.

Keywords— Tigris River, Ni, Zn, Cd, Pb, Baghdad.

I. INTRODUCTION

The man tamed himself to be the primary interface of the uses of heavy metals in his life by finding out heavy metals cycle and re-distributed in the environment, where the natural levels of these metals are not harmful to the environment and living organisms. Arising from mining and the use of various chemicals has increased the metals pollution, as well as, the agricultural and industrial sewage and fossil fuel activities give a high level of these metals in the environment (Tulonen, *et al.* 2006).

Human activities recorded clear excesses for most of the heavy metals such as lead, mercury, cadmium and zinc,which have been accumulated in the various structures of the environment (Atici, *et al.* 2008).

Heavy metals are working as received the electrons interact with the donor material for electrons to be varied markedly different chemical compounds, but biochemically the term (heavy) metals used for indicating a certain order of most metals harmful to the environment, and that density is equivalent to 4-5 times more than the density water (Goel, 2008).

The industrial plants are an important source of heavy metals and some of the flow of it disbursed to surface and ground water, especially if the industrial clay as it is an important source of pollution (Teplyakov & Nikanorov, 1994).

II. MATERIALS AND METHODS

Description of studying stations:

Three stations were selected on the River Tigris(Figure 1), these are:

1-North Baghdad (Altaqi region) This station is located near Al-Muthana bridge. The two sides of the station are almost identical. The width of the Tigris River section of this station up to 250 meters long and 4.8 meters in depth (Iraq Water Resources, 2011).

2-Center of Baghdad (Al-Aathamiah region) This station is located near the Iron Sarafiya bridge. The downstream section of this region like U-shaped and the tendency has a sharp and deep toward the right bank(Iraq Water Resources, 2011).

3-South Baghdad (Al-Zaafraniah region).

This station is located at the convergence of the Diyala River withthe Tigris River, a section width of the river is up to 200 meters. Where abundant agricultural areas and are considered a last resort for the discharge of pollutants flowing from the north and center of Baghdad.

http://dx.doi.org/10.22161/ijeab/2.1.24 *ISSN: 2456-1878*

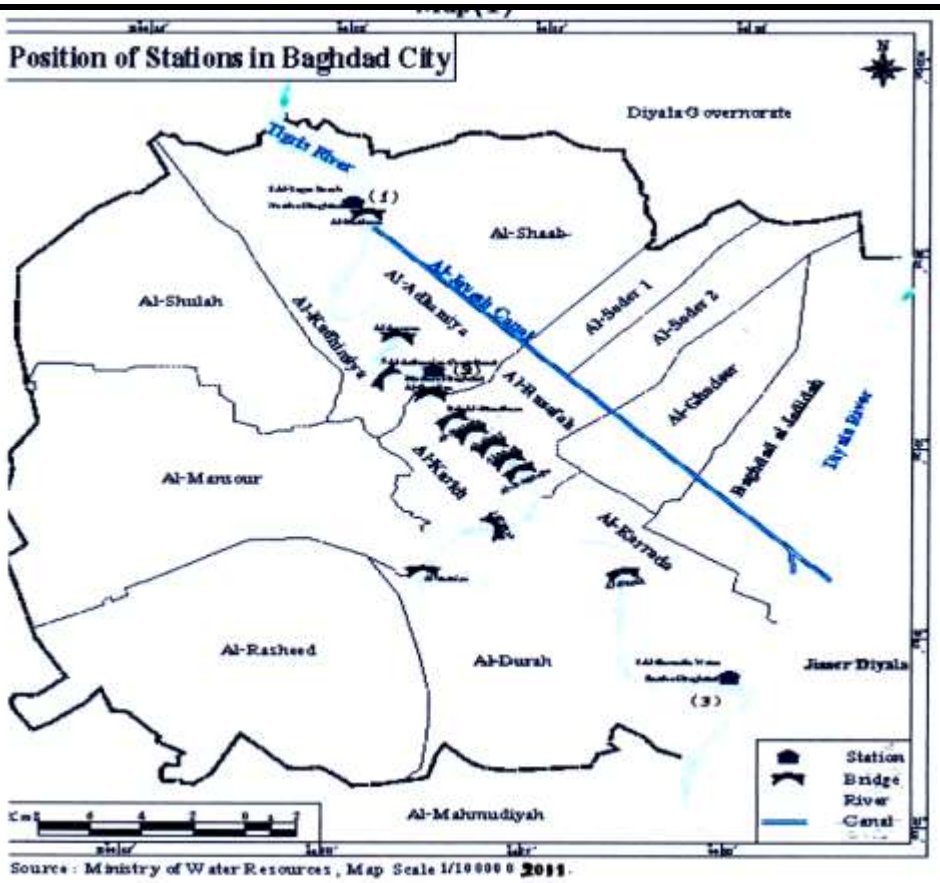


Fig.1: Map of sampling stations (Iraq Water Resources, 2011)
(Source: Ministry of water Resources, Map Scale 1/10000

Sampling collection

Water samples collected from the surface of the water depth (30 cm) for the period from March 2010 until the month of February 2011 and placed in bottles of polyethylene thoroughly washed with river water (2 liter per each station) .

Four heavy metals have been selected which are, Nickel (Ni^{+2}), Znic (Zn^{+2}), Lead (Pb^{+2}) and Cadmium (Cd^{+2}) for the calculating concentrations quantified using Flamless Atomic Absorption Spectrophotometer type Buck, USA),as the most common metals in some industries and perhaps can be inferred in the Tigris River (Al-Saadi,2006), which have been measured in the filtered water river, to estimate the dissolved concentrations of these metals depending on (APHA, 1998).

III. RESULTS AND DISCUSSION

Nickel (Ni^{+2})

The concentration of nickel in three stations were ranged from non-detectable to 0.2 mg / L, with a minimum value recorded of about 0.01 mg / L, which was repeated more than once, as recorded in April 2010 in the stations 1 and 3, and on May 2010 at station (1) in the month of November 2010 at station (2), respectively, while the maximum value was in the month of June 2010 at the station (2) (Table 1) (Figure 2).

Table.1: The minimum and maximum values, mean, SD and analysis of variance for the values of the heavy metals measured in the Tigris River (middle of Baghdad)

	Parameters	Station 1	Station 2	Station 3	Significance
1.	Nickel mg/ℓ	0.01 – 0.17 0.08 ± 0.02**a**	0.01 – 0.2 0.023 ± 0.005**b**	0.01 – 0.04 0.004 ± 0.0002**c**	$P \leq 0.05$
2.	Znic mg/ℓ	0.01 – 0.08 0.045 ± 0.008**a**	0.01 – 0.04 0.017 ± 0.004**b**	0.01 – 0.02 0.022 ± 0.002**b**	$P \leq 0.05$

http://dx.doi.org/10.22161/ijeab/2.1.24 *ISSN: 2456-1878*

3.	Lead mg/ℓ	0.01 – 0.13 0.027 ± 0.009**a**	0.01 – 0.2 0.026 ± 0.005**a**	0.03 – 0.09 0.014 ± 0.001**b**	P ≤ 0.05
4.	Cadmium mg/ℓ	0.01 – 0.19 0.063 ± 0.002**a**	0.01 – 0.07 0.038 ± 0.001**b**	0.01 – 0.03 0.015± 0.005**c**	P ≤ 0.05

Small letters mean no significant differences at the level of probability (P ≤ 0.05)

While significant difference was observed between the three stations at (p≤0.05) between the nickel and cadmium and its value (r=0.964)(Table 2).

Table.2: the correlation coefficient (r) of heavy elements in the studied stations

Mg/L	Ni	Zn	Pb	Cd
Ni	1.0	0.964	-0.219	0.476
Zn		1.0	-0.416	0.167
Pb			1.0	0.091
Cd				1.0

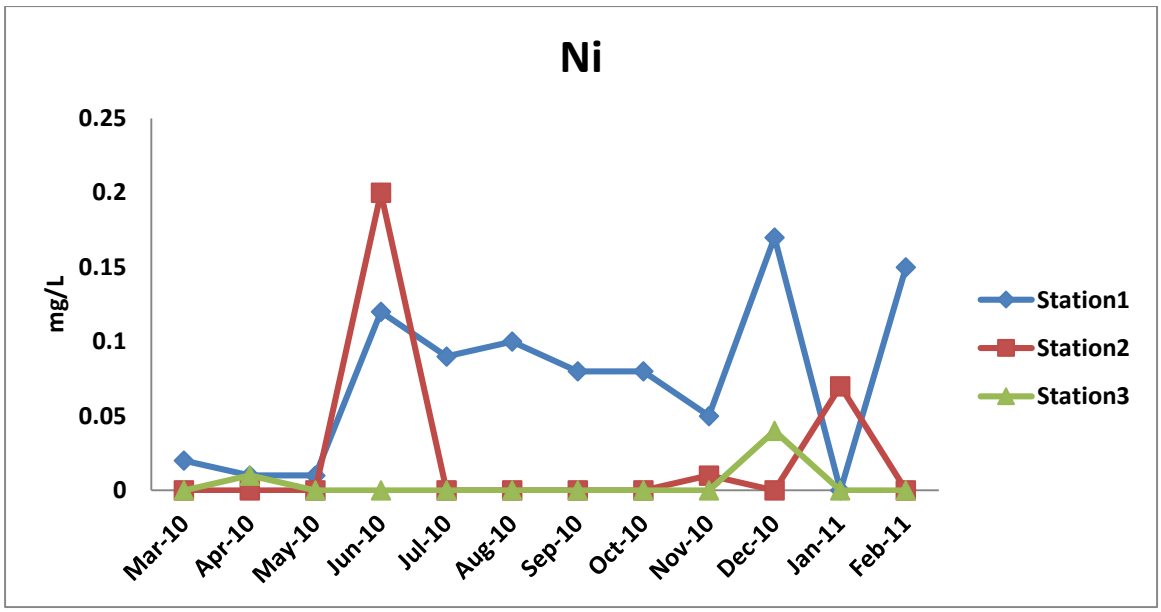


Fig.2: Nickel concentration in the study stations from March 2010 to February 2011.

Zinc (Zn^{+2})

The results showed that the concentration values of zinc ranged from non-detectable to 0.08 mg / L. But adopted the 0.01 mg / L as the value of a minimum which have been repeated more than once, It was in March 2010 at the station (1) In each of April, May, September and October 2010 at the station (2) as well as in June 2010 at a station (3).

While the maximum value recorded in the month of February 2011 at a station (1), also it found a significant difference at the level of probability (p≤0.05) between the study stations, as shown in Figure 3 and Table 1.Statistically, there is a negative correlation (p≤0.05) between the zinc and lead reached (r = -0.416), as in Table 2.

http://dx.doi.org/10.22161/ijeab/2.1.24 ISSN: 2456-1878

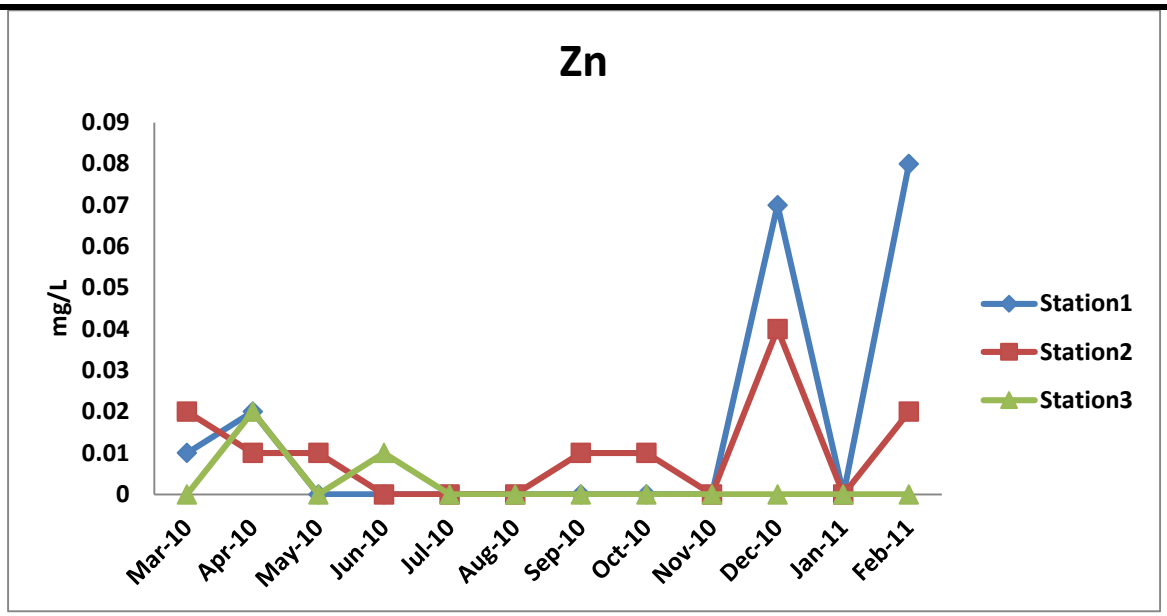


Fig.3: Concentration of zinc in the study stations from March 2010 to February 2011.

Lead (Pb^{+2})

The concentrations of lead variedin all studied stations, as there were values is not detectable in more than once and more than station and are generally recorded a minimum value of about 0.01 mg / L and maximum value of about 0.2 mg / l.,This minimum value have been repeated in more than once, as in May and December 2010 at the station (2) and in January and February 2011 at the station (1), while the maximum value in the month of February 2011 at the station (2).

It was observed a significant difference in the concentrations of lead recorded in the study of different stations at the level of probability ($p \leq 0.05$) as shown in Table 1 and Figure 4.

Statistically a significant negative correlation was found at a level factor ($p \leq 0.05$) between lead and zinc and its value ($r = -0.416$), as shown in Table 2.

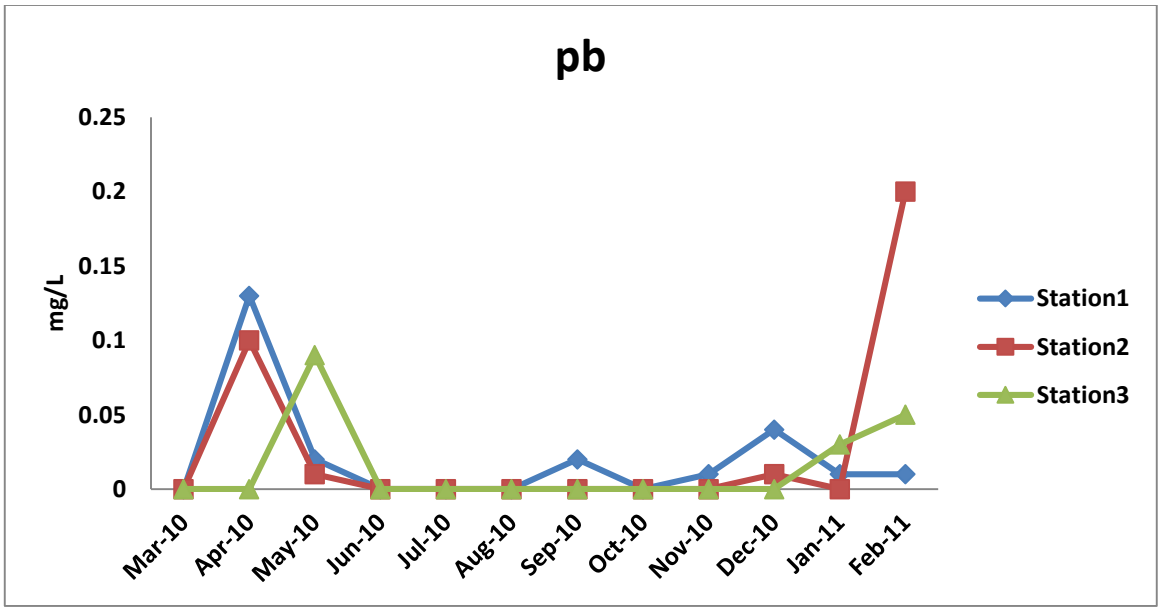


Fig.4: The concentration of lead in the study stations from March 2010 to February 2011.

http://dx.doi.org/10.22161/ijeab/2.1.24 *ISSN: 2456-1878*

Cadmium (Cd^{+2})

Some data recorded for cadmium was intangible, especially at stations 2 and 3, and in general the minimumvalues concentration of cadmium recorded with about 0.01 mg / L and higher concentrations of about 0.19 mg / L. The minimum value repeated the for most of the time, which shown in the station 1 in the months of March 2010 and January 2011,and repeated in the station (2) in the months of May 2010, and the station 3 in the months of April 2010 and January 2011. While, the upper value was recorded in the month of July 2010 at the station (1) (Figure 5).

No significant differences appear at the level of probability ($p \geq 0.05$) between the concentrations of cadmium in the study stations, it was found a statistically significant positive correlation factor ($p \leq 0.05$) between the nickel and cadmium ($r = 0.476$).

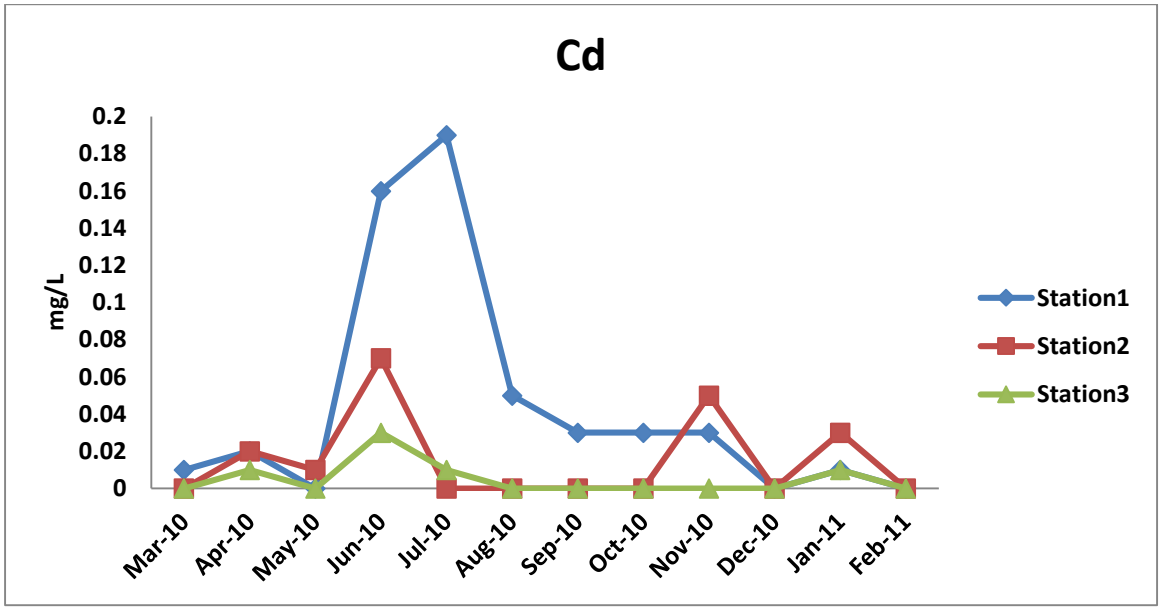


Fig.5: Concentration of cadmium in the study stations from March 2010 to February 2011.

The results of the current study showed that the concentrations of the fourth metals mostly are within the permissible limits by the Iraqi Rivers Maintenance Regulation No. 25 of 1967 and in many of the recorded data were within intangible readings at the low concentration of these metals in the sample.

Nickel scored the highest concentration of it in the month of June 2010 at two stations 1 and 2 and the focus was the highest in Station 1, as well as in the month of December 2010 and February 2011 at the same station.

As for zinc, all recorded concentrations were below the permissible limits, while lead exceeded for all recorded concentration the limits and that was in March 2010 at two stations (1 and 2), and in November 2010 at station (1) and also in February 2011 at station (2).

While, the concentration of cadmium exceeded the permissible limits of this study in June 2010 and the second one which was the highest in August, 2010 at station (1).

The concluding from the foregoing and overview of the figures (16, 17, 18, 19), that the increase in the concentration of the four heavy metals are the highest in station (1) and decreases toward the station 2 and fade sometimes in station (3), this probably indicates the presence of a source poses these heavy metals be the closest to the station (1),the decreases of the concentration of the four metals with downstream, and this reduction in emphasis may by dilution or depositing metal during the flow of the river or combined with organic compounds formingchelating agents turn into sediments. Morel *et al.* (1973) shows that the most metals werein the liquid state, to a free state at a low pH (acidic), especially with aerobic conditions and at increasing of the pH (or about basal) carbon consist of metal first and then to the oxidation view, as well as can be going into silica metal forming at the same time and deposited.

Tulonen, *et al.* (2006) found that the concentrations of metals in the water are much lower than in the humus lakes and there is no relationship between the humus lake waters and the fact that metals are associated with organic matter and humus deposited with them, but the current study was limited to the measurement of heavy metals dissolved in water only.

It also found a significant difference between the current study stations for heavy metals concentrations, this

indicates that the concentrations were varied in the water of the river on the study period and for the three stations, and the proportions of the presence of metals vary depending on the surrounding ecological conditions, and this is what trailed study (Perez and Sumngat, 2001).Drever (1997) in his study also showed, that these metals have common characteristics, where, when present in the oxidizing environment becomes acidic in the form of oxides or carbonate or silicate with iron, and these three metals there among them positive correlation factor it changes according to the same conditions affecting. The lead tends to be associated with various liquid organic ingredients, where, lead comes from human activities, such as pesticides, sewage and industrial batteries and printing processes and fuel (Alloway and Ayres, 1997) and this study is consistent with (Al-Malikey, 2009) and (Abdul-Kareem, *et al.* 2011).

REFERENCES

[1] Abdul-Kareem, B.M.; A.M. Rabee and Y.F. Al-Fatlawy (2011). Monitoring Heavy Metals, Cations and Anions Levels and its Possible Health Risks in Tigris River at Baghdad Region. Iraqi Journal of Science, University of Baghdad.

[2] Alloway, B. and D.C. Ayres, (1997). Chemical principles of environmental pollution (2ed Ed.) Chapman and Hall, London, p: 395.

[3] Al-Malikey, R.N.A. (2009) Biogeochemical Assessment of some heavy metals in Al-Hammar marsh by using GIS. PhD. Thesis, College of Science for Women, University of Baghdad. P: 153.

[4] Al-Saadi H. A. (2006).Basics of environmental science and pollution. Dar Yazouri Scientific Publishing and Distribution, Amman, Jordan. Pp 405.

[5] APHA. (1998). American Public Health Association, Standard Methods for Examination of Water and Wastewater, 20 th ed. Washington. DC.

[6] Atici, T.; A. Seyhan ;A. Ahmet and A. Didem (2008). Ecological effects of some heavy metals (Cd, pb, Hg, Cr) pollution of phytoplanktonic algae and zooplanktonic organisms in Sariyar Dam Reservoir in Turkey. African Journal of Biotechnology Vol. 7 (12). Pp. 1972- 1977.

[7] Drever, J.I. (1997). The geochemistry of natural water surface and groundwater environments. 3rded. Prentice Hall, USA, P: 436.

[8] Goel, P.K. (2008) Water Pollution. Causes, Effects and Control. 2nded. Reprint, New Age international (P) Limited, Publishers, New Delhi.

[9] Iraqi Rivers Maintenance Regulation No. 25 of 1967 and amendments thereto of 1988. The Ministry of Health. Directorate of protecting and improving the environment - environmental legislation.

[10] Iraq Water Resources (2011).Discharges and the levels of the Tigris River in the city of Baghdad.The National Centre for Resource Management Department Control of Water.

[11] Morel, F.; R.E. McC F.F. and J.J. Morgan (1973). Interaction and chemostasis in aquatic chemical systems: Role of PH, PE, Solubility and complexation In (P. C. Singer, ed) Trace Metals and Metal- Organic Interaction in Natural Water, Ann Arbor Science, Michigan.

[12] Perez, T.R. and A.M. Sumugat (2001) Microalgal Heavy Metal indicators in freshwater systems of nonoc and dinagat Islands, Surigao del Norte, Philippines, LSR-SSE. 1: 30-42.

[13] Teplyakov, YuV. and A.M. Nikanorov (1994). Simulation of heavy metal effect on freshwater ecosystems in mesocosms and estimation of water body self purification properties Hydrological, Chemical and Biological processes of Transport of contaminants in Aquatic Environments. IAHS publ. 219: 293-301.

[14] Tulonen, T.; P. Mikacl ; A. Lauri and R. Martti (2006). Concentrations of heavy metals in food web components of small, boreal lakes. Boreal Environment Rescrch, 11: 185-194.

http://dx.doi.org/10.22161/ijeab/2.1.25 *ISSN: 2456-1878*

Morphological and physiological variation of *Fusarium oxysporum* f. sp. *ciceri* isolates causing wilt disease in chickpea

N. Nath[1], A. U. Ahmed[2], F. M. Aminuzzaman[3*]

[1]Scientific Officer, RSRC, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[2]Principal Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute, Gazipur-1701, Bangladesh
[3]Professor, Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh

***Abstract*—** *Nine isolates of Fusarium oxysporum f. sp. ciceri infecting chickpea were collected from major chickpea growing areas of Bangladesh and their cultural, morphological, physiological and pathogenic characteristics were described. The isolates varied significantly in their cultural, morphological and physiological traits, i.e. colony color, shape, margin and texture; mycelial radial growth and spore production. Laboratory studies were conducted to study the effect of different culture media, pH and temperature levels on mycelial growth and sporulation of Fusarium oxysporum f. sp. ciceri. Mycelial radial growth and sporulation of F. oxysporum was maximum for all the isolates at 25°C after seven days of inoculation, which was reduced drastically below 15°C and above 35°C. No growth and sporulation was observed at 5 °C temperature for all the isolates. The most suitable pH level for growth and sporulation of the fungus was at pH 6.0. The fungus grew well on oat meal agar medium among seven culture media tested. No sporulation was observed on WA medium. The highest number of macro spores (3.27 x 10^5 ml^{-1}) and micro spores (4.06 x 10^5 ml^{-1}) were produced on PDA. Among the nine tested isolates, only one isolate (FOC-1) found to be highly virulent (HV) type on reaction on chickpea variety BARI Chola –1.*

Keywords*— *Fusarium oxysporum, variation, morphology, physiology, pathogenicity.

I. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important grain legume crop in the world and first in the Mediterranean basin and South Asia (Saxena, 1990). Chickpea is a cool season food legume crop grown on 10 million ha in 45 countries in the world and producing 93,13,043 tones of grain in the world (FAO, 2008). Chickpea is considered as one of the most important legume crops in Bangladesh. Despite of the large area under chickpea cultivation in the world, the total production and productivity are quite low in most of the chickpea growing areas (Pande *et al*., 2006). The climate and agro-ecological conditions of South Asian countries including Bangladesh favors the rapid growth and development of various plant pathogens (Ahmed, 1996). So, vulnerability of chickpea plant to a number of fungal pathogens from seedling stage to maturity is the primitive cause of low yield. Although a number of biotic and abiotic factors contribute for low chickpea production but endemic occurrence of wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri* is of significant importance. Chickpea is reported to be affected by more than 52 pathogens (Nene *et al*., 1984). Among these, wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is a wide spread soil borne diseases, and is reported from many parts of India with intensity ranging from 10 to 100 percent (Singh *et al*., 1986). *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* is an important disease in many chickpea growing areas. This fungus is able to survive in the soil for long period of time by forming resting spores, thick walled reproductive structures. The wilt pathogen is soil-borne and survives through chlamydospores in seed and dead plant debris in soil (Haware *et al*., 1978). Since, the fungus can survive in the soil for several years; it is not possible to control the disease through normal crop rotations. Although a number of chickpea lines have been reported as resistant to wilt from different countries of the world (Nene *et al*., 1981), but their success has been highly localized due to location-specific races of the pathogen (Singh and Reddy, 1991). It is important to know which isolate to use in the screening process, how the resistance is expressed and inherited. In view of the above facts, the present research work was aimed to carry out comprehensive investigation on the cultural, morphological, physiological and pathogenic variation of *Fusarium oxysporum* f. sp. *ciceri*.

II. MATERIALS AND METHODS

Isolation and identification of the pathogen

Wilt infected plant samples were collected from nine locations covering four chickpea growing districts of Bangladesh. The pathogens that causes wilt disease in chickpea were isolated using tissue culture techniques. The infected chickpea roots were washed and placed into petriplates containing PDA media and incubated at 25 °C under near ultraviolet (NUV) light following ISTA rules (ISTA, 1996). Seven days after incubation, the fungal culture were studied under stereoscopic (Model: Olympus, SZ 61, Japan) and compound microscope (Model: Olympus, CX 21 FSI, Tokyo, Japan) for identification of the desired pathogens. Then the pathogen purified by single spore culture technique, preserved in PDA slants at 4 °C for further study.

Morphological variability

Nine isolates of *Fusarium oxysporum* f. sp. *ciceri* isolates were observed on PDA medium after 7 days of inoculation on the basis of colony color, shape, texture, margin, conidial color, size, shape and color of conidiophores.

Effect of culture media on radial mycelial growth of Fusarium oxysporum f. sp. ciceri

Seven culture media viz. potato dextrose agar (PDA) medium (Slice potato–200 g, dextrose–20 g, agar–20 g and distilled water– 1000 ml), Czapek'sdox agar (CDA) medium (Sucrose – 30 g, sodium nitrate – 2 g, di-potassium phosphate – 1 g, magnesium sulphate – 0.5 g, potassium chloride – 0.5 g, ferrous sulphate – 0.01 g, agar – 15 g and distilled water – 1000 ml), malt extract dextrose agar (MDA) medium (Malt extract – 20 g, peptone – 2 g, dextrose – 20 g, agar – 20 g, and distilled water – 1000 ml), corn meal agar (CMA) medium (Corn meal infusion form–50 g, agar–15 g and distilled water–1000 ml), oat meal agar (OMA) medium (Oat meal – 60 g, agar – 12.5 g and distilled water – 1000 ml), V_8 juice agar (V_8JA) medium [V_8 juice (100 ml) – 8.3 g, L-asparagine – 10 g, yeast extract – 2 g, calcium carbonate –2 g, glucose –2 g, agar –20 g and distilled water – 1000 ml)] and water agar (WA) medium (Agar – 20 g and distilled water – 1000 ml) were used to find out the most suitable one for the mycelia growth of the fungus.

Effect of temperature on radial mycelial growth and sporulation of Fusarium oxysporum *f. sp.* ciceri

The fungus was inoculated in PDA media using seven different levels of temperature viz., 5, 10, 15, 20, 25, 30 and to determine the temperature effect on radial colony growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri*.

Effect of pH levels radial mycelial growth of Fusarium oxysporum *f. sp.* ceceri

The isolates were inoculated in PDA medium having six pH levels viz., 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 in 9 cm diameter glass petriplates and incubated at 25±0.5 °C with alternating 12 hours of light and 12 hours of dark period in an incubator. The different level of pH were maintained by adding 0.1 N NaOH or 0.1N HCl.

For all the tests, 16 ml of medium were poured into each Petri plates using media dispenser having three replications. The medium autoclaved at 121 °C for 30 minutes at 15 PSI and then allowed for solidification in laminar airflow cabinet. Five mm diameter of mycelial disc were cut from the periphery of 7 days old culture of *Fusarium oxysporum* f. sp. *ciceri* with the help of a flame sterilized cork borer and then transferred into the centre of the petriplates containing solidified PDA medium. Then the plates were placed in an incubator maintaining required temperature level for temperature study. Data were noted on mycelial radial growth of *Fusarium oxysporum* f. sp. *ciceri* after two day of incubation till covering the entire petriplates of any isolates. The number of spores of *Fusarium oxysporum* f. sp. *ciceri* on different temperature and pH levels were counted using haemacytometer after 7 days of incubation. One (1) ml distilled water was poured in each test-tube and 5mm block of *Fusarium oxysporum* f. sp. *ciceri* isolates were put into the test-tube. Then the test-tubes were shaken by vortex shaker. After shaking, spores were counted using haemacytometer. The spore counting process was repeated 10 times of each replication.

Pathogenic variability

Plastic pots (8×10 cm) were used to grow chickpea plants in the pot house of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur. The pots were filled with 500 gm sterilized soil with well decomposed organic matter. In order to get a huge amount of inocula of *Fusarium oxysporum* f. sp. *ciceri*, isolates were sub-cultured on PDA medium and incubated for 10 days. One pertriplate (90 mm) full of inocula (mycelial mat and spores) were scraped by a plastic scrapper, wrapped with alluminium foil and preserved in the room temperature. Previously prepared inocula were incorporated into the sterilized soil. Five seeds of BARI Chola-1 were sown in each pot having three replications. Prior to sowing seeds were surface sterilized with Clorox (0.1% available chlorine) for 50 seconds and were rinsed by sterile distilled water for three times. The pots were kept in the net house of the Plant Pathology Division, BARI. Wilt incidence were recorded at 30, 45 and 60

http://dx.doi.org/10.22161/ijeab/2.1.25 *ISSN: 2456-1878*

DAI but aggressiveness of the tested isolates were measured considering wilt incidence only at 60 DAI (days after inoculation). Koch's postulates were proved and pathogenic nature of each isolate was established.

III. RESULTS AND DISCUSSION

Morphological variability

Fusarium oxysporum f. sp. *ciceri* exhibited variations in colony characteristics such as color, shape, margin and texture. Colony colors were purplish white, whitish orange, creamy white, cottony white. Colony shapes were irregular, regular, regular with sector, regular without sector. Colony margins were irregular, entire and wavy. Colony textures were fluffy, flat/velvet (Table 1). In past studies various type of pigmentations (yellow, brown, crimson) in culture has been recorded (Saxena and Singh, 1987). Chauhan (1962) found variation among 22 isolates with respect to their mycelium type, colony colour, toxin production and pathogenicity. *Fusarium* wilt isolates were highly variable in their colony growth pattern, size of colony and pigmentations. The current findings were well supported by Dubey *et al*., (2010). In this experiment it was observed that the length of micro conidia varied from 5.00-14.00 µm. The breadth of micro conidia was 1.00-4.00 µm. Micro conidia was 0-2 septed. The length and breadth of macro conidia ranged from 9.00-26.00 µm and 1.00-5.00 µm respectively. The number of septation of macro conidia ranged from 1-5. *F. oxysporum* f. sp. *ciceri* showed variations in the size of micro and macro-conidia of 9 isolates with three replications was also studied. The largest size of the micro-conidia was obtained from the isolate Foc-14 (3.7× 4.5, 3.1 × 5.0 µm) and the smallest size was from isolates FOC-21 (3.0 × 3.7 µm). Whereas, the biggest size 7.5× 20.10 µm of the macro-conidia was obtained from the isolates Foc-25 and the smallest size of 3.5× 22.5 µm conidia were obtained from isolates Foc-11(Table 1).

Effect of temperature on mycelial radial growth and sporulation

As evident from Fig. 1, the fungus grew at the temperature range of 10–35°C. Maximum growth was found between 25°C and 30 °C for all the 9 isolates after 7 days of incubation. At 25°C maximum colony diameter (78.00 mm) was obtained in isolate FOC-2 followed by FOC-9 (76.67 mm). The lowest colony growth (9.66 mm) was noted at 35 °C in FOC-2. The present findings agreed with the findings of Farooq *et al*., (2005). They reported that the growth of the *F. oxysporum* f. sp. *ciceri* was drastically reduced below 15 °C and started to decline above 35 °C, as these temperatures did not favor for growth of the fungus. It was observed that at 25°C and 30°C, the fungus attained the maximum growth of 76.8 and 85.4 mm while at 15°C, it was 59.3 mm after seven days of inoculation. No growth was observed at 5 °C. The highest (6.78 x 10^5 ml^{-1}) sporulation of micro conidia was observed in FOC-3 at 25 °C followed by FOC-6 (6.00 x 10^5 ml^{-1}); FOC-1 (5.13 x 10^5 ml^{-1}) and FOC-7 (3.70 x10^5 ml^{-1}) after seven days of incubation period. The minimum (3.30 x 10^3 ml^{-1}) sporulation was observed in FOC-4 at 15 °C. Spore production was not observed in isolates at 5 °C in FOC-1 and FOC- 2 at10°C, in FOC-9 at 15°C and in FOC-4, FOC-5, FOC-7 at 35 °C (Table 2).The maximum (3.43 x 10^6 ml^{-1}) sporulation of macro conidia was observed in FOC-1 followed by FOC-6 (6.66 x 10^5 ml^{-1}) and FOC-9 (5.58 x 10^5 ml^{-1}) at 25 °C after seven days of incubation period. The minimum (1.66 x 10^3 ml^{-1}) sporulation was observed in FOC-5 and FOC-8 at 15 °C and FOC-2 (1.66 x 10^3 ml^{-1}) at 35 °C. All the nine isolates failed to produce any spore at 5 °C temperature (Table 3). Abundant sporulation of this fungus was found after seven days of incubation at 27±2 °C on potato dextrose agar medium (Barhate, 2006). This observation supports the result obtained from this study. Khilare and Rafi Ahmed (2012) stated the highest growth of pathogen was recorded at 30 °C with higher sporulation 27.90 conidia μl^{-1} and after seven days of incubation, which was reduced drastically below 15 °C and above 35 °C. Chauhan (1963) and Desai et al., (1994) found that 25 °C is the optimum temperature for growth of Fusarium wilt. Similarly, Sharma et al., (2005) verify that a temperature around 25 °C is optimum for disease development. While, Mina and Dubey (2010) observed maximum colony diameter (85 mm) at 28 °C. From this experiment, it appeared that 25 °C temperature is suitable for mycelial radial growth and spore production of *Fusarium oxysporum* f. sp. *ciceri*.

Table.1: Morphological variability in the isolates of Fusarium oxysporum f. sp. ciceri

Isolates	Cultural characters				Dimension and septation					
					Micro conidia			Macro conidia		
	Color	Shape	Margin	Texture	Length (µm)	Breadth (µm)	Septation	Length (µm)	Breadth (µm)	Septation
FOC 1	Purplish white	Irregular	Irregular	Fluffy	6-14	2-4	0-1	12-25	1.5-5	3-5

http://dx.doi.org/10.22161/ijeab/2.1.25 *ISSN: 2456-1878*

FOC 2	Whitish orange	Regular	Entire	Fluffy	6-14	1.5-3	0-2	11-25	2-4	2-5
FOC 3	Creamy white	Regular	Entire	Flat/Velvet	5-10	1-3	0-1	9-15	2-3	1-4
FOC 4	Creamy white	Regular without sector	Wavy	Flat/Velvet	5-9	1.5-3	0	10-18	2-3	1-4
FOC 5	Cottony white	Regular without sector	Wavy, entire	Flat/Velvet	6-8	1.5-3	0-1	11-25	2-4	1-4
FOC 6	Creamy white	Regular	Wavy	Flat/Velvet	6-11	1.5-3	0-1	12-25	1-4	2-5
FOC 7	Whitish orange	Irregular	Irregular	Fluffy	5-12	1-3	0-1	15-26	2-5	2-5
FOC 8	Cottony white	Regular with sector	Wavy, entire	Fluffy	7-11	1-3	0-1	12-25	2-4	2-5
FOC 9	Cottony white	Irregular	Irregular	Fluffy	5-10	1-3	0-1	11-16	2-3	1-3

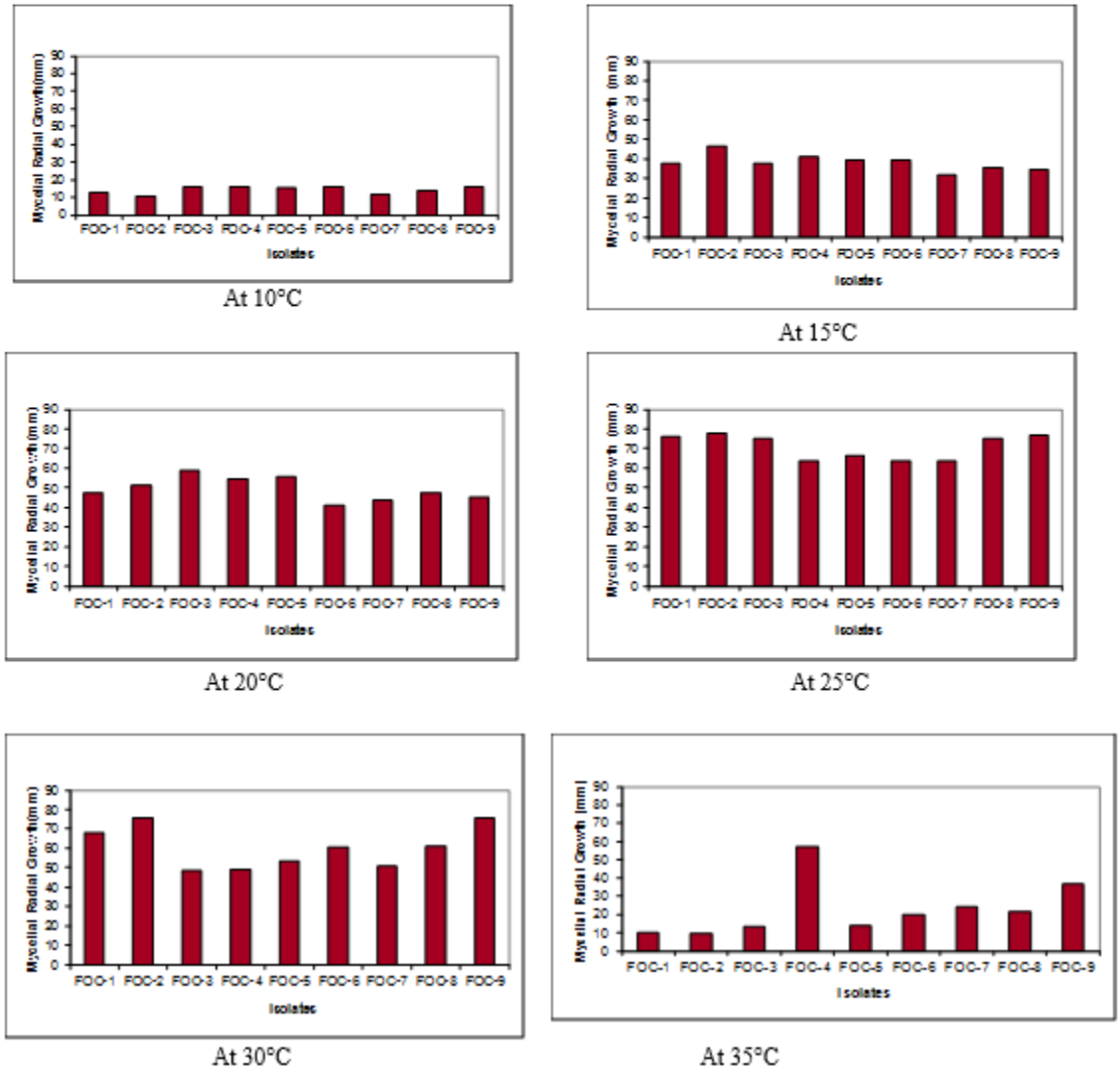


Fig.1: Radial mycelial growth of Fusarium oxysporum f. sp. ciceri at different temperature (°C) levels.

http://dx.doi.org/10.22161/ijeab/2.1.25 *ISSN: 2456-1878*

Table.2: Effect of temperature on production of micro conidia of nine Fusarium oxysporum f. sp. ciceri isolates

Isolates	Production of micro conidia (ml^{-1}) at different temperature levels (°C)						
	5	10	15	20	25	30	35
FOC-1	*	*	3.33 $x10^4$	6.38 $x10^4$	5.13 $x\ 10^5$	1.12 $x\ 10^5$	3.38 $x\ 10^4$
FOC-2	*	*	2.61 $x10^4$	7.33 $x10^4$	1.66 $x\ 10^5$	9.38 $x\ 10^4$	5.61 $x\ 10^4$
FOC-3	*	**	6.27 $x10^3$	8.25 $x\ 10^3$	6.78 $x\ 10^5$	3.86 $x\ 10^4$	2.71$x10^4$
FOC-4	*	**	3.30 $x\ 10^3$	1.00 $x10^4$	1.25 $x\ 10^5$	1.83 $x10^4$	*
FOC-5	*	**	4.00 $x10^4$	1.36 $x\ 10^5$	3.63 $x\ 10^5$	2.98 $x\ 10^5$	*
FOC-6	*	**	3.66 $x10^4$	1.01 $x\ 10^5$	6.00 $x\ 10^5$	3.60 $x\ 10^5$	1.83 $x10^5$
FOC-7	*	**	5.33 $x\ 10^4$	2.85 $x\ 10^5$	3.70 $x10^5$	1.38 $x10^5$	*
FOC-8	*	**	4.16 $x10^4$	7.66 $x\ 10^4$	2.61 $x\ 10^5$	8.50 $x10^4$	3.83 $x\ 10^4$
FOC-9	*	**	*	1.16 $x\ 10^4$	9.66$x10^4$	5.16 $x\ 10^4$	8.33 $x\ 10^3$

* No sporulation
** Very few sporulation

Table.3: Effect of temperature on production of macro conidia of nine Fusarium oxysporum f. sp. ciceri isolates

Isolates	Production of macro conidia (ml^{-1}) at different temperature levels (°C)						
	5	10	15	20	25	30	35
FOC-1	*	**	2.11×10^4	5.22×10^4	3.43 $x\ 10^6$	1.64 $x\ 10^5$	1.33 $x\ 10^4$
FOC-2	*	**	2.44 $x\ 10^4$	7.33 $x\ 10^4$	1.56 $x\ 10^5$	1.03 $x\ 10^5$	1.66 $x\ 10^3$
FOC-3	*	*	6.44 $x\ 10^3$	1.06 $x\ 10^4$	4.33 $x\ 10^4$	1.97 $x\ 10^4$	*
FOC-4	*	**	2.50 $x\ 10^4$	8.66 $x\ 10^4$	1.33 $x\ 10^5$	2.66 $x\ 10^4$	1.66 $x\ 10^4$
FOC-5	*	*	1.66 $x\ 10^3$	3.33 $x\ 10^3$	2.00 $x\ 10^4$	1.00 $x\ 10^4$	*
FOC-6	*	*	*	6.66 $x\ 10^3$	6.66 $x\ 10^5$	1.66 $x\ 10^3$	*
FOC-7	*	**	*	1.00 $x\ 10^4$	2.70 $x\ 10^5$	3.33 $x\ 10^3$	3.33 $x\ 10^3$
FOC-8	*	**	1.66 $x\ 10^3$	3.33 $x\ 10^3$	3.83 $x\ 10^4$	*	*
FOC-9	*	*	5.33 $x\ 10^4$	1.73 $x\ 10^5$	5.58 $x\ 10^5$	2.61 $x\ 10^5$	6.33 $x\ 10^4$

* No sporulation
** Very few sporulation

Effect of pH on mycelial radial growth and sporulation

The results of this experiment indicated that luxuriant radial growth exhibited in all of the isolates at pH 6.0 and pH 6.5 (Fig. 2). The highest colony diameter was noted for the isolate FOC-2 at pH 6.0 (87.83 mm) followed by FOC-1 (86.17mm) at pH 6.0 and FOC-8 (84.50 mm) at pH 6.0. The lowest mycelial radial growth was recorded in isolate FOC-1 (24.83mm) at pH 4.5. Farooq *et al*., (2005) reported that *F. oxysporum* f. sp. *ciceri* can grow well at pH 7 where the radial growth was 80 mm after seven days of inoculation. They also observed that the growth of the fungus decreased by increasing or decreasing the pH level from the neutral level. Imran Khan *et al*., (2011) showed optimum pH for growth of *F. oxysporum* f. sp. *ciceri* ranged from pH 6.5 to 7.0. *F. oxysporum* f. sp. *ciceri* has ability to tolerate pH 5.0–6.5, at a wide range (Shaikh, 1974). Maximum (3.03 x 10^5 ml^{-1}) micro conidia was produced by FOC-7 at pH 6.0 followed by FOC-5 (1.86 x 10^5 ml^{-1}) and minimum sporulation was observed on FOC-3 (8.87x 10^3 ml^{-1}) at pH 4.5 after seven days of incubation period (Table 4). Maximum (7.06 x 10^5 ml^{-1}) macro conidia were produced by FOC-9 at pH 6.0 and minimum sporulation was observed on FOC-6 (1.66 x 10^3 ml^{-1}) at pH 4.5. No macro conidia was produced by FOC-9 at 4.5; FOC-7 and FOC-8 at pH 6.5 and FOC-4, FOC-5 and FOC-8 at pH 7.0 (Table 5). Khilare and Rafi Ahmed (2012) reported that the highest sporulation of *F. oxysporum* f. sp. *ciceri* was 24.70 conidia μl^{-1} at pH 6.0. T. Swati and P. Rajan (2014) found that the Maximum sporulation of the macro conidia and micro conidia was observed at pH 6.5 (5.06 and 122.4 spore/100 mL of medium respectively) and minimum sporulation occurred in pH 2.0 (0.47 and 2.42 spore/100 mL of medium respectively). Chaudhary (1971) and Prasad *et al*. (1992) reported 6.0 pH level as the best for the growth and sporulation of *Fusarium moniliforme v subglutinanse*.

http://dx.doi.org/10.22161/ijeab/2.1.25 ISSN: 2456-1878

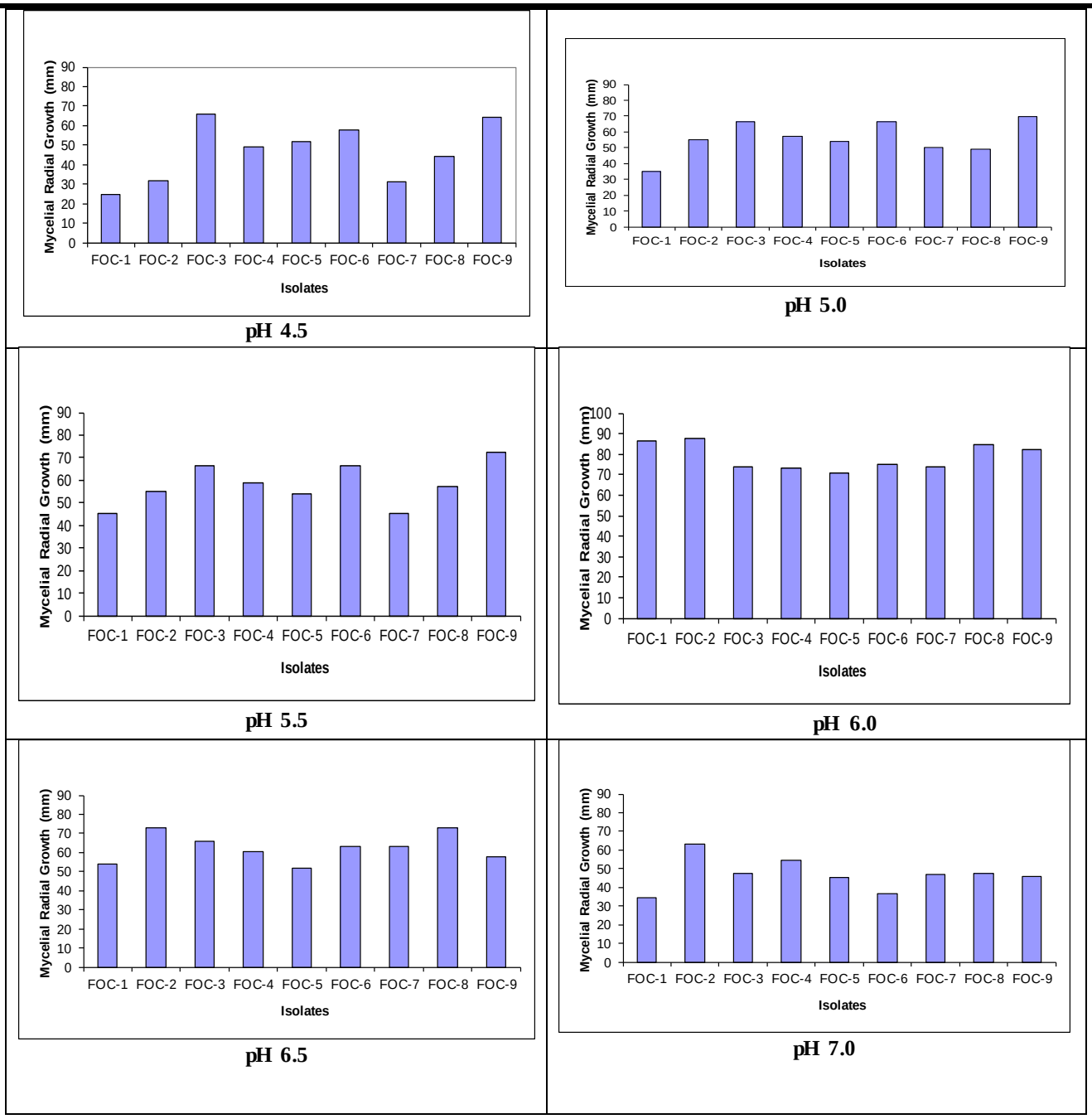


Fig.2: Effect of pH on mycelial radial growth of Fusarium oxysporum f. sp. ciceri isolates.

Table.4: ***Effect of*** *pH* ***on production*** *of micro conidia of Fusarium oxysporum f. sp. ciceri isolates*

Isolates	Production of micro conidia (ml^{-1}) at different pH levels					
	4.5	5.0	5.5	6.0	6.5	7.0
FOC-1	5.61x 10^4	5.77x 10^4	6.72 x 10^4	8.00 x 10^4	6.72 x 10^4	5.27 x 10^4
FOC-2	3.94x 10^4	4.72x 10^4	7.50x10^4	7.50 x 10^4	5.88 x 10^4	5.77 x 10^4
FOC-3	8.87x 10^3	1.06 x 10^4	1.33x 10^4	8.03 x 10^4	1.61 x 10^4	1.31 x 10^4
FOC-4	4.50x 10^4	5.00x 10^4	6.00 x 10^4	1.51 x 10^5	9.50 x 10^4	5.00 x 10^4
FOC-5	3.66x 10^4	3.83x10^4	5.83 x 10^4	1.86 x 10^5	6.16 x 10^4	6.00 x 10^4
FOC-6	2.50x 10^4	4.00 x 10^4	7.16 x 10^4	1.56 x 10^5	8.83 x 10^4	7.33x 10^4
FOC-7	6.16x 10^4	7.33 x 10^4	1.03 x 10^5	3.03 x 10^5	1.61 x 10^5	1.58x 10^5

http://dx.doi.org/10.22161/ijeab/2.1.25 *ISSN: 2456-1878*

FOC-8	1.16x 10^4	3.50x 10^4	3.66 x 10^4	7.33 x 10^4	6.66 x 10^4	6.83 x 10^4
FOC-9	1.16x 10^4	2.33 x 10^4	3.00 x 10^4	1.01 x 10^5	8.83 x 10^4	6.50x 10^4

Table.5: Effect of pH on production of macro conidia of 9 Fusarium oxysporum f. sp. ciceri isolates

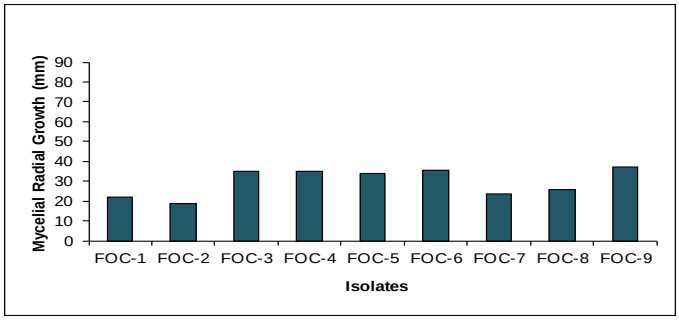
Isolates	Production of macro conidia (ml^{-1}) at different pH levels					
	4.5	**5.0**	**5.5**	**6.0**	**6.5**	**7.0**
FOC-1	2.50x10^4	5.72 x 10^4	1.72 x 10^5	3.90 x 10^5	1.38 x 10^4	1.00 x 10^4
FOC-2	2.00 x 10^4	5.05 x 10^4	8.61 x 10^4	4.76 x 10^5	8.33 x 10^4	1.33 x 10^4
FOC-3	1.00 x 10^4	1.35 x 10^4	5.47 x 10^4	1.50 x 10^5	2.30 x 10^4	1.16 x 10^4
FOC-4	5.00 x 10^3	6.66 x 10^3	3.25 x 10^5	3.80 x 10^5	6.66 x 10^3	*
FOC-5	3.50 x 10^4	9.33 x 10^4	6.66 x 10^3	2.30 x 10^5	1.66 x 10^3	*
FOC-6	1.66 x 10^3	1.66 x 10^3	5.00x 10^3	7.00 x 10^4	1.00 x 10^4	8.33 x 10^3
FOC-7	3.33 x 10^3	8.33 x 10^3	9.83 x 10^4	1.65 x 10^5	*	2.00 x 10^4
FOC-8	1.66 x 10^3	3.33 x 10^3	5.66 x 10^4	8.66 x 10^4	*	*
FOC-9	*	1.42 x 10^3	5.21 x 10^5	7.06 x 10^5	6.00 x 10^4	3.33x 10^3

* No sporulation

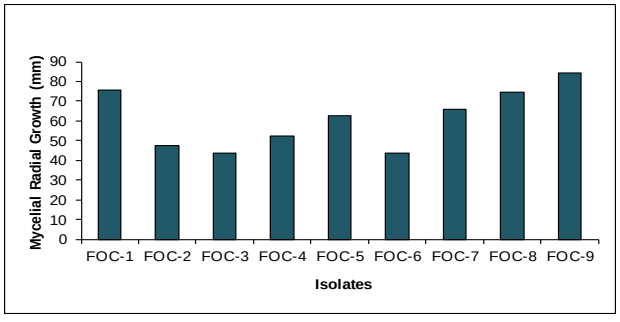
Effect of culture media on mycelial radial growth and sporulation

The results of the experiment revealed that the most effective medium supporting the growth of the fungus was oat meal agar medium (OMA) followed by Czapek's dox agar (CDA) medium which gave 90.00 mm and 84.50 mm mycelium colony growth of *F. oxysporum* f. sp. *ciceri* after an incubation of seven days respectively (Fig. 3). The results of the present study are in agreement with those achieved by Farooq *et al*., (2005). He mentioned that Minimum fungal growth was observed on PDA and the Czapeck's dox agar and CSMA media were the best for the radial growth of *F. oxysporum* as this fungus gave maximum growth of 85 and 80 mm, respectively. Maximum (4.06 x 10^5 ml^{-1}) micro conidia was produced by FOC-5 at PDA medium and minimum (2.41 x 10^3 ml^{-1}) sporulation was observed on FOC-3 at CDA medium. No micro conidia were produced by FOC-4 at V_8 JA and all isolates of WA medium (Table 6).

The highest sporulation of macro conidia was observed on FOC-1 (3.27 x 10^5 ml^{-1}) at PDA medium and the lowest sporulation was observed on FOC-3 (1.08 x 10^3 ml^{-1}) at V_8 JA medium. No sporulation was observed on FOC-8 at CDA; FOC-5, FOC-6, FOC-8 at MDA; FOC-6 at OMA and all isolates of WA medium (Table 7). Recently Imran Khan et al., (2011) studied effect of media on *F. oxysporum* f. sp. *ciceri* and found that PDA is best for the growth of different isolates. Khilare and Rafi Ahmed (2012) found that the fungus grew the best on Czapek's dox agar and PDA media among six culture media were tested. Jamaria (1972) reported maximum growth and sporulation of *F. oxysporum* f. sp. *vanillae* on potato dextrose agar, Richard's agar and Czapek's Dox agar. Khare *et al*., (1975) reported maximum growth of *Fusarium oxysporum* f. sp. *lentis* on PDA followed by lentil extract and Richard's Agar.



PDA



CDA

http://dx.doi.org/10.22161/ijeab/2.1.25 *ISSN: 2456-1878*

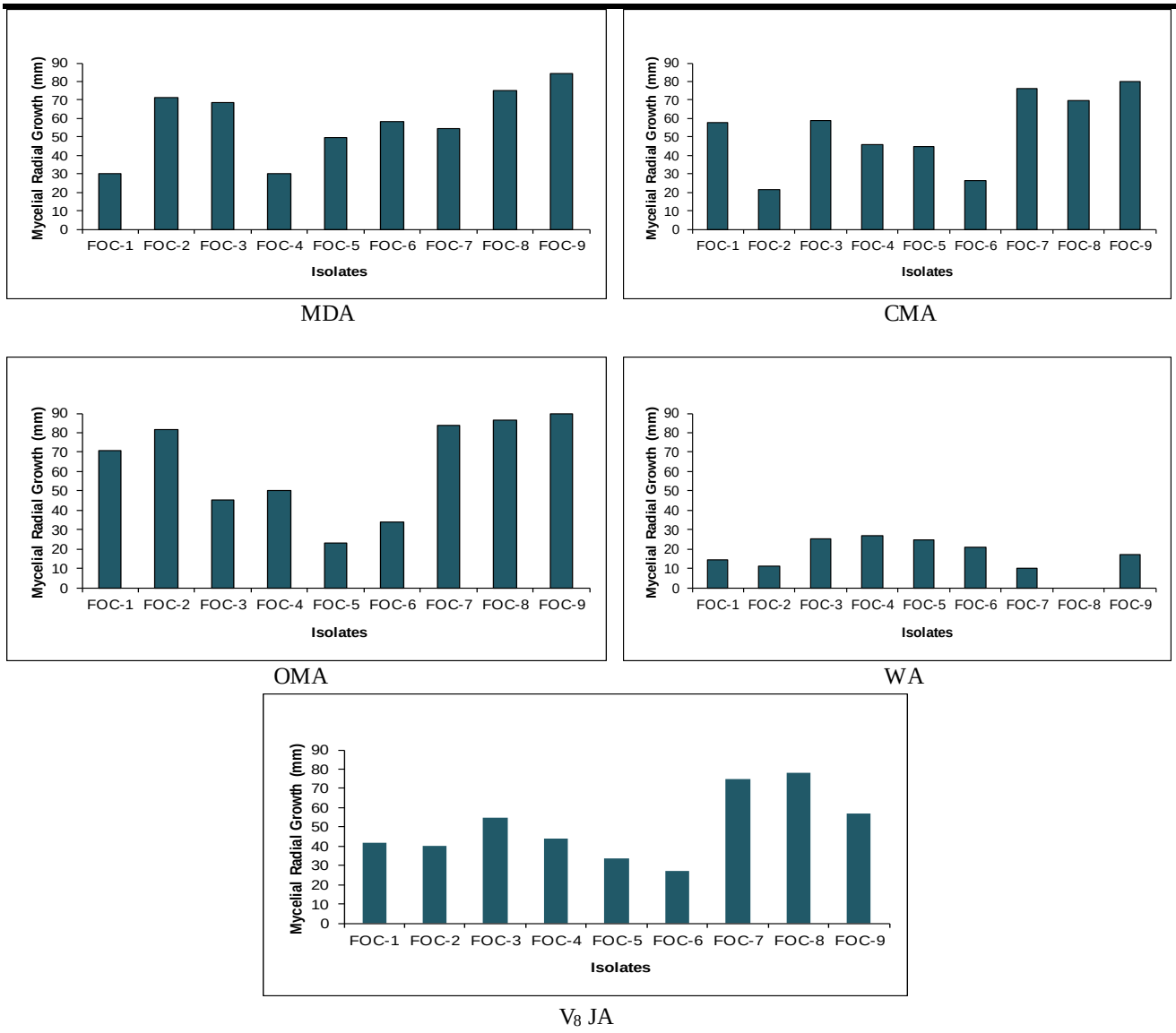


Fig.3: Effect of culture media on mycelial radial growth and sporulation of nine Fusarium oxysporum f. sp. ciceri isolates.

Table.6: Effect of culture media on production of micro conidia of nine Fusarium oxysporum f. sp. ciceri isolates

Isolates	Production of micro conidia (ml^{-1}) on different media						
	PDA	CDA	MDA	CMA	OMA	WA	V_8 JA
FOC-1	1.82x 10^5	1.33x 10^4	1.27x 10^4	1.55x 10^4	2.16x 10^4	*	7.22x 10^3
FOC-2	1.13x 10^5	1.33x 10^4	1.88x 10^4	1.00x 10^4	2.05x 10^4	*	6.11x 10^3
FOC-3	3.28x 10^4	2.41x 10^3	5.25x 10^3	4.32x 10^3	1.11x 10^4	*	4.32x 10^3
FOC-4	3.83x 10^4	2.83x 10^4	3.33x 10^3	8.33x 10^3	1.33x 10^4	*	*
FOC-5	4.06x 10^5	1.16x 10^4	8.33x 10^3	1.00x 10^4	2.16x 10^4	*	1.16x 10^4
FOC-6	4.66x 10^4	1.00x 10^4	6.66x 10^3	2.00x 10^4	1.33x 10^4	*	8.33x 10^3
FOC-7	5.50x 10^4	2.00x 10^4	1.83x 10^4	1.16x 10^4	1.00x 10^4	*	5.00x 10^3
FOC-8	7.50x 10^4	6.66x 10^3	2.33x 10^4	3.66x 10^4	2.00x 10^4	*	2.66x 10^4
FOC-9	5.83x 10^4	5.16x 10^4	8.33x 10^3	5.00x 10^3	2.33x 10^4	*	3.33x 10^3

* No sporulation

http://dx.doi.org/10.22161/ijeab/2.1.25 *ISSN: 2456-1878*

Table.7: Effect of culture media on production of macro conidia of nine Fusarium oxysporum f. sp. ciceri isolates

Isolates	Production of macro conidia (ml $^{-1}$) on different media						
	PDA	CDA	MDA	CMA	OMA	WA	V_8 JA
FOC-1	$3.27x10^5$	$4.38x10^4$	$2.44x10^4$	$5.55x\ 10^3$	$3.94x\ 10^4$	*	$1.11\ x\ 10^3$
FOC-2	$9.07x10^4$	$4.50x\ 10^4$	$3.50x\ 10^4$	$3.33x\ 10^3$	$3.77x\ 10^4$	*	$6.66\ x\ 10^3$
FOC-3	$1.75x10^5$	$1.02x\ 10^4$	$4.46x\ 10^3$	$1.49x\ 10^3$	$4.96x\ 10^3$	*	$1.08\ x\ 10^3$
FOC-4	$1.58\ x\ 10^5$	$1.50x\ 10^5$	$1.16x\ 10^4$	$1.66x\ 10^3$	$2.66x\ 10^4$	*	$5.00\ x\ 10^3$
FOC-5	$9.00\ x\ 10^4$	$5.00x\ 10^3$	*	*	$8.33x\ 10^3$	*	$1.66\ x\ 10^3$
FOC-6	$5.00\ x\ 10^4$	$5.00x\ 10^4$	*	$8.33x\ 10^3$	*	*	$3.33\ x\ 10^3$
FOC-7	$3.33x10^3$	$5.00x\ 10^4$	$5.33x\ 10^4$	$1.66x\ 10^3$	$2.00x\ 10^4$	*	$3.33\ x\ 10^3$
FOC-8	$8.33\ x\ 10^3$	*	*	$6.66x\ 10^3$	$1.00x\ 10^4$	*	$6.66\ x\ 10^3$
FOC-9	$1.06\ x\ 10^5$	$1.55x\ 10^5$	$6.16x\ 10^4$	$1.66x\ 10^3$	$7.50x\ 10^4$	*	$3.33\ x\ 10^3$

* No sporulation

Pathogenic variability

In the present study it was observed that *Fusarium* wilt infected seedlings collapse and lies flat on the ground surface retaining their dull green color. Adult plants showed typical wilt symptoms of drooping of petioles, rachis and leaflets. The roots of the wilted plants did not show any external rotting but when split open vertically, dark brown discoloration of internal xylem was observed. According to these observations it was confirmed that *F. oxysporum* f. sp. *ciceri* is pathogenic to chickpea, which has also been supported by the findings of Nene (1980), who after making detailed symptomatolgical studies observed diagnostic symptoms of wilt at seedling stage (3-5 weeks after sowing). The present study indicates that wilt incidence at 30 DAI and 60 DAI varied from 0% to 13.33%, at 45 DAI it was 6.67% to 53.33% whereas at 60 DAI it ranged from 13.33% to 86.67% (Table 8). The most virulent isolates were Foc-1 (86.67% wilt incidence), Foc-7 (73.33%) and Foc-8 (73.33%) while, the least virulent isolate was Foc-6 (13.33% wilt incidence). The remaining isolates showed intermediate response of variation in virulence. Ahmad (2010) noted that the pathogenic variability of 27 isolates against differential chickpea cultivars, the most virulence isolates was observed Foc-2 (AZRI, Bahawalpur), whereas, the least virulence was Foc-4 (Chakwal). Shehabu *et al.*, (2008) studied 24 isolates for wilt resistance on 10 chickpea lines and eight improved varieties and found F13, F20 and F22 most virulent isolate. Haware *et al.*, (1992) also found pathogenic diversity among chickpea wilt isolates.

Table.8: Wilt incidence and aggressiveness of nine Fusarium oxysporum f. sp. ciceri isolates on BARI Chola-1 at 30, 45 and 60 DAI

Isolates	Wilt incidence (%) at different days after inoculation (DAI)			Aggressiveness
	30 DAI	45 DAI	60 DAI	
FOC-1	13.33	53.33	86.67	HV
FOC-2	0.00	13.33	40.00	MV
FOC-3	6.67	13.33	20.00	LV
FOC-4	0.00	13.33	20.00	LV
FOC-5	13.33	40.00	46.67	MV
FOC-6	0.00	6.67	13.33	LV
FOC-7	6.67	53.33	73.33	V
FOC-8	6.67	53.33	73.33	V
FOC-9	0.00	13.33	20.00	LV
Control	0.00	0.00	0.00	-

REFERENCES

[1] Ahmed, A. U. (1996). Variation of twenty seven isolates of *Bipolaris sorokiniana* from wheat. MS Thesis, Institute of Postgraduate Studies in Agriculture (IPSA) Salna, Gazipur, Bangladesh.

[2] Ahmad, M. A. (2010). Variability in *Fusarium oxysporum* f. sp. *ciceris* for chickpea wilt resistance in Pakistan. Ph.D Thesis. Quaid-i-Azam University, Islamabad, Pakistan.

[3] Barhate, B. G.; Dake, G. N.; Game, B. C. and Padule, D. N. (2006).Variability for virulence in *F.*

oxysporum f. sp. *ciceri* causing wilt of chickpea. *Legume Res. 29 (4):* 308 – 310.

[4] Chaudhary, S. K. (1971). Studies of the Physiology of the *Fusarium oxysporum f. udum* (Butler) Snyder and Hansen (Causal Organism of Wilt of Cajanus cajan (Linn.) Millsp) Ph.D. Thesis, Deptt. of Bot. Ranchi University Ranchi, p. 297.

[5] Chauhan, S. K. (1962). Physiologic variation in *F. orthoceras* App. Wr. Var. *ciceris* causing wilt of gram. *Proc. Natn. Acad. Sci. Sec. B. 22*:78-84.

[6] Chauhan, S. K. (1963). Influence of different soils temperatures on the incidence of *Fusarium* wilt of gram (*C. arietinum* L.) *Proc. Indian. Acad. Sci. 8, 33*:552-554.

[7] Desai, S.; Nene, N. L. and Ramachandra Reddy, A. G. (1994). Races of *Fusarium oxysporum* causing wilt in chickpea. *Indian Journal of Mycology and Plant Pathology 24:* 120 – 127.

[8] Dubey S. C.; Singh, S. R. and Singh, B. (2010). Morphological and pathogenic variability of Indian isolates of *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. *Archives of Phytopathology and Plant Protection 43(2)*: 174 – 190.

[9] FAO (2008). Food and Agriculture Organization Yearbook Production. Rome, Italy: 46: 105-115.

[10] Farooq, S.; Iqbal, S.M. and Rauf, A. (2005). Physiological studies *of Fusarium oxysporum* f. sp. *ciceri. Int. J. Agri. Biol. 7(2):* 275-277.

[11] Haware, M. P.; Nene, Y. L. and Rajeshware, R. (1978). Eradication of *Fusarium oxysporum* f. sp. *ciceri* transmitted in chickpea seed. *Phytopathology 68*: 1364–7.

[12] Haware, M. P.; Nene, Y. L.; Pundir, R. P. S. and Narayana, J. (1992). Screening of world chickpea germplasm for resistance to *Fusarium* wilt. *Field Crops Res. 30*: 147– 154.

[13] Imran Khan, H. S.; Saifulla M.; Mahesh, S. B. and Pallavi, M. S. (2011). Effect of different media and environmental conditions on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing *Fusarium* wilt of chickpea. pp 67-78.

[14] Jamaria, S. L. (1972). Nutritional requirement of *Fusarium oxysporum* f. sp. *niveum. Indian Phytopathology 25*: 29 – 32.

[15] Khare, M. N.; Agarwal, S. C.; Dhyra O. D. and Kushwaha, L. S. (1975). Variability in the growth of light strains of *Fusarium oxysporum* f. sp. *lentis* as different solid media. *Indian Phytopathology 28*: 126-128.

[16] Khilare, V. C. and Rafi Ahmed (2012). Effect of different media, pH and temperature on the growth of *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. *Int. J. of Advance Biological Research 2(1):* 99-102.

[17] Mina, U. and Dubey, S. C. (2010). Effect of environmental variables on development of *Fusarium* wilt in chickpea (*Cicer arietinum*) cultivars. *Indian J. Agric. Sci. 80(3):*231-234.

[18] Nene, Y. L. and Haware, M. P. (1980). Screening chickpea for resistance to wilt. *Pl. Dis. 64:* 379–380.

[19] Nene, Y. L.; Haware, M. P. and Reddy, M. V. (1981). Chickpea diseases: resistance screening techniques. Information Bulletin No. 10, *Int. Crop Res. Inst.* for the Semi Arid Tropics, Patancheru. 1–10.

[20] Nene, Y. L.; Shiela, V. K. and Sharma, S. B. (1984). World list of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan*) pathogens. ICRISAT. *Pulse Pathology Programme report, 32*: 23 pp.

[21] Pande, S.; Galloway, Gaur, P.M.; Siddique, K. H. M.; Tripathi, H. S.; Taylor, P.; MacLeod, M. W. J.; Basadrai, A. K.; Bakr, M. A.; Joshi, S.; Kishore, K. G.; Isenegger, D.A.; Narayana, J. and Sharma, M. (2006). Botrytis gray mold of chickpea: a review of biology, epidemiology and disease management. *Australian J. Agril. Res. 57*: 1137-1150.

[22] Prasad, A.; Chaudhary, R. S. and Chaudhary, S. K. (1992). Effect of different pH levels on growth and sporulation of *Fusarium moniliforme V. subglutinans* Wr. and Rg., *the causal organism of wilt of maize. Bio. J. 4(1 and 2):* 75-78.

[23] Saxena, M. C. and Singh, K. B. (1987). The chickpea published by *C.A.B. Int.* ICARDA. pp 250-252.

[24] Saxena, M. C. (1990). Problems and potential of chickpea production in the nineties. p. 13–27. In H. A. van Rheenen and M. C. Saxena (ed.) Chickpea in the nineties: Proceedings of the Second Interna-tional Workshop on Chickpea Improvement. 4–8 Dec. (1989). ICRI- SAT, Patancheru, India. Chickpea diseases and their con RAPD markers. pp. 116.

[25] Shaikh, M. H. (1974). Studies on wilt of gram (*Cicer arietinum* L.) caused by *F. oxysporum* f. sp. *ciceris* in Marathwada region. M.Sc (Agri.) *Thesis*, Marathwada Krishividysapeeth, Parbhani, India. pp. 78-89.

[26] Sharma, K. D., Chen, W. and Muehlbauer, F. J. (2005). Genetics of chickpea resistance to five races of *Fusarium* wilt and a concise set of race differentials for *F. oxysporum f. sp. ciceriss.* Pl. *Dis. 89*:385-390.

[27] Shehabu, M.; Ahmed, S. and Sakhuja, P. K. (2008). Pathogenic variability in Ethiopian isolates of *F. oxysporum* f. sp. *ciceris* and reaction of chickpea

http://dx.doi.org/10.22161/ijeab/2.1.25 *ISSN: 2456-1878*

improved varieties to the isolates. *Int. J. Pest Management. 54(2):*143–149.

[28] Singh, F.; Singh, N. and Gupta, P. K. (1986). *Fusarium* wilt of chickpea - Current status of the methods of study and measures to reduce crop losses. In: P. K. Gupta and J. Bahel (eds.) Genetics and Crop Improvement. Rastogi and Co., Meerut, pp. 133-148.

[29] Singh, K. B. and Reddy, M. V. (1991). Advances in disease resistance breeding in chickpea. Advances in *Agron.45:* 191–222.

[30] Swati, T. and Rajan, P. (2014). Effect of different pH on the growth and sporulation of *Fusarium oxysporum*: the causal organism of wilt disease of tomato. *International Journal of Basic and Applied Biology (IJBAB) 2 (1):* 103 – 106.

http://dx.doi.org/10.22161/ijeab/2.1.26 *ISSN: 2456-1878*

Forms and Distribution of Potassium along a Toposequence on Basaltic Soils of Vom, Jos Plateau State of Nigeria

M. A. Tijjani *, A. A. David

Department of Soil Science, Allahabad School of Agriculture, Sam Higginbottom University of Agriculture, Technology & Sciences, Allahabad, 211007 U.P. India.

***Abstract*—** *The study was conducted in Vom, Jos Plateau state in the Southern Guinea Savanna zone of Nigeria to accentuate the forms of potassium distribution associated with topographic positions. The study area lies between longitudes 08^0 45' 01" and 8^0 47' 56'' E, latitudes 9^0 43' 17'' and 9^0 45' 15'' N, with an elevation of about 1270m above sea level. A stratified purposive sampling procedure was adapted, where four landscape positions were identified using Global Positioning System (GPS). The crest, upper slope, middle, and lower slope positions were identified, each representing changes in geomorphology. Two pedons were georeferenced at each topographic position, where they were sunk and described. Result show that the forms of K varied with topographic positions. Potassium distribution varied from surface to subsurface in different topographic positions. Water soluble K was higher at crest surface (0.0569 $cmolkg^{-1}$) and decreased with soil profile depth. Exchangeable K has highest value of 0.1317 and 0.1308 $cmol/kg^{-1}$ at both lower slope positions in general. Non exchangeable K values where higher at all surfaces than the subsurfaces of topographic positions. HCl soluble K values were higher at lower and upper slopes surface, moderately at middle and least at crest slope positions. Total K values were higher at upper slope subsurface, middle, and lower slope surface with low variations at the crest positions. However, the distribution of the K forms did not shown a well – defined trend with respect to topographic positions.*

Keywords*— *Potassium forms, topographic positions, Basaltic soil

I. INTRODUCTION

Potassium is the major nutrient and also a most abundant element in soils but the K content of the soil varies from place to place based on physicochemical properties of soil **(Lalitha and Dhakshinamoorthy 2013)**. It plays significant roles in translocation of photosynthates, imparting vigour to plants, stimulating the growth of legumes, increasing the availability of other elements like nitrogen and potash **(Sahai, 2011; Lakudzala, 2013)**. Soil potassium exists in four forms: solution, exchangeable, non-exchangeable, and total K **(Al-Zubaidi *et al.* 2011)**. The distribution of K forms differs with the soil depth and space depending on some overriding environmental and soil factors **(Reza *et al.* 2013)**. These forms, however, are in dynamic equilibrium with one another and change from one form to another. Exchangeable K, is held through electrostatic charges present on organic matter and on clay particles, non-exchangeable constitutes the fraction held between adjacent tetrahedral layers of dioctahedral and trioctahedral micas, vermiculite and intergrade minerals that is sparingly or moderately available to plants while mineral K as a portion of total K is present in such K-bearing minerals as muscovite, biotite, feldspars, microcline and orthoclase **(Conyers and Mc Clean, 1967; Sadusky *et al.* 1987; (Sparks, 2000); Uzoho and Ekeh 2014; (Uzoho et al. 2016).**

Topography generally modifies the development of soil in pedogenesis as a result of microclimate and drainage **(Pidwirny, 2006)**. It is a factor that causes properties differentiation along hillslope and among horizons thereby evaluating the interaction of pedogenic and geomorphic processes **(Gessler *et al.* 2000)**. The Soil formation, mineral weathering, geomorphological conditions have resulted in significant variation in total, non-exchangeable and exchangeable K along different topographic slope positions **(Rezapour *et al.* 2010); Samndi and Tijjani, 2014)**. Variations in slope positions, soil depth and clay mineralogy are some aspect of soil K distribution **(Koné *et al.* 2014)**. The soil at the crest and upper slope position has higher pH values compared to the lower slope position **(Sohotden *et al.* 2015)**. While on the other hand,

significantly higher surface pH values on the foot slope were recorded, moreover the acidic pH might be due to the effect of erosion and leaching of nutrients down the slope **(Tsui *et al*. 2004)**.

In Nigeria, **Obi *et al*. (2016)** studied the effect of land use on soil K forms reported that the amount of total K, non-exchangeable K, exchangeable K and water soluble K as well as pH differed along topographic positions from up to middle to lower positions. **Osodeke *et al*. (2014)** reported a strong relationship between topographic positions on Coastal Plain Sand parent material in Amaeba-Imo Area of Southeastern Nigeria, however this relationship with respect to basaltic parent materials of Vom Jos Plateau, particularly with respect to potassium distribution and its interrelationship has not been adequately published for sustaining crop production, particularly, root and tuber crops. This is because potassium imparts resistance to diseases and insects as well as drought tolerance **(Rehm and Schmitt, 2002).**

II. MATERIALS AND METHODS

Study Location: The study location was Vom, Jos Plateau State situated between longitude 08^0 45' 01 to 8^0 47' 56E'' and latitude 9^0 43' 17 to 9^0 45' 15N, with an elevation of about 1270m above sea level. It has a mean annual rainfall of about 1258mm and temperature of 24^0C. The soils of the study area were derived from Newer Basalts material with Ustic soil moisture and Iso hyperthermic temperature regime respectively **(Eswaran *et al*. 1997)**.

Sample Collection and Preparation: Geographic Position System was used to obtain the co-ordinates of the four topographic positions (crest, upper, middle and lower topographic positions) which were indentified and each representing geomorphologic variations among positions using stratified purposive sampling procedure. Two pedons were sunk and described by genetic horizons and was sampled for laboratory analysis.

Laboratory analysis: Soil pH was determined in water, using soil sample to water ratio of 1:5 and read with a glass electrode meter **(Blackmore *et al*. 1987)**. Water soluble K was determined by shaking 2g of soil with 10 mL of deionized water (1.5 w/v), after shaking for 30 minutes on mechanical shaker and later filtered to obtain clear extract according to **Jackson, (1973)**. Exchangeable K was measured by shaking 10g of soil sample in 1 M of NH_4OAC (buffered at pH 7) followed by filtration. Non-exchangeable K was determined using 5.0g of soil sample boiled in 50 mL of 1 M HNO_3 solution and leached with 1 M HNO_3. The difference between K extracted through HNO_3 and exchangeable K was taken as non-exchangeable K as describe by **De Tunk *et al*. (1943)**. Hydrochloric acid soluble K was extracted with 1N HCl using soil-acid ratio of 1:10 **(Piper, 1950)**. Total K was measured by digesting 2g of soil samples with 20 mL of $HClO\text{-}HNO_3$ acid mixture and leached with HCl according to **Rayment and Lyon, (2011)**. Mineral K was calculated by subtracting total K from $HN0_3$ extractable. All K forms extract were analyzed using the flame photometer.

III. RESULTS AND DISCUSSION

Soil pH values with respect to different topographic positions ranged between 5.7 and 7.5 (Table 1). Slightly higher mean value (7.0) was obtained on the crest positions, while for the other topographic positions, mean pH values varied from 6.1 to 6.3. The resultant lower soil pH variations might be due to moderately weathering of soil along the topographic positions. Similar narrow change in soil pH values with topographic positions was observed by **Sanaullah *et al*. (2016)**.

Mean values of soluble K from surface horizon were not significantly ($P > 0.05$) affected by different topographic slope positions (Table 1), however values were higher (0.0569 $cmolkg^{-1}$) on the crest position, this might be due to less runoff with little erosion at the surface than subsurface while the lowest (0.0187 $cmolkg^{-1}$) on lower topographic positions (Table 2). **Tsui *et al*. (2004)** reported that higher available K content on crest with slightly lower variability among different topographic positions. For the subsurface horizons, mean values were also not significant, although slightly higher mean (0.0345 $cmolkg^{-1}$) value was obtained on the middle topographic positions. Water soluble K distribution mean values were irregularly distributed for some profiles (Table 2). **Al-Zubaidi *et al*. (2011)** reported similar pattern of K distribution in some Lebanese soils.

The mean values of the exchangeable K in the overlaying horizons were also not statistically significant, though values were higher (0.1317 $cmolkg^{-1}$) on the upper topographic position, followed by the crest, lower, and middle topographic positions (Table 1). Morealso, the distribution of exchangeable K in the subsurface horizons were significant with respect to topographic positions. The lowest mean value obtained on the lower topographic position was (0.0860 $cmolkg^{-1}$) at middle slope lower than the highest mean (0.1308 $cmolkg^{-1}$) value at crest positions. **Rubio and Gill-Sotres, (1997)** reported that values of exchangeable K were lower at overlying horizons which might attributed to soil forming processes. Generally, values

http://dx.doi.org/10.22161/ijeab/2.1.26 *ISSN: 2456-1878*

of exchangeable K showed an irregular distribution with profile depth at both topographical slope positions.

The mean values of non exchangeable K were significantly affected by topographic positions for both surface and subsurface mean values (Table 1). However, the surface highest (0.7133 cmolkg^{-1}) and the lowest (0.2456 cmolkg^{-1}) mean values were recorded at both upper and crest position respectively, also with moderate (0.4461 and 0.5441 cmolkg^{-1}) mean values at both lower and middle topographic positions respectively. For the subsurface horizons, the highest (0.4060 cmolkg^{-1}) and the lowest (0.2136 cmolkg^{-1}) mean values were recorded on upper and crest topographic positions respectively. Meanwhile moderate (0.2424 and 0.3141 cmolkg^{-1}) mean values were recorded at both middle and lower topographic positions. The distribution of non-exchangeable K also showed an irregular trend with respect to various topographic positions. Generally, the values of non-exchangeable K were higher in surface horizons increased with soil depth across the different topographic positions (Table 3).

The distribution of HCl solution K was significantly affected by topographic positions for both surface and subsurface horizons. In the surface horizons, mean values of HCl soluble K values were higher on the lower topographic positions. The highest mean value (0.5601 cmolkg^{-1}) was recorded on the lower slope while the lowest (0.3315 cmolkg^{-1}) mean value was obtained on crest positions respectively. For the underlying horizons, highest and lowest mean values (0.5300 and 0.3428 cmolkg^{-1}) were both obtained on the middle and crest slope positions respectively. The distribution of both surface and subsurface HCl soluble K showed an irregular trend with increasing profile depth.

The surface distribution of total K was significantly affected by topographic positions. The highest and the lowest mean (1.0749 and 0.8306 cmolkg^{-1}) values were recorded at the middle and crest topographic positions respectively. Meanwhile for the underlying horizon, the highest and the lowest mean (1.2047 and 0.607 cmolkg^{-1}) value were also significantly at both upper and middle topographic positions respectively.

IV. CONCLUSION

The soil pH showed an irregular distribution trends across the various topographic positions. The surface distribution of water soluble K values were higher (0.1374 cmolkg^{-1}) on crest followed by middle, upper and least at the upper topographic positions. For the underlying horizons, water soluble K was lower (0.0205 cmolkg^{-1}) at the crest. Likewise for the surface distribution of exchangeable K, mean values were not significantly affected with respect to topographic positions. However, mean higher values (0.1317 cmolkg^{-1}) were recorded on upper slope, followed by crest, lower and least at middle positions. The underlying surface horizons indicated that the values were significantly affected by different topographic positions with the highest (0.1109 cmolkg^{-1}) on the crest, followed by lower, middle and least at the upper slope. The values of the non exchangeable K for the surface and subsurface horizons were statistically significant, though higher values were obtained on surface than subsurface and irregularly distributed across the horizons irrespective of the topographic positions. The HCl soluble K distribution was significantly influence by the various topographic position for both surface and subsurface horizons. The lowest (0.3315 and 0.3428 cmolkg^{-1}) mean values were obtained on both crests of the two horizons. The effect of topographic positions on total K distribution for the surface and subsurface horizons was statistically significant, with the lowest (0.8306 and 0.7060 cmolkg^{-1}) mean values obtained on the crest of the two horizons.

Table.1: Mean forms of potassium distribution in surface and subsurface soils on various topographic positions of the study area.

	Water soluble K	**Exchangeable K**	**Non exchangeable K**	**HCl solution K**	**Total K**
Variable	**Cmolkg^{-1}**				
Surface topographic positions					
Crest	0.0569	0.1158	0.2456	0.3315	0.8306
Upper slope	0.0205	0.1317	0.7133	0.5068	0.8898
Middle slope	0.0276	0.1086	0.5441	0.4871	1.0749
Lower slope	0.0187	0.1122	0.4461	0.5601	1.0325

http://dx.doi.org/10.22161/ijeab/2.1.26 *ISSN: 2456-1878*

F- test	NS	NS	S	S	S
S. Ed. (±)	0.016	0.023	0.011	0.013	0.065
C. D. (P = 0.05)	0.034	0.049	0.022	0.027	0.138
Subsurface topographic positions					
Crest	0.0225	0.1109	0.2136	0.3428	0.7060
Upper slope	0.0241	0.0131	0.4060	0.4738	1.2047
Middle slope	0.0345	0.0860	0.2424	0.5300	0.6070
Lower slope	0.0205	0.0986	0.3141	0.3960	0.8746
F- test	NS	S	S	S	S
S. Ed. (±)	0.035	0.027	0.015	0.016	0.078
C. D. (P = 0.05)	0.074	0.058	0.032	0.034	0.164

Table.2: Forms of potassium distribution in soil profiles on the crest, upper, middle and lower topographic positions in the study area.

Horizon	**Depth (cm)**	**pH**	**Water Soluble K ($cmolkg^{-1}$)**	**Exchangeable K ($cmolkg^{-1}$)**	**Non Exchangeable K ($cmolkg^{-1}$)**	**HCl soluble K (cmo/kg^{-1})**	**Total K ($cmolkg^{-1}$)**
Crest profile 1							
A	0-14	6.5	0.0605	0.0997	0.3526	0.3101	1.2581
Bt1	14-29	6.4	0.0305	0.0641	0.2403	0.3541	0.5453
Bt2	39-73	7.3	0.0303	0.0713	0.5040	0.3471	0.4034
Bt3	73-120	6.9	0.0232	0.0749	0.2009	0.3219	0.9966
BC	120-143	7.2	0.0142	0.0677	0.1673	0.3242	0.7590
Crest profile 2							
A	0-16	7.1	0.2140	0.1318	0.1385	0.3169	0.4034
AB	16-59	6.9	0.0160	0.2352	0.0621	0.2688	0.7368
Bt1	59-94	7.0	0.0142	0.0818	0.2317	0.2173	0.8068
Bt2	94-137	7.3	0.0214	0.0749	0.2223	0.6442	0.7829
BC	137-180	7.5	0.0305	0.2172	0.0800	0.2651	0.6171
Upper slope profile 1							
A	0-10	6.4	0.0232	0.1815	0.4579	0.3794	0.5932
AC	10-50	6.1	0.0214	0.0818	0.4240	0.4240	1.0923
Cr	50-130	6.1	0.0303	0.1282	0.2118	0.5041	1.2342
Upper slope profile 2							
A	0-14	6.3	0.0178	0.0818	0.9686	0.6342	1.1863
AC	14-39	6.0	0.0285	0.0749	0.3453	0.5022	1.4239
Cr	39-125	6.5	0.0160	0.2387	0.6427	0.4648	1.0684
Middle slope profile 1							
A	0-29	6.1	0.0356	0.0926	0.4487	0.4133	1.4947
B	29-80	6.0	0.0249	0.0713	0.1746	0.4133	0.9504

http://dx.doi.org/10.22161/ijeab/2.1.26 *ISSN: 2456-1878*

Bt1	80-122	6.1	0.0303	0.0356	0.3828	0.5608	0.1658
Bt2	122-147	6.0	0.1060	0.0641	0.1835	0.3973	0.9265
Cr	147-185	6.2	0.0249	0.1567	0.1389	0.3169	0.4752
Middle slope profile 2							
A	0-31	6.6	0.0196	0.1246	0.6394	0.5609	0.6550
AC	31-62	6.1	0.0178	0.0749	0.3135	0.5483	0.8068
Cr1	62-123	6.3	0.0106	0.0785	0.2812	0.5519	0.4752
Cr2	123-167	7.3	0.0267	0.1210	0.2226	0.3579	0.5453
Lower slope profile 1							
A	0-28	6.4	0.0232	0.1354	0.7035	0.5537	1.2581
Bt1	28-77	5.7	0.016	0.0641	0.4537	0.4040	0.7128
Bt2	77-135	5.7	0.0142	0.0785	0.3063	0.3986	1.2103
Cr	135+	5.7	0.0196	0.0713	0.066	0.2794	0.9966
Lower slope profile 2							
A	0-22	6.3	0.0142	0.0890	0.1886	0.5665	0.8068
B	22-64	6.1	0.0214	0.0641	0.2848	0.6124	1.0923
BC	64-93	6.1	0.0305	0.0785	0.2541	0.4325	0.7366
Cr	93+	7.1	0.0214	0.2352	0.5198	0.2490	0.4991

Table.3: Mean values of surface and subsuface forms of potassium distribution in soil profiles on the various topographic positions in the study area.

Horizon	Water soluble K ($cmolkg^{-1}$)	Exchangeable K ($cmolkg^{-1}$)	Non exchangeable K ($cmolkg^{-1}$)	HCl Soluble K ($cmolkg^{-1}$)	Total K ($cmolkg^{-1}$)
CREST PROFILE					
surface	0.0569	0.1158	0.2456	0.3135	0.8380
subsurface	0.0225	0.1109	0.2136	0.3428	0.7070
UPPER SLOPE					
surface	0.0205	0.1317	0.7133	0.5068	0.8898
subsurface	0.0241	0.1308	0.4060	0.4870	1.2047
MIDDLE SLOPE					
surface	0.0276	0.1086	0.5441	0.4871	1.0749
subsurface	0.0345	0.0860	0.2424	0.5300	0.6207
LOWER SLOPE					
surface	0.0187	0.1122	0.4461	0.5601	1.0325
subsurface	0.0205	0.0986	0.3141	0.3960	0.8746

REFERENCES

[1] Al-Zubaidi, A., Bashour, I., Darwish, T. and Safieddine, M. (2011). *Content of Different Forms of Potassium in Lebanese Soils.* Switzerland: International potash Institute.

[2] Blackmore, L. C., Searle, P. L. and Daly, B. K. (1987). Method for Chemical Analysis of Soil. *New Zealand Soil Bureau Scientific Report* , 103.

[3] Conyer, E. and Mclean, L. (1969). Plant Uptake and Chemical Extractions for Evaluating Potassium Release Characteristics of Soil. *Soil Sci. Am. Proc* , 226-230.

[4] De Tunk, E. E., Wood, L. K. and Bray, R. H. (1943). Potassium fixation in corn belt soils. *Soil Science* , 1-12.

[5] Eswaran, H., Almaraz, R., van den Berg, E. and Reich, P. (1997). An assessment of soil resources of Africa in relation to productivity. *Geoderma* , 1-18.

[6] Gessler, P. E., Chamran, F., Althouse, L. and Holmes, K. (2000). Modelling soil-landscape and ecosystem properties using terrain attributes. *Soil Science Society of America* , 2046-2056.

[7] Jackson, M. L. (1973). *Soil Chemical Analysis.* new Delhi: Prentice Hall of India, Private Limited.

[8] Koné, B., Bongoua-Devisme, A. J., Hippolyte, K. K., Firmin, K. K. and Joachim, T. M. (2014). Potassium supplying capacity as indicated by soil colour in Ferralsol environment. *Basic Research Journal of Soil and Environmental Science* , 46-55.

[9] Lakudzala, D. D. (2013). Potassium responce in some Malawi soils. *Internatonal Letters of Chemistry, Physics and Astronomy* , 175-181.

[10] Lalitha, M. and Dhakshinamoorthy, M. (2013). Forms Of Soil Potassium- A Review. *Agri. Reviews* , 64-68.

[11] Obi, J. C., Ibia, T. O. and Eshiet, P. B. (2016). Effect of land use on potassium forms of coastal plain sands of Nigeria. *Chemistry and Ecology* , 1-21.

[12] Osodeke, V. E., Akinmutimi, A. L. and Inyama, C. (2014). Potassium Distribution along a Toposequence of Coastal Plain Sand Parent Material in Amaoba-Ime Area of Southeastern Nigeria. *Nigerian Journal of Soil Science and Environmental Research* , 1-9.

[13] Pidwirny, M. (2006). *"Soil Pedogenesis".Fundamentals of Physical Geography.* Okanagan: Scott Jones University of British Columbia.

[14] Piper, C. S. (1950). *Soil and Plant Analysis.* New York: Inter Science Publishers.

[15] Rayment, G. E. and Lyon, D. L. (2011). *Soil Chemical Method.* Australia: Csiro Publishing.

[16] Rehm, G. and Schmitt, M. (2002). *Potassium for crop production.* Minnesota USA: Regents of the University of Minnesota.

[17] Reza, S. K., Utpal, B., Chattopadhay, T. and Dipak, S. (2013). Distribution of forms of potassium in relation to different different agroecological regions of North-Eastern India. *Archives of Agronomy and Soil Science* , 507-518.

[18] Rezapour, S. A., Samadi, A. A., Jafarzadeh, S. and Oustan, S. (2010). Impact of Clay Mineralogy and Landscape on Potassium Forms in Calcareous Soils, Urmia Region. *Journal Agr. Sci. Tech.* , *12*, 495-507.

[19] Rubio, B., and Gill-Sotres, F. (1997). Distribution of four major forms of potassium in soils of Galicia (N.W. Spain). *Communications in Soil Science and Plant Analysis* , 1805-1816.

[20] Sadusky, M. C., Sparks, D. L., Noll, M. R. and Hendricks, G. J. (1987). Kinetics and mechanisms of potassium release from sandy soils. *Soil Science Society of America Journal* , 1460-1465.

[21] Sahai, V. N. (2011). *Fundamentals of Soil* . New Delhi: Kalyani Printings.

[22] Samndi, M. A. and Tijjani, M. A. (2014). Distribution of Potassium Forms along a Hillslope Positions of Newer Basalt on the Jos Plateau Nigeria. *International Journal of Soil Science* , 90-100.

[23] Sanaullah, A. F., Akhtaruzzaman, M. and Uddin, M. A. (2016). Effect of Topography and Soil Depth on Clay Content, Organic Matter Content, Active Acidity, Reserve Acidity and Cation Exchange Capacity of Some Tea Soils of Bangladesh. *Journal of Scientific Research* , 229-235.

[24] Sohotden, C. D., Vivan, E. L., Ali, A. Y. and Shehu, B. M. (2015). An Assessment Of Landscape Segments Suitability For Agriculture in Kwrang Volcanic Area Of Jos Plateau, Nigeria. *International Journal of Scientific and Technology Research* , 294-297.

[25] Sparks, D. L. (2000). *Bioavailability of soil potassium, D-38-D-52. In M.E. Sumner (ed.).* Florida: Handbook of Soil Science, CRC Press, Boca Raton.

[26] Tsui, C. C., Chen, Z. S. and Hsieh, C. F. (2004). Relationship between soil properties and slope position in a lowland rain forest of southern Taiwan. *Geoderma* , 131-142.

[27] Uzoho, B. U. and Ekeh, C. (2014). Potassium status of soils in relation to land use types in Ngor- Okpala, Souteastern, Nigeria. *Journal of Natural Sciences Research* , 104-114.

[28] Uzoho, B. U., Ihem, E. E., Ogueri, E. I., Igwe, C. A., Effiong, J. A. and Njoku, G. U. (2016). Potassium Forms in Particle Size Fractions of Soils on Toposequence in Mbano, Southeastern Nigeria. *International Journal of Environment and Pollution Research* , 1-11.

http://dx.doi.org/10.22161/ijeab/2.1.27 *ISSN: 2456-1878*

Development of Smart Automated Irrigation System

Sirisha Adamala[1], Krishna Reddy YV[2]

[1]Assistant Professor, Department of Applied Engineering, Vignan's Foundation for Science, Technology and Research University (VFSTRU), Vadlamudi (Guntur), Andhra Pradesh, India
[2]Research Assistant, Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad, Telangana, India

Abstract*—** *This study is designed to develop an automatic irrigation system that switches (ON/OFF) a pump motor by sensing the moisture content of the soil using wireless technology. Through* ***GSM Modem*, the sensed moisture content data will be sent as an SMS to the user. The project uses* ***8051 series microcontroller****, which is programmed to receive the input signal of varying moistures of the soil through sensors. This is achieved by using an* ***op-amp*** *as comparator which acts as interface between the sensing device and the microcontroller. Once the controller receives the signal, it generates an output that drives a relay for operating the water pump. It also sends an SMS to the concerned number using GSM modem. An LCD display is also interfaced to the microcontroller to display the status of the soil and water pump ON/Off condition. The sensing arrangement is made using two stiff metallic rods inserted to the agricultural field required to be in control. Connections from the metallic rods are interfaced to the control unit. This concept can also be enhanced by integrating XBEE/Bluetooth technology, such that whenever the water pump switches ON/OFF, the information is sent to a smart mobile phone or XBEE transceiver module regarding the status of the pump.*

Keywords—Automation, Irrigation, Micro-controller, Bluetooth, GSM Module.

I. INTRODUCTION

Water is a very precious resource and must be properly utilized. Agriculture is one of those areas which consume a lot of water. Irrigation is a time consuming process and must be done on a timely basis. The aim of this study is to develop an auto irrigation system which measures the moisture of the soil and automatically turns on or off the water supply system. The project requires very less human involvement once installed. The circuit is based on PIC microcontroller and also a soil moisture sensor. A properly configured soil moisture sensor can save up to 60 percent of water used in irrigation. Irrigation system uses valves to turn irrigation ON and OFF. These valves may be easily automated by using controllers and solenoids. Automating farm or nursery irrigation allows farmers to apply the right amount of water at the right time, regardless of the availability of labor to turn valves on and off. In addition, farmers using automation equipment are able to reduce runoff from over watering saturated soils, avoid irrigating at the wrong time of day, which will improve crop performance by ensuring adequate water and nutrients when needed. It also helps in time saving, removal of human error in adjusting available soil moisture levels and to maximize their net profits. A lot of research has been done by many authors (Dukes et al. 2003; Suriyachai et al. 2012; Smajstrla and Locascio, 1996; Phene and Howell, 1984; Nogueira et al. 2003; Dursun and Ozden, 2011; Prathyusha and Suman, 2012; Gracon et al. 2010; Dukes and Scholberg, 2005).

Irrigation of plants is usually a very time consuming activity; to be done in a reasonable amount of time, it requires a large amount of human resources. Traditionally, all the steps were executed by humans. Now a days, some systems use technology to reduce the number of workers or the time required to water the plants. With such systems, the control is very limited, and many resources are still wasted. Water is one of these resources that are used excessively. Flood irrigation is one method used to water plants. This method represents massive losses since the amount of water given is in excess of plants need. The contemporary perception on of water is that of a free, renewable resource that can be used in abundance. However, this is not reality; in some parts of India, water consumption is taxed. It is therefore, reasonable to assume that it will soon become a very expensive resource everywhere. In addition to excess cost of water labour is becoming more and more expensive. As a result, if no effort is in invested in optimizing these resources, there will be more money involved in this process. Technology is probably a solution to reduce the costs and prevent loss of resources. The objective of this study is to design a small scale automated irrigation system that would use water in a more efficient way, in order to prevent water loss and minimize the cost of labour.

II. MATERIALS AND METHODS

This study proposed an embedded system for automatic control of irrigation (Fig. 1). This project has wireless

http://dx.doi.org/10.22161/ijeab/2.1.27 *ISSN: 2456-1878*

sensor network for real-time sensing and control of an irrigation system. This system provides uniform and required level of water for the agricultural farm and it avoids water wastage. When the condition of water in the agricultural farm is abnormal then the system automatically switches ON the motor. When the water level reaches normal level the motor automatically switch OFF. In this project we are interfacing microcontroller through temperature sensor, humidity sensor and also interfacing to GSM through wireless network. In this we set specified values of temperature, humidity and the conditioned is uniformly monitored by any programming language.

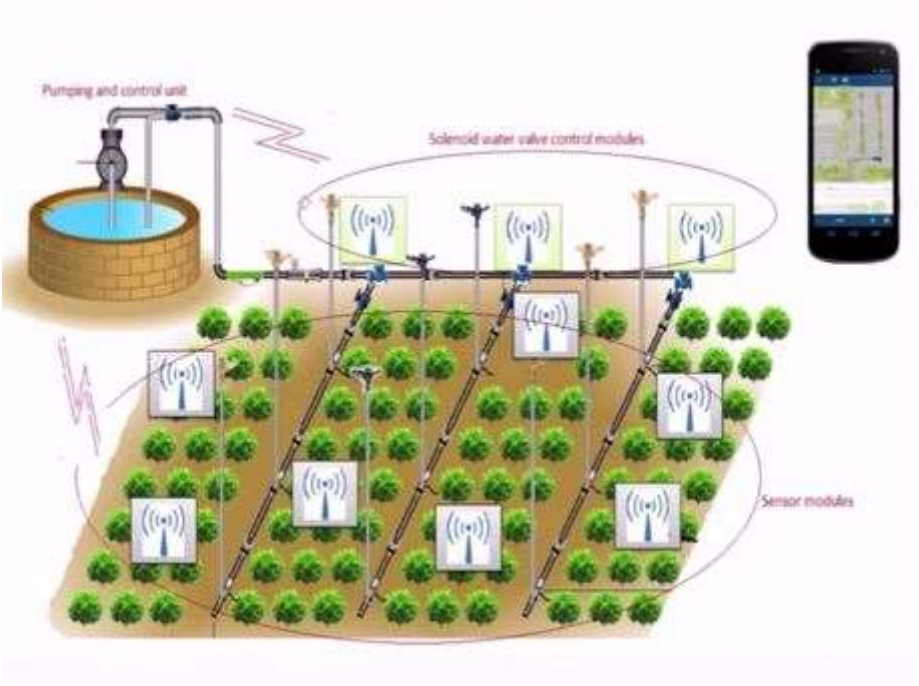


Fig. 1 Automated Irrigation System

The various equipments needed to develop an automated smart irrigation system are listed in Table 1. Hardware requirements are Microcontrollers (AT89C52/S52), opamp, Max232, GSM modem, Crystal oscillator, Switch, LED, Resistors, Capacitors, voltage Regulator, relay driver (ULN2003), relay, DB9 connector. Software requirements are Keil compiler and programming language compiled is embedded C (Or) Assembly.

Table.1.: Detailed components required for designing a smart irrigation system

Component Name	Configuration
Resistors	330R, 1K, 2.2K, 4.7K, 10K and 10K Preset
Capacitors	1000uF/35V, 10uF/63V, 1uF/63V, 33pF Ceramic
Integrated Circuits	7805, 7809, AT89S52, MAX232, LM358
IC Bases	40-PIN BASE, 16-PIN BASE, 08-PIN BASE
Diodes	1N4007
BC547	
CRYSTAL	11.0592MHz
DB9 Male Connector PCB Mounted	
Straight DB9 Cord	
LCD	16x2
LED-RED	
12V Relay	
2 PIN Push Button	
Power Cord	
Transformer	0-12V
Female Burge	0-12V

http://dx.doi.org/10.22161/ijeab/2.1.27 *ISSN: 2456-1878*

Male Burge (Included With LCD)	16 PIN
PCB Connector	2-PIN
Sensor Strip	
Male Relement	2-PIN
Male Burge	2-PIN
Female Relement (Transformer & GSM MODEM)	2-PIN
GSM MODEM	
DC Pin	
Heat Sink	
Screw Nut For Heat-Sink	
Submersible Pump	
Copper Wire for Load	
Plain PCB	
Soldering LED	50 gm
Connecting Wire	

The entire field is first divided into small sections such that each section should contain one moisture sensor (Fig. 2). These sensors are buried in the ground at required depth. Once the soil has reached desired moisture level the sensors send a signal to the micro controller to turn on the relays (Fig. 3), which control the motor. In this system, automated irrigation mechanism turns the pumping motor ON and OFF on detecting the dampness content of the soil. In the domain of farming, utilization of appropriate means of irrigation is significant. The benefit of employing these techniques is to decrease human interference. This automated irrigation project, the soil sensor senses the moisture content by giving input signal to an Arduino board which operates on **8051 series micro-controller (**Fig. 4), is programmed to collect the input signal of changeable dampness circumstances of the earth via dampness detecting system.

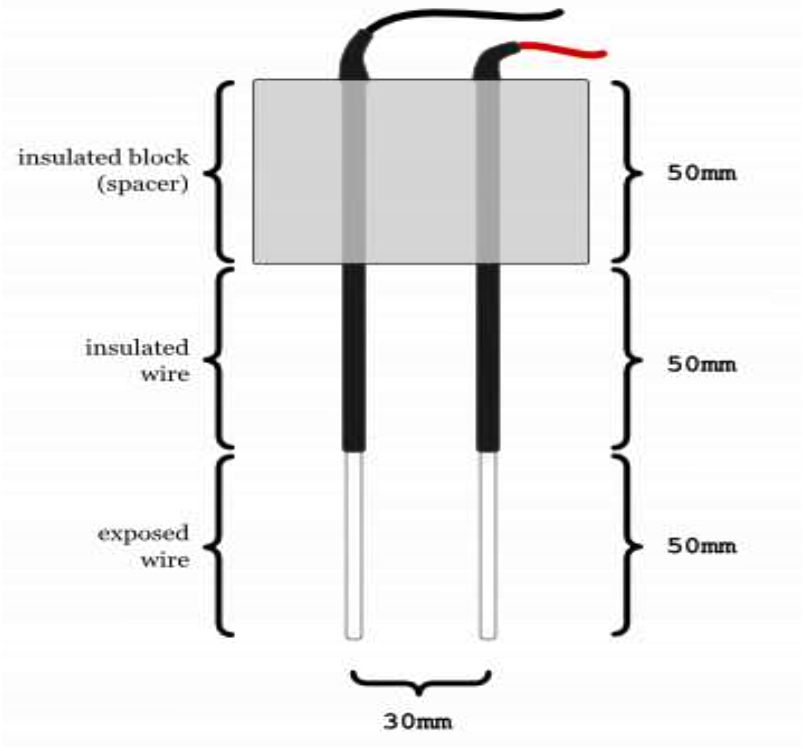


Fig. 2 Soil moisture sensor

http://dx.doi.org/10.22161/ijeab/2.1.27 *ISSN: 2456-1878*

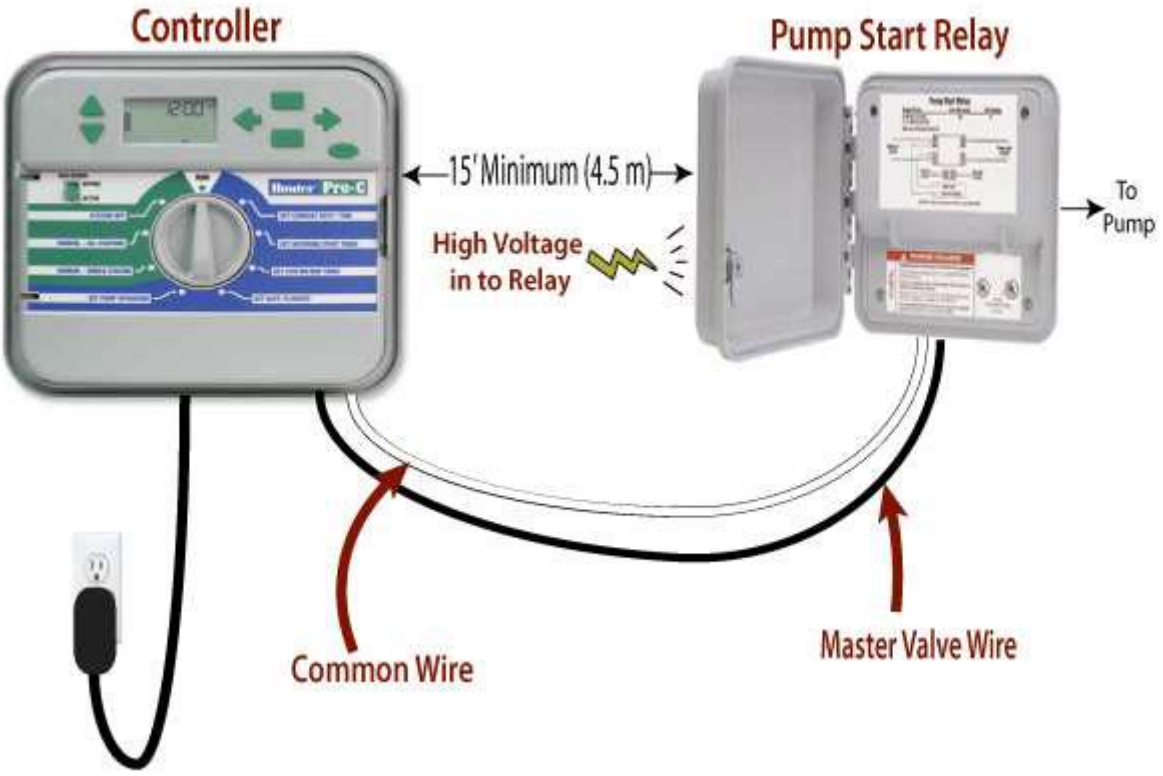


Fig. 3: Op-amp Relay

Fig. 4: 8051 Microcontroller

The idea of the study is to implement an automatic irrigation system by sensing the moisture of the soil. The working of the circuit is shown in Fig. 5. The microcontroller used in the project is 8-bit microcontroller. The main functions of the microcontroller are reading the values from the soil moisture sensor, displaying appropriate messages on the LCD and controlling the relay to the motor. The soil moisture sensor is inserted in the soil. Depending on the quality of the sensor, it must be inserted near the roots of the plant. The soil moisture sensor measures the conductivity of the soil. Wet soil will be more conductive than dry soil. The soil moisture sensor module has a comparator in it. The voltage from the prongs and the

http://dx.doi.org/10.22161/ijeab/2.1.27 *ISSN: 2456-1878*

predefined voltage are compared and the output of the comparator is high only when the soil condition is dry. This output from the soil moisture sensor is given to the analogue input pin of the microcontroller. The microcontroller continuously monitors the analogue input pin. When the moisture in the soil is above the threshold, the microcontroller displays a message mentioning the same and the motor is off. When the output from the soil moisture sensor is high i.e. the moisture of the soil is less. This will trigger the microcontroller and displays an appropriate message on the LCD and the output of the microcontroller, which is connected to the base of the transistor, is high. When the transistor is turned on, the relay coil gets energized and turns on the motor. The LED is also turned on and acts as an indicator. When the moisture of the soil reaches the threshold value, the output of the soil moisture sensor is low and the motor is turned off. The system is also designed to warn when the moisture is very high than the threshold and the soil is too wet, which is dangerous for the plant.

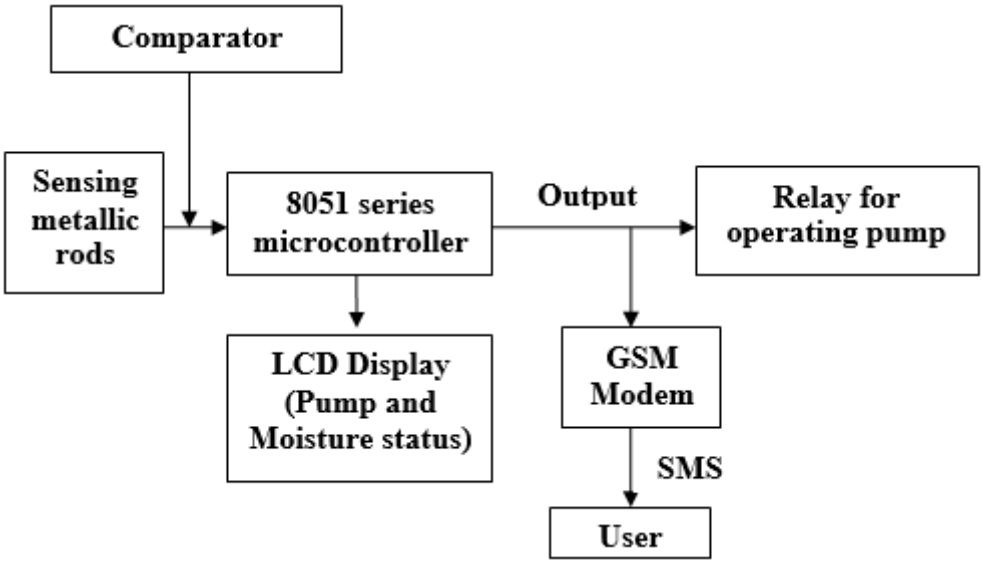


Fig. 6: Methodology diagram

Fig. 7 shows Microcontroller based irrigation system, which proves to be a real time feedback control system that monitors and controls all the activities of irrigation system efficiently.

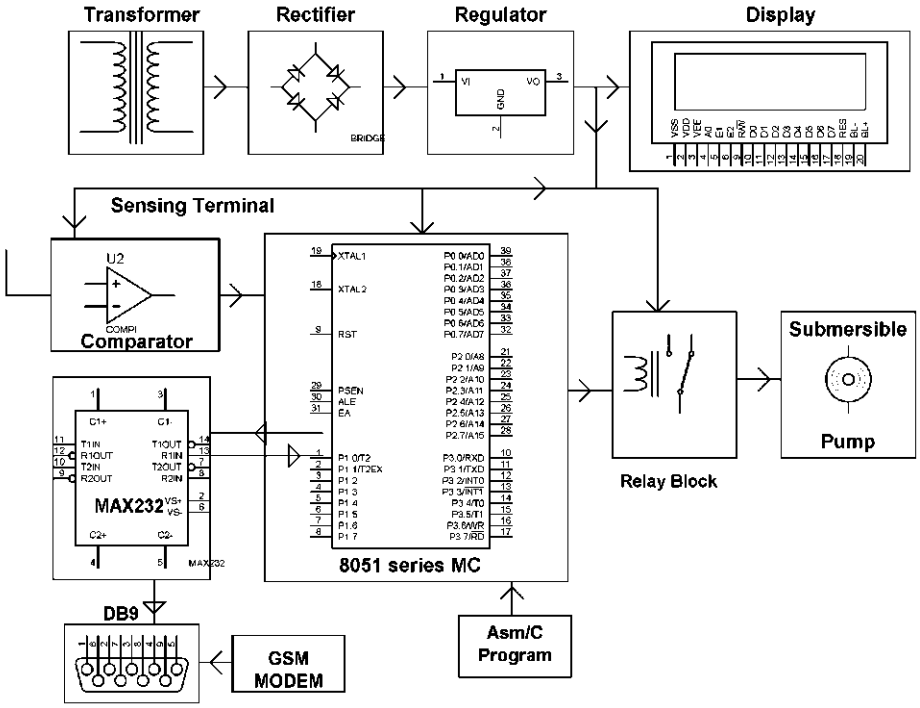


Fig. 7: Block diagram of the system

III. RESULTS AND DISCUSSION

Irrigation becomes easy, accurate and practical with the idea above shared and can be implemented in agricultural fields in future to promote agriculture to next level. The output from moisture sensor and level system plays major role in producing the output. The chosen approach is expected to yield the following results.

- Reduced labour
- Reduced monitoring
- Decrease in water input
- Low maintenance
- Low power consumption

The advantage of using this method is to reduce human intervention and to ensure proper irrigation.

- Minimizes water waste and improves plant growth.
- This system is designed to work automatically and hence, there is no need for any human intervention.

IV. CONCLUSIONS

The primary applications for this project are for farmers and gardeners who do not have enough time to water their crops/plants. It also covers those farmers who waste of water during irrigation. The project can be extended to greenhouses where manual supervision is far and few in between. The principle can be extended to create fully automated farmlands. Combined with the principle of rain water harvesting, it could lead to huge water savings if applied in the right manner. In agricultural lands with severe shortage of rainfall, this model can be successfully applied to achieve great results with most types of soil.

REFERENCES

[1] Dukes, M. D. and Scholberg, J. M. (2005). Soil moisture controlled subsurface drip irrigation on sandy soils. Applied Engineering in Agriculture 21(1): 89-101.

[2] Dukes, M. D., Simonne, E. H., Davis, W. E., Studstill, D. W., Hochmuth, R. (2003). Effect of sensor-based high frequency irrigation on bell pepper yield and water use. Proceedings of 2nd International Conference on Irrigation and Drainage, May 12-15. Phoenix, AZ. pp. 665-674.

[3] Dursun, M. and Ozden, S. (2011). A wireless application of drip irrigation automation supported by soil moisture sensors. Scientific Research and Essays, 6(7), pp. 1573-1582.

[4] Gracon H. E. L. de Lima, Lenardo C. e Silva, and Pedro F. R. Neto (2010). WSN as a tool for supporting agriculture in the precision irrigation, Sixth International Conference on Networking and Services,137-142.

[5] Nogueira, L. C., Dukes, M. D., Haman, D. Z., Cholberg, J. M., Cornejo, C. (2003). Data acquisition and irrigation controller based on CR10X datalogger and TDR sensor. Proceedings Soil and Crop Science Society of Florida 2002, 62:38-46.

[6] Phene, C. J. and Howell, T. A. (1984). Soil sensor control of high frequency irrigation systems. Transactions ASAE, 27(2):392-396.

[7] Prathyusha, K. and Suman, M. C. (2012). Design of embedded systems for the automation of drip irrigation. IJAIEM 1(2).

[8] Smajstrla, A. G. and Locascio, S. J. (1996). Tensiometer-controlled drip irrigation scheduling of tomato. Applied Engineering in Agriculture, 12(3):315-319.

[9] Suriyachai, P., Roedig, U. and Scott, A. (2012). A survey of MAC protocols for mission-critical applications in wireless sensor networks. Commun. Surveys Tuts., 14(2), 240–264.

http://dx.doi.org/10.22161/ijeab/2.1.28 *ISSN: 2456-1878*

Analysis and Determinants of Profit Efficiency of Cassava Farmers in Cross River State, Nigeria

Ettah O. I.[*1], Kuye O.O.[2]

*[1,2]Department of Agricultural Economics and Extension, Cross River State University of Science and Technology, Obubra Campus, Calabar, Nigeria.

Abstract**—** *A study on the analysis and determinants of profit efficiency of cassava farmers in Cross River State, Nigeria was carried out using the stochastic frontier profit function of Cobb-Douglas functional form. Data for the study were collected from primary sources with the aid of a set of structured and pre-tested questionnaires. For the determinants of profit efficiency, the minimum and maximum profit efficiency was 0.14 and 0.91 respectively with mean profit efficiency of 0.65. The mean profit efficiency implies that farmers were able to obtain 65% of their potential profit from a unit mix of inputs. In other words, about 35% of the profit is lost to inefficiency of management. Thus in the short run, there is a scope for increasing profit from cassava production by 35%. Age (0.37), education (0.67) and household size (0.58) had positive impact on profit inefficiency. The analysis of profit inefficiency effect showed a significant gamma (γ = 0.86). This implies that 86% deviation from maximum profit obtainable was as a result of inefficiency of the farmers rather than random error or variability. The signs and significance of the estimated coefficients in the inefficiency model have important implication on profit efficiency of the farmers. It is recommended that farmers should be encouraged to invest in cassava production for its profitability and economic value, inputs should be made available and at affordable prices especially improved varieties of cassava cuttings and cassava farmers should be encouraged to receive training on proper agronomic practices and usage of inputs to enhance profit efficiency of input use.*

Keywords**— Cassava, profit efficiency, profit function, farmers and determinants.**

I. INTRODUCTION

Profit is the excess of revenue over costs. There are basically two concepts of profits. These are the accounting and economic profits. In arriving at the accounting profit, only explicit costs are considered while the economic profit concept accounts for both implicit and explicit costs (Kolawole, 2006). Thus economic profit concept records higher amount of total cost and lower total profit relative to accounting profit. Arene (2000) noted that no alternative hypothesis explains and predicts the behaviour of firms better than the profit maximizing assumption. Olayide and Heady (1982) had earlier affirmed that all other objectives are secondary to profit maximization in the multi-dimensional and/or multivariate motives of enterprise objective. One of the methods of calculating profit is the Gross Margin Analysis.

Efficiency means the production situation where there is no waste. Thus, production efficiency occurs at the point where there is minimum cost of production Olayide and Heady (1982) and Ettah and Nweze (2016) noted that profit efficiency is a concept used in assessing whether an input is expending an optimally balanced level of rent for the use of such a capital. It is an economic performance measure of farms (Adesina & Djato, 1997). Output that provide insufficient returns to the input used are said to be profit inefficient and such inputs should be moved to alternative investments where the perceived returns is higher. Profit efficient farmers are those paying the minimum profit to owners of inputs (Ettah and Nweze, 2016)

Cassava *(Manihot esculenta*) is a perennial woody shrub of the *euphorbiaceae* family. It is grown principally for it tuberous root but it leaves are also eaten in some parts of Africa and used as animal feeds as well. In terms of it nutritional value, cassava roots contains about 60 percent of water and are rich in carbohydrates (Yakassai, 2010). The roots are low in protein and lipids but reasonably rich in calcium and vitamin. Products from cassava when consumed with energy dense protein and nutrient rich supplementary foods such as beans and oil seeds, fishes and pulses provides energy in adequate diet (FAO, 2009). In Nigeria, cassava production is well developed as an organized agricultural crop. It has a well- established multiplication and processing technique for food products and livestock feeds. Though the crop is produced in 24 of the 36 states in the country, cassava production dominates the southern parts of the country, both in terms of area covered and number of farmers growing the crop. The

http://dx.doi.org/10.22161/ijeab/2.1.28 *ISSN: 2456-1878*

major states of Nigeria which produce cassava are Benue, Cross River, Anambra, Delta, Edo, Imo, Oyo and Rivers States and to lesser extent Kwara and Ondo States.

Cassava displays an exceptional ability to adapt to climate changes. It is tolerant to low soil fertility, resistant to drought conditions, pests and disease and suitability to store it root for long periods underground even after maturity, this could be the reason why the crop is the most favourite among the farmers in the area. Hence it is grown throughout the year making it preferable to the seasonal crops of yam, beans, pea, etc. Use of fertilizer is limited and it is also grown in fallow lands. The land holding for farming in Nigeria is between 0.5 - 2.5 hectares (1.2 – 6.2 acres), with about 92 percent of producers being small scale farmers, as in many other crops (Yakassai, 2010). In Cross River State, Nigeria, cassava is widely cultivated in the state and grows in all the 18 local government areas. The study therefore seeks to achieve the following objectives: assess profit efficiency of cassava farmers; examine the determinants of profit efficiency; and make policy recommendations.

II. MATERIALS AND METHODS

2.1 The study

The study area is Cross River State, Nigeria chosen purposively for this study because of the peculiarity of this research problem in the area and the familiarity of the researcher to the area, factors that facilitated data generation. The state lies between latitude $4^{o}15^{1}$ North and $7^{o}00^{1}$ North and longitude $7^{o}15^{1}$ East and $9^{o}30^{1}$ East. (Cross River Ministry of Lands and Survey Bulletin, 2010). The land area of Cross River State is about 7,782 square miles or 20,156 square kilometres (Federal Office of Statistics (FOS), 2007) and the population estimated at 2,888,966 persons (NPC, 2006). Cross River State, Nigeria has a climate made up of two distinct seasons-the dry and wet seasons. The dry season spans from November to late March, while rainy season spans from April to October with a short break in August called "August break". The mean annual rainfall is between 1,300mm to 3,000mm, which varies from place to place across the state (Cross River State Tourism Guide, 2010). According to the tourism guide, highest temperature is recorded between February and March and does not exceed $37^{o}C$ and the lowest between May and October and does not go below $15^{o}C$ and also varies from place to place. The vegetation of the state includes the following: Mangrove Swamp (wetland), rainforest, derived savannah and parkland (Cross River Tourism Bulletin, 2010). Deep laterite fertile and dark clayey basalt soil is found in the area.

2.2 Sampling Procedure

Cassava farmers from Cross River State, Nigeria formed the population for this study. A three stage random sampling technique was used to select respondents for the study. The three agricultural zones (Calabar, Ikom and Ogoja) of the state where covered. Three local government areas each were selected randomly from each of the three agricultural zones in the first stage. This gave a total number of nine local government areas in the sample.The second stage involved the random selection of three cassava farming communities from each of the nine local government areas previously selected making a total of twenty seven cassava farming communities. The third stage involved a random selection of four cassava farmers from each of the twenty seven cassava farming communities making a total of 108 respondents for the study.

2.3 Data Collection and Analysis

Data required for this study was generated from primary sources. The primary data was collected using a set of pre-tested structured questionnaires. The questionnaire captured information on the socio-economic characteristics of respondents. The questionnaires were administered by well-trained enumerators, who were conversant with the selected locality. Primary data were also obtained through personal contact, oral interviews, etc. The stochastic frontier profit function using Cobb-Douglas functional form was used for the analysis.

2.4 Validation and Reliability of Questionnaire.

The instrument for data collection in this study was validated by passing them through erudite scholars to ensure that it possessed both face and content validity. In other to check the consistency of the measuring instrument over time, reliability test was conducted using the test-retest method. A coefficient of 0.79 was obtained using the Cronbach Alpha Technique indicating the suitability of the instrument for use. A pilot study was then conducted where enumerators were used for pre-testing of the questionnaire. This was to avoid inconsistency and incomplete response and also ensure clear understanding of the instrument.

2.5 Model Specification

The stochastic frontier profit function using Cobb-Douglas functional form used for the analysis is specified below as:

$$l_nC = \beta_o\ Y^* + \beta_1 l_n\ x_1 + \beta_2 l_n\ x_2 + l_3 L_n x_3 + \beta_4\ l_n x_4 + \beta_5\ l_n x_5 + \beta_6 l_n x_6 \quad (1)$$

ln = Logarithm to base

C= Gross margin (₦)

Y*=Cassava output (Kg)

X_1 =Land rent per ha

X_2= Cost of hired labour used in Cassava production per ha

X_3= Price of cassava cuttings

X_4= Price of Agrochemical per litre
X_5= Price of fertilizer per kg
X_6 = Price of capital inputs (₦)
U_1 = error term
β_o = Constant term
β_1-β_2 ..... β_6 = Regression coefficients
U_i = are random variables which are assumed to be independent and normally distributed with zero mean and constant variance Vi- N (0, δ^2), which are non –negative random variables and are assumed to account for technical inefficiency in production (Aigner, Lovell & Schmidt, 1977).

The determinant of profit inefficiency is defined by:

$$Ui = \delta_0 + \delta, z_1 + \delta_2 z_{2i} + \delta_{3i} z_{3i} + \delta_4 2_{4i} + \delta_5 z_{5i} + \delta_6 z_{6i} + \delta_7 z_{7i} + \quad (ii)$$

Where:
Ui = profit inefficiency
Z_1= Farmers' age (years)
Z_2=Farming experience (years)
Z_3= Education (years)
Z_4=Training (1 if received training, 0 otherwise)
Z_5 = Membership of farmers' association (1, yes, 0, no)
Z_6= Household size
Z_7= Sex (1, Male, 0, Female)
δ_0-δ_7 = parameters. (Aigner, Lovell & Schmidt, 1977).

III. RESULTS AND DISCUSSIONS

3.1 Determinants of Profit Efficiency of Cassava Farmers

The profit efficiency of the respondents is shown in table 1. 7.4 % of the respondents had a profit efficiency of less than or equal to 0.2, 27.7% had 0.21-0.40, 41.6% had 0.41-0.60, 13.8% had 0.61-0.80 and 9.3% had 0.81-1.0. The minimum and maximum profit efficiency was 0.14 and 0.91 respectively with mean profit efficiency of 0.65. The mean profit efficiency implies that farmers were able to obtain 65% of their potential profit from a unit mix of inputs. In other words, about 35% of the profit is lost to inefficiency of management. Thus in a short run, there is a scope for increasing profit from cassava production by 35%. This result is in consonance with the findings of Otitoju (2011) who reported a mean profit efficiency of 0.67 for crop farmers in southwestern Nigeria. The result is lower than the result of Iorlamen (2015) who reported a mean profit efficiency of 0.59 for sesame farmers in Benue State, Nigeria.

Table.1: Profit Efficiency

Profit efficiency range	**frequency**	**percentage**
<0.2	8	7.4
0.21-0.40	30	27.7
0.41-0.60	45	41.6
0.61-0.80	15	13.8
0.81-1.00	10	9.3
Total	**108**	**100**
Minimum	0.14	
Maximum	0.91	
Mean	0.65	
Mean of best ten	0.81	
Mean of worst ten	0.16	

Source: field Survey, 2016.

3.2 Factors Influencing Profit Efficiency of Cassava farmers

The parameter estimates of the influence of socio-economic factors on profit inefficiency of cassava farmers are presented in the lower section of Table 2. The analysis of profit inefficiency effect showed a significant gamma (γ = 0.86). This implies that 86% deviation from maximum profit obtainable was as a result of inefficiency of the farmers rather than random error or variability. The signs and significance of the estimated coefficients in the inefficiency model have important implication on profit efficiency of the farmers. The estimated coefficient for age (0.37) was positive and significant at 1% level. The positive relationship implies that as age of farmers increases, the level of profit inefficiency tends to increase thereby decreasing profit efficiency.This could be that as the farmers get older, the less efficient his supervision. This finding is in line with the work of Abu *et al.*, (2012) and Arene, (2000) where age positively contributed to profit inefficiency among sesame farmers in Nassarawa and Benue States of Nigeria respectively.

A direct and significant relationship was found between education (0.67) at 1% probability level and profit inefficiency. This implies that an increase in the level of

education increases the level of profit inefficiency (i.e. decrease profit efficiency). The positive value obtained is unexpected as farmers may go in search of white collar jobs thereby neglecting the farming sector or paying little or no attention to it. This findings disagrees with the work of Tanko, Ajani &Adeniyi (2012) that education decreases profit inefficiency in rice farming. The finding agrees with that of Iorlamen (2015) that education increases profit inefficiency in rice farming. The estimated coefficient for farming experience (-0.21) significant at 5% level of probability was negative and significant implying that, increase in farming experience tends to decrease the level of profit inefficiency (i.e. increase profit efficiency).This findings is in consonance with the findings of Kolawole (2006) and Abu and Abah (2012) who found that increase in farming experience decrease profit inefficiency of small rice farmers in Nigeria and female small holder farmers in Atiba local government area of Oyo State, Nigeria respectively.

Household size (0.8) had positive and significant relationship on profit inefficiency at 5 % probability level. This implies that, increase in household size increases profit inefficiency (i.e. decrease profit efficiency). This result is in congruence with findings of Arene (2000) who observed a positive relationship between household size and profit efficiency in sesame production in Benue State. This is contrary to the findings of Nwaru & Iheke (2012) who found household size to increase profit efficiency among catfish farmers that used kitchen/animal waste. Number of training (5.61) was positively related to profit efficiency at 1% probability level; this is because training enhances farmers' knowledge about innovations in agricultural production and ease of access to agricultural aids (Adeniji *et al.,* 2005).

The result also showed a negative and significant relationship (-8.06) between membership of association and profit inefficiency at 1% significance level. Membership of association decreases profit inefficiency and increases profit efficiency. This is expected as farmers membership of association could afford them the opportunity of interacting with other farmers thereby exchanging information on improved technology in farming. Although the result disagrees with the finding of Nweze & Pamwal (2006). In conclusion, age, education and household size had positive impact on profit inefficiency and this is contrary to *apriori* expectation regarding the roles of these factors.

Table.2: Factors affecting profit Efficiency of Cassava farmers

Variable	Coefficient	Standard error	t- ratio
Constant	9.22	1.29	7.13*
Output	0.57	0.07	8.34*
Land rent per ha	$-0.94x10^{-2}$	0.072	-0.13**
Hired labour per ha	$-0.72x10^{-2}$	0.001	-0.69*
Cuttings per Kg	0.68x10-2	0.035	0.19**
Agrochemical price per ha	-0.07	3.5	-0.02*
Inefficiency Model			
Constant	-38.40	6.26	-6.13*
Age	0.37	0.092	4.03*
Farming Experience	-0.21	0.047	2.08**
Education	0.67	0.058	12.54*
Number of training	5.61	1.021	5.73*
Member of Association	-8.06	2.420	3.33*
Household size	0.58	0.243	1.81**
Sex	0.16	0.197	0.81
Sigma Squared	18.66	1.842	10.13
Gamma	0.86	0.52x10-2	163.19
Likelihood function	-188.34		

Source: field survey, 2016

*, ** = t ratio significant at 1% and 5 % level respectively.

IV. CONCLUSION AND RECOMMENDATIONS

The study analysed the determinants of profit efficiency of cassava farmers in Cross River State, Nigeria. The specific objectives were to: assess their profit efficiency and examine the determinants of profit efficiency. A three -stage (multistage) random sampling technique was adopted in the selection of 108. Inferential statistical tool used was the stochastic frontier profit model. Analysis

shows that the mean profit efficiency of cassava farmers was 0.65 with minimum and maximum of 0.14 and 0.91 respectively. This implies that the farmers are not fully profit efficient. Cassava production in the study area is undergoing an increasing return to scale and by this a profitable venture. Factors affecting profit efficiency were age of farmers and education which were positive and significant at 1%. Farming experience and membership of association were negative and significant at 5% and 1% respectively. Household size was positive and significant at 5%.

Based on the findings of this study, the following recommendations were made: farmers should be encouraged to invest on cassava production for its profitability and economic value, inputs should be made available and at affordable prices especially improved varieties of cassava cuttings and cassava farmers should be encouraged to receive training on proper agronomic practices and usage of inputs to enhance profit efficiency of input use.

REFERENCES

[1] Abu, G.A., Ater, P.I & Abah, D. (2012). Profit efficiency among sesame farmers in Nassarawa State, Nigeria. *Journal of Social Sciences*, 4(4), 261-268.

[2] Adeniji, A. A., Ega, L.A., Akoroda, M.O., Adeniji, B.O., Ugwu, A. & Balogun, D.A. (2005). Cassava Development in Nigeria. Department of Agriculture, Federal Ministry of Agriculture and Natural Resources, Nigeria. F.A.O. *Retrieved 22 September, 2013*

[3] Adesina, A. A. & Djato, K.K. (1997). Relative efficiency of women as farm managers; profit Functional approach in Cote d'voire. *Journal of Agricultural Economics* 4(12) Pp. 23-30

[4] Aigner, D. K., Lovell, A. K. & Schmidt, P. (1977). Formulation and estimation of stochastic frontier production function models. *Journal of Econometrics*, 6(9), 21-37.

[5] Arene, C. J. (2000). Profit function analysis of small ruminants' enterprises in Nsukka L.G.A. of Enugu State; Nigeria. *Economic Affairs*, 47(4), 209-214.

[6] Cross River State Ministry of Lands and Survey Yearly Bulletin (2010). Handbook for visitors to Cross River State, Calabar, Nigeria.Pp 2-4 Retrieved from *www.crs govt.org.ng*

[7] Cross River State Tourism Bulletin (2010). Annual publication of tourism potentials of Cross River State, Calabar, Nigeria Pp 3-9.

[8] Ekubika, E. (2010). Economic analysis of cassava production farming in Akwa Ibom State, Nigeria. *Agriculture. Biology and Animal science. 4*(2).

[9] Ettah, O. I. & Nweze, N. J. (2016). Analysis and determinants of technical efficiency among cassava farmers in Cross River State, Nigeria. *International Journal of Innovative Research and Development*, 5(10), 109-113

[10] Federal Office of Statistics, (2007). Annual abstract of Nigeria's statistics 2007, Federal Ministry of Finance, Abuja, Nigeria Pp 27-32.

[11] Food and Agricultural Organisation (2009). Statistical data base: F.A.O. Quarterly bulletin of 2009. pp. 42-47.

[12] Iorlamen, T. (2015). Resource use efficiency of Sesame farmers in Benue State, *Unpublished Ph.D. thesis* submitted to the Department of Agricultural Economics, University of Nigeria, Nsukka.

[13] Kolawole, O. (2006). Determinants of profit efficiency among small scale rice farmers in Nigeria: a profit function approach. Poster paper presented at the International Association of Agricultural Economists Conference, Gold Coast, Australia August 12-18.

[14] National population commission, (2006). Census enumeration survey N. P. C. Abuja, Nigeria. *Pp* 6-8. Nigeria.

[15] Nwaru, J. C. & Iheke, R. O. (2012).Comparative analysis of resource use in rice production systems in Abia State, Nigeria. *International Journal of Agriculture, Environment*, 2(4), 8-20.

[16] Nweze, N. J. & Panwal E. F. (2006).Resource- use and productivity among rainfed and irrigated Irish Potato Producers in Plateau State, Nigeria. A paper presented at 20th Annual National Conference of Farm Management Association of Nigeria, held at Forestry Research Institute of Nigeria, Federal College of Forestry, Jos Plateau, 18th-21stSeptember.

[17] Olayide, S. O. & Heady, E.O. (1982). *Introduction to agricultural production economics.* Ibadan University Press, Ibadan, Nigeria.

[18] Otitoju, A. (2011). Influence of climate change adaptation strategies on efficiency in food crop production in Southern Nigeria. *An unpublished Ph.D. thesis* submitted to the Department of Agricultural Economics, University of Nigeria, Nsukka.

[19] Tanko, L. Ajani, R. & Adeniyi, (2012).Relative technical efficiency of credit and non-credit users in rice-based enterprises in Lavun LGA of Niger State, Nigeria. *International Journal of Agricultural Economics and Rural Development*, 5(4) 20-32.

[20] Yakassai, M. T. (2010). Economic contribution of cassava production (a case study of Kuje area council F.C.T. Abuja Nigeria. *Bayero Journal of Pure and Applied Sciences, 3*(1), 215-219.

http://dx.doi.org/10.22161/ijeab/2.1.29 *ISSN: 2456-1878*

Farmers' Knowledge of Cassava Streak Virus Disease in Selected Districts of Central Uganda

B. Bua

Department of Agriculture, Kyambogo University, Po Box 1, Kyambogo Kampala (U)

***Abstract*—** *Cassava brown streak disease is one of the latest outbreaks of diseases threatening cassava production in Uganda. Although, previously reported in some parts of east African coast, CBSD was not a common problem in Uganda until over a decade ago. Since, its first reported outbreak in mid 2000s, CBSD has continued to spread in many cassava growing districts of Uganda. Cassava brown streak disease manifests as a syndrome characterised by leaf chlorosis, stem and root necrosis. The infected root tubers are unfit for human consumption. Therefore, the study was conducted to assess farmers' knowledge of CBSD in the selected districts in central Uganda. Semi-structure questionnaires were used to gather information from 180 respondents from the districts of Mukono, Masaka and Wakiso on the knowledge and perception of CBSD. The findings revealed that cassava was widely grown in the three districts. However, a number of constraints including pests and diseases were reported to be affecting cassava growing. Of the diseases, CBSD was ranked as the most widespread and devastating. In fact, 75% of the respondents had good knowledge of CBSD and perceive it as responsible for the declining cassava production in the districts. The most common symptoms associated with CBSD leaf chlorosis, rotting and necrosis of the root tubers. Both the old and newly introduced cassava varieties were susceptible to CBSD. Accordingly, CBSD was thought of as responsible for food insecurity, livelihoods and the loss of cassava biodiversity among others.*

***Keywords*— Chlorosis, necrosis, rotting, susceptible, varieties.**

I. INTRODUCTION

Cassava (*Manihot esculentum* Crantz) is an important root crop in many countries of the world including Uganda. Traditionally a subsistence crop, cassava has gained prominence as a potential source of income and food security for the poor and marginalised farming communities in many parts of the world including sub-Saharan Africa (Dixon *et al*., 2003). According to FAO (2009), world production of cassava was estimated at 184 million tonnes in 2002, rising to 230 million tonnes in 2008. The world leading producer and exporter are Nigeria and Thailand, respectively. In Uganda, recent production statistics showed a decline in total production by more than 4.5% from 1999 to 2009. The major cause of this tragedy are mainly the biophysical factors of which pests and diseases are most the disastrous followed by lack of improved varieties, inadequate support services, weeds and dynamic weather changes (FAO, *1999*). According to IITA (2009), the major pests of cassava in sub Saharan Africa are the cassava green mite and the variegated grasshopper while the main diseases are cassava mosaic disease (CMD), cassava bacterial blight, cassava anthracnose disease and root rot. Cassava mosaic disease (CMD) alone accounted for an estimated 47% of east and central Africa's cassava production losses during a serious outbreak beginning in the early 1990's until 2006. However, in Uganda the major hindrances to cassava production included insect pests like whiteflies, cassava mealy bugs and the elegant grasshoppers among others. The disease of marked significance is cassava mosaic (CMD) and cassava brown streak disease (CBSD) (Alicai *et al*., 2007).

Cassava brown streak disease was first reported and distinguished from the cassava mosaic disease (CMD) in Tanzania during the 1930's (Storey, 1936). Cassava brown streak disease (CBSD) was found to be endemic in all east African coastal cassava growing areas from Kenya to Ruvuma River that marks the southern borders between Tanzania and Mozambique. The disease also occurred at lower altitude in Malawi (Nicholas, 1950). However, recent surveys have confirmed that the disease occurs throughout the coastal strip surrounding Lake Malawi (Shaba *et al*., 2003), coastal Kenya (Bonk, 1994; Muga and Thresh, 2002), and Mozambique (Hillocks *et al*., 2002; Thresh and Hillocks, 2003). Indeed, the disease is very devastating as it renders the edible roots unsuitable for human consumption (Hillocks and Jennings, 2003). Higher incidences of CBSB in these districts are reported to be closely associated with high whiteflies population (Anon, 2005).

Cassava brown streak disease manifests in a variety of ways, on leaves, it causes yellowing/chlorosis of the leaf margins coalescing into yellow patches whereas on the young stems, the disease appears as brown lesions along the nodes resulting into death of the buds and the

branches die downwards and on roots, CBSD causes rotting of the edible roots. Estimates of the yield losses attributed to CBSD is scanty and limited because the extent of loss is governed by many factors e.g, susceptibility of the cultivars and stage of harvest. In general, CBSD is insidious, causing mild or no leaf distortion making it hard to notice because the plant looks healthy but the tubers of the plant become yellow/ brown with a corky necrosis making it unfit for consumption by man or animal (Hillocks and Jennings, 2003). However, the symptoms have been noted to be less distinct from other infections and disorders like senescence and those that are as a result of diverse environmental conditions such as prolonged drought that may result in leaf chlorosis (ITTA, 2009). According to Hillocks *et al*. (2003), the CBSD leaf symptoms are the most distinct indicators of the disease compared to brown streaks on stems as the name suggests and root necrosis which may not occur in some varieties. Therefore, this study was conducted to assess farmers' knowledge and diversity of CBSD symptoms in the selected districts of central Uganda.

II. METHODOLOGY

The study was conducted in three selected districts of central Uganda including Mukono, Wakiso and Masaka in 2013. The three districts were chosen because of the outcry about the devastating and widespread reports of cassava brown streak disease (CBSD) as well as the long history of cassava growing in the areas. Multi-stage random sampling technique was used to identify the sub countries, parishes, villages and the respondents to be interviewed. Two to three leading cassava growing sub counties identified from each district were Nama, Seeta Namuganga and Kyampisi (Mukono); Busabala, Kakiri and Busukuma (Wakiso); Kyanamukaka and Kabonera (Masaka), respectively. From each sub county, two major cassava growing parishes were selected depending on the size and intensity of cassava production. In general, 2-3 villages were surveyed per parish depending on the population in the area giving a total of 20 farmers per sub county and 60 farmers per district including one technical staff per sub county. Semi- structured questionnaires were used to gather information from 180 respondents in the three districts. For each of the farmer interviewed, the cassava field was visited to assess the incidence and severity of cassava brown streak diseases using visual symptoms. Disease incidence was assessed as the number of plants diseased expressed as the percentage of the total number of plants assessed per field.

Disease severity was visually assessed as the percent leaf area affected (PLAA) using a scale of 1-5 where 1=no symptoms, 2=slight symptoms, 3=foliar mosaic, mild stem lesions, no die back, 4=foliar mosaic, severe stem lesions, no die back and 5=defoliation, severe stem lesions and die back or 1=no apparent necrosis, 2=less than 5 % root necrosis, 3=5-10% root necrosis, 4=10-25% root necrosis and 5= more than 25% root necrosis and severe root constriction for root symptoms (IITA, 1995). Diseased cassava plant samples were also collected for laboratory identification of cassava brown streak virus species associated with CBSD and viral characterization. All the data collected was edited, coded and entered into an excel spreadsheet (version 2007). The data was analyzed using descriptive statistics of the SPSS computer package (version 14.0).

III. RESULTS

Close to 100% of the respondents was involved in cassava growing (Table 1). However, the acreages grown vary from 0.25 to 2 acres and above. In fact, close to 90% grew less than one acre whereas only, slightly above 10% grew between one acre and above (Table 2). Among the food crops encountered during the survey, cassava was one of the crops reportedly grown for a variety of purpose including consumption, sale and brewing. However, in terms of cash crops, cassava and other crops feature less compared to coffee and banana (Table 3). Overall, cassava has been grown in these districts for over 25 years and above although slightly over 60% have been growing cassava for between 5 and 10 years (Table 4). The major planting seasons of cassava is presented in Table 5. Slightly over 60% of the respondents planted cassava in both seasons whereas about 20% was not sure of the planting seasons. The cassava planting materials used by the respondents were obtained from various sources including own fields, neighbours, NGOs and Government. The varieties grown also varied and ranged from the old and newly introduced varieties (Table 6). Interestingly, the older varieties seem to be more popular than the newly introduced varieties for a number of reasons including inability to access the improved varieties, taste and preferences among others (data not shown). However, a multitude of constraints affecting cassava production were highlighted as shown in Table 7. The most important constraints reported were diseases, drought and pests. In fact, 75% of the respondents were knowledgeable about cassava brown streak virus disease (Table 8). *Cassava brown streak disease was reportedly widespread and devastating in these areas*. Cassava brown streak disease was associated with different symptoms as shown in Table 9. The most obvious symptoms of cassava brown streak disease reported were leaf chlorosis and rotting and necrosis of the tubers. However, 50% of the respondents were not able to tell the causes of cassava brown streak disease although 28.9 and

20.9% associate cassava brown streak to soil and insect related causes (Table 10). Close to 50% of the respondents attribute the effects of cassava brown streak disease to low yield (Table 10). However, 95% of the respondents indicated that they don't report the outbreak of cassava brown streak disease to the relevant authority for appropriate action as evidenced by no action reported by 98% of the respondents (Table 11). Moreso, only negligible percentage of the respondents reported, they were trained on cassava brown streak disease recognition, means of spread and control. Similarly, negligible proportions of the respondents reported that they get information on cassava brown streak disease from the relevant authorities (data not shown). Nevertheless, various attempts for controlling and managing the disease were reported by the respondents. The most commonly reported method of control was roguing as opposed to the use of resistant varieties (Table 12).

IV. DISCUSSION

The study was conducted to assess the farmers' knowledge and diversity of CBSD symptoms in the selected districts of central Uganda. In fact, the study has shown that 75% of the respondents were familiar with the disease but, the majority does not report the outbreak to any authority. Similarly, the study has also shown that cassava brown streak disease is a syndrome characterised by leaf chlorosis, streak on the stem and root necrosis. In fact, tubers of the affected plant become yellow/ brown with corky necrosis occurring in the starch bearing tissues, making it unfit for consumption by man or animal (Hillocks and Jennings, 2003). Cassava brown streak disease was also reported to be widespread and devastating in most of the areas visited. This is not strange because like the other diseases farmers always find it difficult to recognize diseases unlike pests they can easily see. Moreover, even those who reported had nothing done to save their crop. Cassava brown streak disease (CBSD) was first reported and distinguished from cassava mosaic disease (CMD) in Tanzania in the 1930's but was confirmed in Uganda by 2000s in highland regions spreading to low land regions like lake Victoria basin (Alicai *et al*.,2007; Shores, 2011). However, the symptoms have been noted to be less distinct from other infections and disorders like senescence and those that are as a result of diverse environmental conditions e.g. prolonged drought that may result in leaf chlorosis (ITTA, 2009). According to Hillocks *et al*. (2003), the CBSD leaf symptoms are the most distinct indicators of the disease compared to the brown streaks on stems as the name suggests and root necrosis which may not occur in some varieties. In fact, leaf symptoms present an important tool in reporting the prevalence of the incidence amongst a diversity of cassava varieties in many places. In fact, the outbreak of cassava brown streak disease is a serious threat to food security, livelihoods and loss of cassava biodiversity. This is because whilst considerable efforts have been devoted to come up with varieties resistant to cassava mosaic disease (CMD), these are the same varieties succumbing to CBSD.

ACKNOWLEDGEMENTS

Funding for this study was provided by Kyambogo University for which I am very grateful. The research assistants who collected the data during the survey are also acknowledged. The cooperation of the farmers during data collection is gratefully acknowledged.

REFERENCES

[1] Alicai, T. Omongo, C.A,. Maruthis, M.I.V, Hillock, R.C.T. Baguma Y.Kawuki R Otim-G W and Colvin J. (2007). Re-emergence of cassava brown streak disease Uganda. Plant Disease Vol.91:24-29.

[2] Bock, K.R. (1994). Studies on cassava brown streak disease in Kenya. Tropical Science 34, 134-145.

[3] Food and Agriculture Organisation Statistics (FAOSTAT), 2009, production statistics for cassava. Accessed on internet 25th -01-2912

[4] Gondwe, F.M.T., Mahungu, N.M, Hillocks, R.J, Raya M.D, Moyo, C.C, Soko, M.M, Chipungu, F and Benesi, I.R.M (2003). Economic losses experienced by small-scale farmers in Malawi due to cassava brown streak virus disease.

[5] Hillocks, R.J, Raya, M.D and Thresh, J.M. (1996). The association between root necrosis and above ground symptoms of brown streak virus infection of cassava in southern Tanzania. Internationa Journal of Pest Management 42:285-289.

[6] Hillocks, R.J and Jennings, D.L (2003). Cassava brown streak disease; a review of present knowledge and research needs. International Journal of Pest Management 49,225-234

[7] Hillocks, R.J, Raya, M.G. and Thresh, J.M.2002. Distribution and symptoms expression of cassava brown streak disease in Southern Tanzania. African Journal of Tuber crops 3:57-67

[8] Hillocks, R.J. Raya, M.G., Mtunda, K. and Kioza, H. (2008). Effects of brown streak virus disease on yield and quality of cassava in Tanzania, Journal of photopathology

[9] Mohammmed, I.U., Abarshi, M.M., Muli, B., Hillocks, R.J. and. Genetic Diversity of cassava Brown Streak Virus infecting cassava in East Africa, Hindawi Publishing Corporation, volume 2012, Article ID 795697, 10 pages

[10] Jennings, D.L. (1960). Observation on virus disease of cassava in resistant and susceptible varieties II. Brown streak disease. Empire Journal of experimental Agriculture 28:261-269.
[11] Mbanzibwa, D.R., Tian Y.P.Tugume, A.K., Mukasa, S.B Tairo, F., Kyamanya, S., Kalluya, A., Valkonen, J.P.T (2009). Genetically distinct strains of cassava brown streak virus in the Lake Victoria basin and the Indian Ocean coastal areas of East Africa. Achieves of Virology, Vol. 154:353-359.
[12] Monger, W.A, Seal, S, Isaac, A.M and Foster, G. (2007). Molecular characterization of the cassava brown streak virus coat protein. Plant pathology vol. 50: 527-534.
[13] Nicholas, R.F, (1950). The brown streak disease of cassava, distribution climate effects and diagonistic symptoms, East African Agricultural Journal 5:154-160
[14] Storey, H.H. (1936). Virus disease of East African plants; VI.A progress report on studies of the disease of cassava. East African Agricultural Journal 2:34-39.
[15] Storey, H.H. (1939). Report for 1939 of the cost Agricultural research station, P.9 Teri Shors, (2011). Understanding virus Jones and Bolott publisher copyright-
[16] Uganda National Bureau of Statistic (UBOS). (2009). Production statistics for agricultural products accessed on internet 25th -01-2012

Table.1: involvement in cassava growing in Mukono, Wakiso and Masaka districts, 2013

Cassava growing	**Frequency (%)**
Yes	99.4
No	0.6
Total	**100**

Table.2: Acreages of cassava cultivation in Mukono, Wakiso districts, 2013

Acreages	**Frequency (%)**
0.25	31.1
0.5	35.0
0.75	22.8
1.0	05.0
1.5	03.9
2.0	01.1
2+	01.1

Table.3: Food and cash crops grown in Mukono, Wakiso and Masaka districts, 2013

Crops	**Frequency (%)**
Food	
Maize	2.8
Banana	18.9
Beans	1.7
Groundnuts	0.6
Root crops	59.4
All	16.6
Cash	
Coffee	38.9
Banana	19.4
Beans, maize and groundnuts	17.2
Banana and coffee	07.2
Passion fruit	06.7
Vegetables	05.6
Root crops	05.0
Total	**100**

Table.4: Years of involvement in cassava cultivation in Mukono, Wakiso and Masaka districts, 2013

Seasons	**Frequency (%)**
Less 5	28.3
5-10	34.4
10-15	02.2
15-20	12.2
20-25	05.1
25+	17.8
Total	**100**

Table.5: Planting seasons of cassava in Mukono, Wakiso and Masaka districts, 2013

Seasons	**Frequency (%)**
Rainy seasons	09.9
Dry seasons	10.6
All seasons	62.8
Not sure	17.2
Total	**100**

Table.6: Source of cassava planting materials and varieties grown in Mukono, Wakiso and Masaka districts, 2013

Source	**Frequency (%)**
Own field	61.7
Neighbors	28.3
NGO	09.4
Government	0.6

Varieties/cultivars	
Bukalasa	35
TME	24
Improved	15
Others	26
Total	**100**

Table.7: Constraints to cassava growing in Mukono, Wakiso and Masaka districts, 2013

Constraints	**Frequency (%)**
Diseases	78
Drought	71
Pests	67
Shortage of land	55
Low price	47
Lack of transport	38
Soil infertility	45
Lack of planting materials	17

Table.8: Knowledge of cassava brown streak disease in Mukono, Wakiso and Masaka districts, 2013

Knowledge	Frequency (%)
Yes	75
No	25
Total	**100**

Table.9: Symptoms of cassava brown steak disease in Mukono, Wakiso and Masaka districts, 2013

Symptoms	**Frequency (%)**
Leaf chlorosis	42.2
HL	15.6
Rotting and necrosis	39.1
Leaf chlorosis and root necrosis	03.1
Total	**100**

Table.10: Causes and effects of cassava brown streak disease in Mukono, Wakiso and Masaka districts, 2013

Characteristics	**Frequency (%)**
Causes	
Soil	28.9
Insects	20.9
Do not know	50.2
Effects	
Low yield	48
Low plant population	27
Do not know	25
Total	**100**

Table.11: Reporting and action on the presence of cassava brown streak disease in Mukono, Wakiso and Masaka districts, 2013

Characteristics	**Frequency (%)**
Report	
Yes	02.8
No	97.2
Action	
No action	98.3
Action	01.7
Total	**100**

Table.12: Control of cassava brown streak disease in Mukono, Wakiso and Masaka districts, 2013

Method	**Frequency**
Roguing	52
Spraying	03
Roguing and spraying	09
Use of resistant varieties	01
Do nothing	35
Total	**100**

http://dx.doi.org/10.22161/ijeab/2.1.30 *ISSN: 2456-1878*

Study of two fungal species of *Tulostoma* genus encountered for the first time in Morocco: *Tulostoma melanocyclum* Bres. and *Tulostoma kotlabae* Pouzar

Saifeddine El Kholfy, Anas Nmichi, Ahmed Ouabbou, Mohamed Ajana, Nadia Belahbib, Amina Ouazzani Touhami, Rachid Benkirane, Allal Douira

Université Ibn Tofaïl, Faculté des Sciences, Laboratoire de Botanique, Biotechnologie et de Protection des Plantes, B.P. 133, Kenitra, Maroc.

Abstract**—** *In Morocco, the works on mushrooms are rare and no complete list that lists the species in a given region is not yet available. Surveys in the mobile dunes of Mehdia (North West) and Tifnit (South West Morocco) have allowed us to determine for the first time in Morocco two species of the genus Tulostoma, Tulostoma melanocyclum Bres., (1904) and Tulostoma kotlabae Pouzar (1958). This study is part of the contribution to the determination of the fungal diversity in Morocco that it remains incomplete.*

Keywords**— Mushrooms, Morocco, Mehdia, Tifnit, Dunes, Tulostoma melanocyclum, Tulostoma kotlabae.**

I. INTRODUCTION

The genus *Tulostoma* belongs to the Gasteromycetes, order Tulostomatales and family Tulostomataceae (Courtecuisse & Duhem, 2000; Sesli *et al.*, 2000; Gerault, 2005; Kirk, 2005; Karadelev *et al.*, 2006). However, some species of this genus are currently considered to have affinities with the family Agaricaceae (Agaricales, Agaricomycetidae, Agaricomycetes, Agaricomycotina, Basidiomycota, Fungi) (Kirk *et al.*, 2008).

The genus *Tulostoma* was proposed by Persoon (1794), its representatives have a worldwide distribution, with a common presence in temperate and sandy areas (Wright, 1987). According to Kirk *et al.*, (2008), this genus includes 267 species, characterized by stipitate basidiocarps (Courtecuisse, 2000), a stem inserted at the base of the endoperidium (Internal peridium) that opens at the top by a perforation, a well developed peristome (Roger, 1981; Romagnesi, 1995; Courtecuisse & Duhem, 2000 and Gerault, 2005), a septated capillitium (Baseia *et al.*, 2002) and verrucous spores.

In this work, two species of *Tulostoma*' genus (*Tulostoma melanocyclum* Bres. And *Tulostoma kotlabae* Pouzar) were studied. Similarly, the affinity of the Tulosmatales with the Agaricales was discussed.

II. MATERIAL AND METHODS

Prospecting, carried out in the mobile dunes of Tifnit in August 2010 (Southwest of the Souss region) and Mehdia in January 2015 (North West of Morocco), allowed us to encounter two species of the genus *Tulostoma*. Specimens of these two species were collected and brought back to the laboratory.

The macroscopic descriptions are based on the morphological characteristics (shape, color, size, aspect, ...) as well as other peculiarities related to peridium and stipe (odor, flavor, ...). This study is completed by a microscopic description of the spores, capillitium and stipe. The dimensions of spores and capillitium are measured via a micrometer 10 × (18 mm) with a scale of 10 mm divided into 100 graduations (0.1 mm). Microscopic observations were made using an optical microscope (magnification × 400). The mounting liquid is tap water. The shape of the spores is obtained from the calculation of the quotient of Bas (1969) according to the following ratio, Q = length (L) / width (l).

The identification of the species was carried out by consulting the work of Malençon (1952 & 1956); Malençon and Bertault (1955 to 1969); Roger (1981) ; Wright, (1987); Courtecuisse and Duhem (2000); Poumarat (2001 & 2003); Gerault (2005) ; Kirk *et al.*, (2008) and Outcoumit (2011).

III. RESULTS

Two species (*Tulostoma melanocyclum* and *Tulostoma kotlabae*) are described for the first time in Morocco.

Tulostoma melanocyclum Bres. (1904)

Terricultural species harvested on 28/01/2015 on sandy soil among the mosses under Juniper (*Juniperus phoenicea* L.) in the dunes of Mehdia.

The head is spheroidal, 1.2 cm in diameter, ochraced white (Figure 1, A (1)). **The stipe** is very thin, 2.5 to 3 cm long and 0.3 cm wide, cylindrical, fistulous, its surface is brown ochraced, with fine concolores scales and whitish

color on the inside (Figure 1, A (3)). **The peristome** is placed in the center, circular 1 mm at margin a little toothed and surrounded by a brownish aureole (Figure 1, A (2)).

The spores are brownish, verrucous, globose to subglobulous (1 ⟨ Q ⟨1.05), of 4.66 to 6 µm (Figure 1, D). **The spore-print** is brownish. **The gleba** at maturity is converted into brownish dust (Figure 1, B). **The capillitium** is very thin, of 3.33 µm, inflated at the level of bulkhead (9.99µm), with presence of loops (Figure 1, C (1 and 2)).

Tulostoma kotlabae Pouzar (1958)

Terricultural species harvested on 04/08/2010 on sandy soil in the dunes of Tifnit.

The head is spherical, from 1.5 cm to 2 cm in diameter, pale to ochraced white. **The stipe** is hard and tenacious, thin, striped 5 to 6 cm long and 0.4 to 0.6 cm wide, cylindrical, fistulous, bulbous and whitish in color (Figure 2, A). **The peristome** is central, oval of 2 to 6 mm and of light color (Figure 2, B).

The spores are globose (1 ⟨ Q ⟨1.05), slightly verrucous, measuring 4-5.5 µm and light brown in color (Figure 2, E). **The spore-print** is rusty brown. The gleba becomes a brownish dust at maturity (Figure 2, D). **The capillitium**, thick-walled, is light brown, thin, 3.33-6.66 µm, swollen at bulkheads, unfilled (Figure 2, F).

IV. DISCUSSION

In Morocco, Malençon harvested in 1952 and 1956 two species of the genus *Tulostoma*, *Tulostoma tortuosum* Ehrenb., (1829) and *Tulostoma volvulatum* Kalchbr., (1881) which have been preserved in the National Herbarium of the Rabat Scientific Institute (El-Assfouri *et al.,* 2003). The *Tulostoma* genus is represented by 5 species (*Tulostoma brumale* Pers. (1794), *Tulostoma campestre* Morgan (1889), *Tulostoma mammosum* P. Micheli ex Fr. (1829), *Tulostoma tortuosum* Ehrenb. (1829) and *Tulostoma volvulatum* Kalchbr. (1881), I.G. Borshch. (1865)). Malençon and Bertault have reported *Tulostoma campestre* Morgan, (1889) and *Tulostoma mammosum* P. Micheli ex Fr., (1829), *Tulostoma tortuosum* Ehrenb., (1829) in the 7th list of Fungi of the Rif (1961a) and *Tulostoma brumale* Pers., (1794) in the 5th list of Fungi of Tangier (from 1955 to 1969) Middle Atlas, (1967). Similarly, these species are also cited in other bibliographic works (El Kholfy *et al.,* 2011; Ouabbou *et al.,* 2012; Haimed *et al.,* 2013 and Outcoumit *et al.,* 2014). Otherwise, *Tulostoma brumale* Pers., (1794) is the only species of the genus *Tulostoma* which was described in Morocco (Middle Atlas) by Larouz (2007).

The genus *Tulostoma* is little studied in Morocco, this requires important studies to be carried out concerning the ecological requirement, the time of push and carpophores distribution mode in different localities and regions of Morocco.

The species of *Tulostoma* genus present a great morphological similarity, origin of several taxonomic problems (Fries, 1921; Fischer 1900, 1933; Petri, 1909; Pouzar, 1958 ; Wright, 1987; Courtecuisse & Duhem, 2000; Sesli *et al.,* 2000; Esqueda *et al.,* 2004; Gerault, 2005; Karadelev *et al.,* 2006; Calonge, 2007 and Tomaszewska *et al.,* 2011). Otherwise, various taxonomic approaches have been adopted in monographic studies on *Tulostoma* species (Luszczynski, 2000; Calonge, 2007 and Tomaszewska *et al.,* 2011). The latter is subdivided initially into two sections (*Eutylostoma* and *Schizostoma*), distinguished by the morphology of the peristome (Petri 1909; Fries 1921; Fischer 1900, 1933). Pouzar (1958) subdivided the genus *Tulostoma* into four sections (*Brumalia*, *Poculata*, *Fimbriata* and *Volvulata*), differentiated on the basis of morphology, rupture of exoperidium, and morphology of peristome and stipe (Calonge, 2007 and Tomaszewska *et al.,* 2011).

Mycologists have reported that the differentiation between *Tulostoma brumale* var. *brumale* and *T. melanocyclum* is done on the basis of peristome characteristics (Fries 1921; Fischer, 1900, 1933; Petri, 1909; Pouzar, 1958 ; Wright, 1987). A peristome comparison of these two species showed that Tulostoma brumale var. Brumale is characterized by a light colored peristome. By cons, *T. melanocyclum* presents a peristome surrounded by a brownish aureole (Tomaszewska *et al.,* 2011). This description of peristome is identical to that given to our species, *T. melanocyclum* (Figure 1, A (1)). The latter species is also mainly recognized by similar macroscopic characteristics with *Tulostoma brumale* Pers .: Pers, but with different microscopic characteristics: capillitium colorless lightly branched, with thickening around partitions (Esqueda *et al.,* 2004), and echinulate spores (5-6,5 µm diam) with large spines fused at the apex which are similar to the spore ornamentation of *T. squamosum* (Luszczynski, 2000).

In the literature, we find T. fimbriatum Fr. 1829, with fimbriated peristome, plan or slightly elevated, *T. fimbriatum* var. *compestre* (Morgan) G. Moreno 1980, with flattened peristome and *T. fimbriatum* var. *heterosporum* J.E. Wright 1987, with peristome forming a bead (Cheype, 2014).

Similarly, *Tulostoma brumale* var. *brumale* and *T. kotlabae* have very similar characteristics (Pale, white, beige or ochraced endoperdium, yellow to light brown to squamous stipe). However, *T. kotlabae* is characterized by significantly smaller spores with small warts, capillitium without crystals and bulkheads not widened. By cons, *T. brumale* var. *brumale* Developed warts spores,

crystallized capillitium, and enlarged bulkheads (Tomaszewska *et al.*, 2011).

V. CONCLUSION

Species of the genus *Tulostoma* are secotioides, that is to say, morphogenetic and ontogenetic type corresponding to gasteroides species manifest affinity to Agaricomycetideae (Courtecuisse and Duhem, 2000). They are characterized by the presence of a stipe, the glebe, the capillitium and the release of the spores is effected by an active action (Bessey, 1952 et Reijnders, 2000). The biomolecular studies recently carried out on the secotioid species have transferred these taxa according to their anatomical affinities with the Agaricales in the Agaricomycetideae (Moncalvo *et al.*, 2002 ; Justo *et al.*, 2004 and Vellinga, 2004).

The two species studied *Tulostoma kotlabae*, harvested in August 2010 on sandy soil in the dunes of Tifnit (Northeast of the central plateau) And *Tulostoma melanocyclum*, encountered under Juniperus (*Juniperus phoenicea* L.) in the dunes of Mehdia in January 2015, were described for the first time in Morocco.

REFERENCES

[1] Baseia I. G. & Galvão T. C. O., 2002. Some Interesting Gasteromycetes (Basidiomycota) In Dry Areas From Northeastern Brazil. Acta bot. bras. 16(1): 1-8.

[2] Bas C., 1969. Morphology and Subdivision of *Amanita* and a monograph on its section *Lepidella*, Persoonia, 5(4): 285-579.

[3] Calongel F.D., Llimona x. y., MartinM.P., 2007. Nuevos datos sobre el género *Tulostoma* (Gastéromycètes) en Espana. IV. Revista Catalana de Micologia, 29: 1 1-16.

[4] Cheype J-L., 2014. Contribution à la connaissance des champignons de la haute vall de l'Arve (Haute-Savoie), 7ème note « espèces intéressantes des glariers et terrains vegues de l'Arve ». Bull. myco.bot. Dauphiné-Savoie, ISSN 1771-754X, N° 214: 11-27.

[5] Courtecuisse R. & Duhem B., 2000. Guide des champignons de France et d'Europe. Ed. Delachaux et Niestlé, S. A. Lausane (Switzerlande), Paris, 476 p.

[6] El kholfy S, Aït Aguil F, Ouazzani Touhami A, Benkirane A & Douira A., 2011. Bibliographic Inventory of Moroccan Rif's fungi: Catalog of rifain fungal flora, Journal of Animal & Plant Sciences. Vol. 12, Issue 1: 1493-1526.

[7] Esqueda M., Moreno G., Pérez-Silva E., Sánchez A., Altés A., 2004. The genus Tulostoma in Sonora, México. Mycotaxon, 90: 409–422.

[8] Fischer E., 1900. Phallineae, Hymenogastrineae, Lycoperdinaeae, Nidulariineae, Plectobasidiineae. (In:) A. Engler & K. Prantl (eds). Die Natürlichen Pflanzenfamilien. I Teil. Abteilung 1. Verlag von Wilhelm Engelmann, Leipzig, 276-346.

[9] Fischer E., 1933. Gastromyceteae. (In:) A. Engler, K. Prantl (eds). Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigen Arten insbesondere den Nutzpflanzen. 2nd. ed. Bd. 7a, Verlag von Wilhelm Engelmann, Leipzig, 72 pp.

[10] Fries Th. C. E., 1921. Sveriges Gasteromyceter. Arkiv för Botanik 17 (9): 1-63.

[11] Jeppson M., 2005. Åtgärdsprogram för bevarande av vit stjälkröksvamp (*Tulostoma niveum*). Naturvårds-verket rapport 5512. 28p.

[12] Gerault A., 2005. Florule evolutive des basidiomycotina du finistere. Hétérobasidiomycètes, Agaricales. Version 2.1, 183 p. Projet.aulnaies.free.fr/Flourules/AGARICALES.pdf.

[13] Haimed M., Nmichi A., Ouazzani Touhami A., Benkirane R. et Douira A., 2013. Bibliographic inventory of Moroccan Central Plateau fungi. Journal of Animal &Plant Sciences, 18(2): 2723-2749.

[14] Hawksworth D. L., Kirk P. M., Sutton B. C. & Pegler D. N., 1995. Ainsworth & Bisby's Dictionary of the fungi. Surrey, 8th edn., International Mycological Institute, 412 p.

[15] Karadelev M., Rusevska K., 2006. Ecology and distribution of species from genus Tulostoma (Gasteromycetes) in the Republic of Macedonia. Scientific Area D. Fungal diversity, 4p.

[16] Kirk P., 2005. Ainsworth and Bisby's Dictionary of the Fungi, 9th Edition. CABI Bioscience, UK Centre, Egham, UK; P.F. Cannon, CABI Bioscience, UK Centre, Egham, UK; J C David, CABI Bioscience, UK Centre, Egham, UK; J A Stalpers, Central bureau voor Schimmelcultures, Utrecht, The Netherlands, 624 p.

[17] Kirk P.M., Cannon P.F., Minter D.W., Stalpers J.A., 2008. Dictionary of the Fungi. 10th edn. CABI, Wallingford, UK.

[18] Larouz B., 2007. Biodiversité fongique du Maroc: Etude des champignons supérieur du Moyen Atlas. Thèse de Doctorat, Université Ibn Tofaïl, Faculté des Sciences, Kénitra, Maroc. 332 p.

[19] Luszczynski J., 2000. The first record of *Tulostoma melanocyclum* in Poland. Acta Mycol., 35 (1): 37-40.

[20] Ouabbou A., El-Assfouri A., Ouazzani Touhami A., Benkirane R. and Douira A., 2012 b. Bibliographic catalog of the forest of Mamora (Morocco) fungal flora. Journal of Animal &Plant Sciences, 2012. Vol.15, Issue 3: 2200-2242.

[21] Outcoumit A., 2011. Contribution à l'étude de la diversité fongique du Maroc et mise en évidence de quelques espèces fongicoles et de l'importance des espèces sécotoïdes dans la systématique des Basidiomycètes. Thèse de Doctorat, Université Ibn Tofaïl, Faculté des Sciences, Kénitra, Maroc. Volume I et II, 497 p.

[22] Outcoumit A., El Kholfy S., Ouazzani Touhami A. & Douira A., 2014. Bibliographic Inventory of Tangier fungi: Ctalogue of the Basidiomycetes fungal flora, International Journal of Plant, Animal and Environmental Sciences (IJPAES). Vol. 4, Issue 1:205- 256.

[23] Poumarat S., 2001. Clé des Gastéromycètes épigés d'Europe (Lycoperdales, Nidulariales, Phallales, Sclérodermatales, Tulostomatales). Monographie Mycologique de la FAMM. Edition FAMM n°2, Nice, 96 pp.

[24] Poumarat S., 2003. Clé des Gastéromycètes épigés d'Europe. Phallales : Geastraceae, Hysterangiaceae, Phallaceae ; Agaricales : Lycoperdaceae, Mycenastraceae, Nidulariaceae, Phelloriniaceae, Tulostomataceae ; Boletales : Sclerodermataceae (genres sécotioïdes exclus). Monographies Mycologiques de la FAMM, n° 2, 2ème édit. Revue et augmentée, Edit. FAMM, Nice, 100 p.

[25] Pouzar Z., 1958. 1. rod Tulostoma Pers. ex Pers. – Palečka. (In:) A. Pilát. (ed.). Flora ČSR, Gasteromycetes, Houby – břichatky. Ser. B. Československé Akademie Věd, Praha, 589–613.

[26] Reijnders A.F.M., 2000. A morphogenetic analysis of the basic characters of the gasteromycetes and their relation to other basidiomycetes. Mycological Research, 104: 900-910.

[27] Roger P., 1981. Les champignons. Edition du Club Français Loisirs, Paris, avec l'autorisation des Editions Solar, 288p.

[28] Romagnesi H., 1995. Atlas des champignons d'Europe, Bordas, Paris. 290 p.

[29] Sesli E., 1995. *Tulostoma brumale* Pers.: Pers. : A New Record of Gasteromycetes for Turkey, Tr. J. of Botany, 19: 599-600.

[30] Sesli E., Wrigh, J. E., 2000. The Genus *Tulostoma* Pers.: Pers. (Gasteromycetes) in Turkey. Turk J. Bot., 24: 269-272.

[31] Singer R., 1986. The Agaricales in Modern Taxonomy (4th Ed.). Königstein im Taunus, Germany: Koeltz Scientific Books, 981p.

[32] Tomaszewska A., Łuszczynski J., Łuszczynska B. and Jaworska J., 2011. Taxonomic notes on the Polish Tulostoma species. Acta Mycologica, 46 (2): 179–186.

[33] Vellinga Else C., 2004. Genera in the family Agaricaceae: evidence from nrITS and nrLSU sequences, The British Mycological Society, Mycol. Res., 108 (4): 354–377.

[34] Wright, J. E., 1987. The genus *Tulostoma* (Gasteromycetes) – A World Monograph. J. Cramer. Berlin, Stuttgart, 338 p.

Fig.1: (1) Basidiocarpes, (2) Peristome circular and (3) Stipe (A), Gleba and fistulous Stipe (B), (1) Capillitium (2) Knots (C) and Spores (D) of Tulostoma melanocyclum.

http://dx.doi.org/10.22161/ijeab/2.1.30 ISSN: 2456-1878

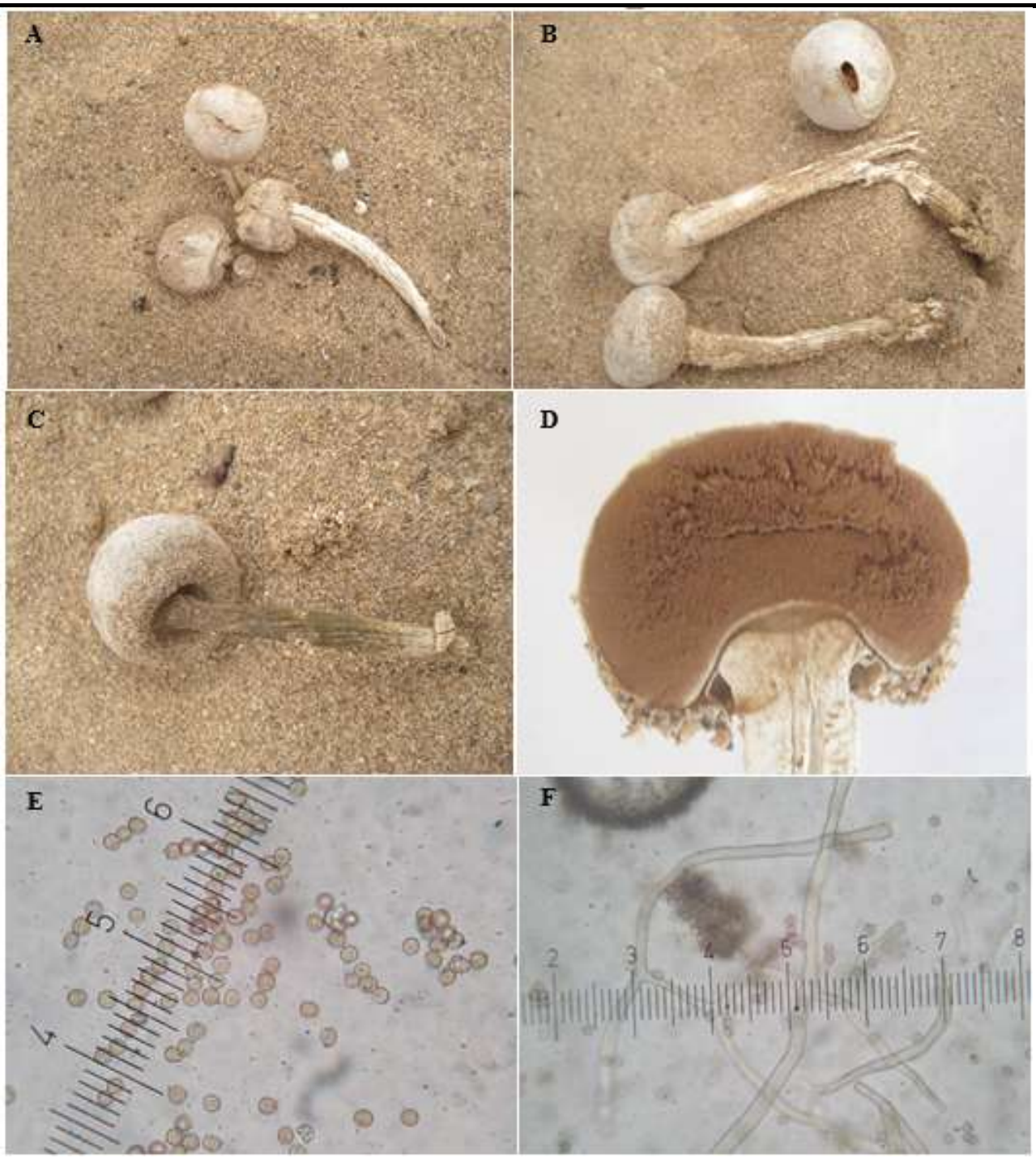


Fig.2: Basidiocarpes and stipe (A), Peristome (B), Insertion of the stipe (C), Gleba (D), Spores (E) and Capillitium (F) of Tulostoma kotlabae.

http://dx.doi.org/10.22161/ijeab/2.1.31 *ISSN: 2456-1878*

Effects of Climate Change on Vegetation in Mediterranean Forests: A review

A.D. Solomou*, N.D. Proutsos, G. Karetsos, K. Tsagari

Hellenic Agricultural Organization "DEMETER", Institute of Mediterranean Forest Ecosystems,
N. Chlorou 1, Ilisia, 11528, Athens, Greece.

***Abstract*—** *A systematic literature review was undertaken to analyze the effects of climate change concerning the forests in the Mediterranean region as it is a climate and a global hot spot of biological diversity and the richest biodiversity region in Europe. Climate change threatens several ecosystems (e.g. forests) with ecological and socioeconomic importance. It is noteworthy that all warming scenarios in the Mediterranean predict an increase of drought and heat events, and a reduction in precipitation within the next hundred years in the Mediterranean basin with important consequences in local vegetation communities. Forests can therefore be used as a tool in developing solutions to the problem of climate change. Nowadays, is considered necessary firstly to continue monitoring and research concerning climate change patterns and impacts on regional scales and secondly to implement management strategies in order to preserve Mediterranean habitats.*

***Keywords*—Forest, vegetation, management, climate change, adaptation.**

I. INTRODUCTION

Mediterranean is considered as global biodiversity hotspot [1,2]. Expanding between temperate-rainy (South Europe) and arid regions (Africa), constitutes essentially, a transitional zone, werevarious types of ecosystems and species co-exist, but in a delicate balance [3].

Climate change effects have already begun to be felt throughout the Mediterranean. Prolonged periods of drought, frequent and severe storms, flooding, increased extreme heat events and more mega-fires are a testimony to this change. The rapid and acute changes in climatic conditions within the next 100 years is expected to produce an important impact on the Mediterranean forests [4].

Mediterranean ecosystems are characterized by contrasting plant functional types competing for water [5]andare sensitive to warming and alsotochanges in water availability [6]. They have undergone numerous climatic changes in the past, responding with various ways (tolerance to environmental changes as a result of phenotypic plasticity of certain species, adaptation by changing physiological procedures, exploitation of genotypes, immigrationetc) [7]. Further temperature increase and water availability reduction is expected to cause Mediterranean biodiversity loss in the future [8] and have notable impacts on natural vegetation.

Hence, the aim of the present activated review is to present comprehensive information about the effects of climate change concerning the forestsin the Mediterranean region, which has been identified as "climate change hot spot"[9,10].

II. METHODOLOGY

In order to review and consolidate existing research on the climate change effects on Mediterranean Forest vegetation, a literature search was conducted using Scopus, Web of Science and Google scholar. A systematic methodology was implemented in order to ensure that a rigorous and repeatable method was applied to each synthetic of the effects of climatic change on vegetation in Mediterranean Forests. The methodology consisted of two stages: (i) the generation of keywords and (ii) a systematic search [11].

III. RESULTS

Environmental conditions play an important role in defining the function and distribution of vegetation, in relation with other factors. Changes in long term environmental conditions that can be collectively coined climate change have significant effects on vegetation community structure, composition and distribution pattern in the future [12].

Mediterranean regions are passing climate regions where it has been presumed that climatic changes may have the greatest impacts. Mediterranean regions are also predicted to have minutely intense feedbacks from the earth to the atmosphere [13].

Climate changes

Climate constitutes a constantly changing system due to both anthropogenic and natural factors. Recent past records indicate a temperature increaseby about 0.85ºC globally and about 1.3ºC in the Mediterranean area compared to the levels of the time period of 1880-1920. Cook et al. (2016) [14] refer in their study that the 1998-2012 period was the driest of the last 500 years.

Future climate patterns foresee a further increase of air temperature.It is noteworthy the fact that the predicted

future changes in temperature over the next period (2016-2035) are expected to be in the range of 0.3-0.7°C [15] under medium confidence levels.

In the Mediterranean basin, models also predict, increases in temperature and heat stress and reduction in precipitation and water availability [16,17]with increases in extreme heat and precipitation events [18]. Extreme temperature events are provided to become more regularly, intense and longer duration than present [19].Generally, all warming scenarios in the Mediterranean predict worse future conditions compared to the global pattern, with warming to exceed 2^{o}C at the end of the century.Drier conditions are also expected to threat the Mediterranean habitats [9,10,20].

Land use changes

Land use changes in the Mediterranean are significant when studying the effects of climate change. Petit *et al.* (2001) [21] mentions contradicting changes in the basin with deforestation, abandonment and intense use co-existing. Though, the extensive reductions of forests by intense land use [22], wildfires and grazing are the key factors that shaped todaysMediterranean landscape [23].The changes in climate along with those in land uses (conversion of wildlands to agricultural lands and urban areas) are expected affect negatively ecosystems biodiversity [24,2].

Changesinplant growth

Warming, increase of drought and heat events and drastic reduction in precipitation is likely within the next hundred years in the Mediterranean basin with important consequences in photosynthesis, growth and survival of local vegetation [25,26].

It has been observed that increasing atmospheric CO_2 concentration influence plants photosynthesis, consistently the increases in plant water use efficiency enhancing the photosynthetic capacity and favoring the plant growth [27]. Specifically, rising concentrations of CO_2 in the atmosphere increase photosynthesis rates and vary with plant nitrogen status and species [28]. For example, mature *Fagussylvatica* and *Quercuspetratea* responded more than *Carpinusbetulus*, *Prunusavium*, and *Tiliaplatyphyllos* in a central European free air experiment enrichment [29]. Tree growth rate might not increase proportionally with increase in photosynthesis because of other limiting factors such as nutrient availability[30,31].

Although experiment enrichment or short – term CO_2 increase can lead to higher net primary productivity [32], tree ring analysis in the Mediterranean shows the opposite [33] probably due to limitations in water and nutrients availability [34,35]. This is in line, with the recorded tree growth reduction [36], increased growth variability [37] and defoliation in Mediterranean forests the last decades.

The higher atmospheric CO_2 concentration (increase from 280ppm in the pre-industrial age to 400 ppm at present, Kennedy 2015 [38]) is not expected to lead to increase in carbon assimilation by natural vegetation in the Mediterranean, mainly because of the impact of drought to metabolic limitation to photosynthesis [39] and limitations in water availability and nutrients [40, 28]. Thus sclerophyllous vegetation, that dominates the Mediterranean, will not be favored by CO_2 changes, while thermophilous species will have to deal with better climatic conditions mainly because of the warmer winters[26].

The Mediterranean species are established to temperature zones where temperature is near its optimum values for photosynthesis [40,41]. An increase in temperature (near or beyond its critical values) combined with low water availability, especially in summer, is expected to lead to photosynthesis decline, reduction in CO_2 assimilation and stomatal conductance, cell dehydration and necrosis [42]. Though, there are species tolerant to high temperatures with specific morphological characteristics (small thick or trichom covered leaves, small leaf angles with the shoot, etc.) and adaptation strategies (such as completion of biological stages before the drought ignition, intraspecific variability, phenotypic plasticity, local adaptation, e.tc. (seereviews [26,3]) that allow them to grow and survive to warm environments. An interesting review of the adaptation mechanisms of Mediterranean heat tolerant species to drought was presented by Bussotti*et al.* (2014) [26], who also mention extensively reported tree dieback events in southern Europe and in Mediterranean regions and suffering of sclerophyllous Mediterranean vegetation due to severe drought events.

Changes in vegetation patterns

Many studies foresee habitat reduction due to climate change though, with different habitat loss rates [2]. The habitat loss [43] and seed production [44] will be affected by climate change, with direct effects to plant communities. Drought [15] and extreme cold events [46] are also found to affect fauna.

The most sensitive vegetation zones in the Mediterranean are those extended to the southern limits of the Mediterranean basin. Changes in atmospheric CO_2 concentration (reaching 600ppm at the end of the century [25], will have severe impacts on plant populations (Lenoir *et al.* 2008), by affecting plant productivity and water use efficiency [48, 49].

Habitat migration to regions with more favorable climate conditions will also occur as a climate change adaptation strategy of vegetation. Though, many plant species cannot meet the needs of velocity transition requirements in order to establish new plant communities in new areas [50]. Tinner and Lotter (2006) [51] calculated that in order to ac-

complish a 100 km migration transition, species will need about 250-1000 years, when climate change occurs much faster (according to A1B scenario mean temperature increase velocity will be 42 km per 100 years and in many regions will reach 100-1000 km per 100 years, [52]).

The spatial climate change shifts will occur with different regional velocities, higher at lowlands and lower in mountainous regions [52,53]. Also, different immigration rates are expected among species with respect to their reproductive dynamics and dispersion strategy. For example, Clark *et al.* (2001) [54] found migration rates varying from 300m per year for boreal spruce to 0.1-1 m per year for animal-disperse species, when Higgins *et al.* (2003) [55] estimated much higher rates for specific weeds and shrubs reaching 2186 m per year. In general, Davis *et al.* (2005) [56] estimated local adaptation times from decades to century for herbs and 100-1000 years for trees.

Altitudinal upward shifts of vegetation have also occurred during past along with immigration to southern (cooler) areas. Bussotti*et al.* (2014)[26] states that tree species will follow a migration natural pattern from south to north and from low to high altitudes. Lenoir *et al.* (2008) [47] found upward shift rates in 171 forest plant species in France of about 29m per decade, when warming and elevation lapse rates were much higher (about 75 m per decade).

The evergreen species are generally slower to adaptation in changing environments [57]. Bussotti*et al.* (2014) [26] states that these species in the Mediterranean, are not expected to respond to the fast climate change rates by evolutionary adaptation, but probably will survive by migration and that the evergreen tree species, in the future will extend to xeric regions that nowdays are covered by deciduous oaks and mountains, while mountain conifers and temperate deciduous species will be limited to their southern extension ranges.

Reduce in frost injuries of plants [58] and increase in winter photosynthesis [59] are expected due to warmer winters in the Mediterranean, with regard to plant species [60]. In general the sensitive to cold species will be favored over the existing cold-tolerant and this will increase species-competition and affect forests structure, population dynamics with possible results the conversion of forests to shrublands[60].

The Mediterranean mountains are considered as extremely vulnerable to climate change [9,10]. It is predicted that will undergo warming, precipitation decrease and interannual variability more intense than other mountains [20] with higher species losses [61]. Ruiz-Labourdette*et al.* (2013)[62] forecast for the Mediterranean mountains' vegetation that xerphylous vegetation will considerably increase and dominate low mountain areas and perennial sclerophyllous species will also increase, while moderate-tolerant to water availability vegetation will notably decrease. At higher altitudes vegetation will up-shifted, the semiarid forests will expand, the broadleaf forest will reduce and cold gymposperm forest will radically reduce their expansion ranges.

Changes in phenology

Beyond its impact on vegetation composition and species ranges, climate change affects also species phenology and reproductive process. Phenology is affected both by precipitation and temperature [63,64] and can be considered as a reliable index to track climate change impacts to the species ecology [65].

Changes in phenophases have already being tracked the last few decades [66] with advancement of flowering date and increase in the length of the growing season. Parry et al. (2007) [67] found a rate of spring onset advance by 2.3-5.2 day per decade, since 1970s. Gordo and Sanz (2010) [68] conducted an extensive research in Spain (29 species from 1500 sites) and found advancement rates of 4.8, 5.9 and 3.2 days per decade in leaf-out, flowering and fruiting, respectively and a rate of 1.2 days per decade delay in leaf abscission since 1970s. Morin *et al*. (2010) [63] conducted experimental warming and found advancing leaf-out of 8-13 days both for evergreen and deciduous oaks, while Cleland *et al.* (2006) [69] found advanced flowering by 2-5 days for annual species though with phenological responses variations among groups to elevated CO_2 and N manipulations.Richardson *et al.* (2013)[70] consider that climate change will result to further advancement of vegetation's growing period in winter-spring and also earlier onset and longer summer drought period.

Drought also affects phenology especially to species sensitive to water availability such as shrubs [71] or grasses. Peñuelas et al. (2003)[72] addresses rainfall and water availability changes, as important factors leading to significant phenological changes in Mediterranean species of bushes such as *Erica multiflora*and*Globulariaalypum* in Catalonia with subsequent changes in the structure, composition and operation of their communities. Though trees are more tolerant because of the structure of their rooting system that allows to exploit soil water from deeper [73].

Changesin wildfires

Fires are a key factor in the Mediterranean, with their numbers to have increased the last decades [74] and further increase is expected due to climate warming [75]. Additionally, under future climate change patterns, wildfires are expected to be more aggressive and not easily to manage with current fire-suppressing strategies [76].

The forest fires have significant effect on vegetation dynamics in the Mediterranean which is mainly dominated by non-resilient, to fire, species with low regeneration ability [77]. Increases in fires frequency and/or intensity will

impose the succession by oaks, shrublands and grasslands [78], with high risk for other native species not to succeed seeders regeneration [79] and the risk to increase the invasion of non-native species [3]. In all cases the wildfires frequency and specifically the length of the period between fire events is crucial. According to Valdecantos (2008) [80] if the period between two consecutive fire incidents is too small, is rather unlikely to achieveproper seed-regeneration, with consequences to future post-fire succession and rehabilitation of the ecosystem, especially for exclusively seed-regenerated species such as *Pinus sp.*, *Ulexparviflorus*, *Cistus sp.* etc.

Changes in soils

Soil processes are affected by precipitation [81]. Climate change impacts on vegetation is expected also to affect soils due to both climate change [82] and vegetation changes [83]. These soil changes will again adversely affect vegetation dynamics as already occurred during the Holocene [84]. Johnstone and Chapin (2003) [85] mention that the local expansion of pines against spruce, increased fire incidents and reduced soil carbon.Both changes in soils and vegetation regimes will have impacts in local hydrology and water chemistry e.g. lakes [86]. Important is the effect of soil depth on climate change impacts, mainly because it affects evapotranspiration and runoff dynamics [5].

IV. CONCLUDED REMARKS

Climate change scenarios predict massive impacts on Mediterranean forests. Though, changes in climate have also occurred in the past and plants managed to adapt to the new established environments through morphological, anatomical, physiological and molecular mechanisms and processes [57]. In the Mediterranean plants adopted survival mechanisms in order to avoid the winter frost or summer drought. Webb (1986) [87] estimates that vegetation adaptation will occur fast enough, so to accomplish equilibrium with climate.

Doblas-Miranda (2016) [3] mentions that different climate change factors, when combined, can alter the effect of others, changing the impacts of global change, especially in the Mediterranean, where many contradicting factors coexist. They also state that "although global change is unavoidable in many cases, change does not necessarily mean catastrophe, but adaptation" and consider as a challenge the conservation of Mediterranean ecosystems.

Under this point of view and in order to meet climate change challenge, it is considered necessary a) to continue monitoring and research concerning climate change patterns and impacts on regional scales and b) to implement management strategies in order to preserve Mediterranean habitats and improve vegetation's adaptation to the new established environments.

REFERENCES

[1] Myers, N., Mittermeier, R.A., Mittermeier, C.G, da Fonseca, G.A.B. and Kent, J. (2000). Biodiversity hotspots for conservation priorities. Nature 403: 853-858.

[2] Beltrán, B.J., Franklin, J., Syphard, A.D., Regan, H.M., Flint, L.E. and Flint A.L. (2014) Effects of climate change and urban development on the distribution and conservation of vegetation in a Mediterranean type ecosystem. International Journal of Geograph. Information Science, 28(8): 1561-1589.

[3] Doblas-Miranda, E., Alonso, R., Arnan, X., Bermejo, V., Brotons, L., de lasHeras, J., Estiarte, M., Hódar, J.A., Llorens, P., Lloret, F., López-Serrano, F.R., Martínez-Vilalta, J., Moya, D., Peñuelas, J., Pino, J., Rodrigo, A., Roura-Pascual, N., Valladares, F., Vilà, M., Zamora, R. and Retana, J. (2017). A review of the combination among global change factors in forests, shrublands and pastures of the Mediterranean Region: Beyond drought effects, Global and Planetary Change 148: 42–54.

[4] Regato, P. &Korakaki, E. (2010). The Mediterranean forests against Global Climate Change. Publications: WWF Greece pp 106.

[5] Montaldo, N., Corona, R. and Albertson, J. (2016). The Climate change impact on the water balance and use efficiency of two contrasting water limited Mediterranean ecosystems in Sardinia. Geophysical Research Abstracts, Vol. 18, EGU2016-8647, EGU General Assembly 2016.

[6] Guiot, J. & Cramer, W. (2016). Climate change: The 2015 Paris Agreement thresholds and Mediterranean basin ecosystems. Science 354(6311): 465-468.

[7] Thompson, I., Mackey, B., McNulty, S. and Mosseler, A. (2009). Forest Resilience, Biodiversity, and Climatic Change. A synthesis of the biodiversity/resilience/stability relationship in forest ecosystems. Secretariat of the Convention on Biological Diversity, Montreal. Tech. Series no. 43, pp. 67.

[8] Keenan, T., Serra, J.M., Lloret, F., Ninyerola, M. and Sabate, S. (2011). Predicting the future of forests in the Mediterranean under climate change, with niche- and process-based models: CO_2 matters! Glob. Chang. Biol. 17: 565-579

[9] Giorgi, F. (2006). Climate change hot-spots. Geophys. Res. Lett. 33: L08707.

[10] IPCC (2007). Climate Change 2007: Impacts, Adaptation and Vulnerability: Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cam-

bridge University Press, Cambridge, U.K. and New York, NY.

[11] Harrison, P.A., Berry, P.M., Simpson, G., Haslett, J.R., Blicharska, M., Bucur, M., Dunford, R., Egoh, B., Garcia-Llorente, M., Geamănă, N., Geertsema, W., Lommelen, E., Meiresonne, L. and Turkelboom, F. (2014). Linkages between biodiversity attributes and ecosystem services: A systematic review. Ecosystem Services 9: 191–203.

[12] Sahney, S., Benton, M.J. and Falcon-Lang, H.J. (2010). Rainforest collapse triggered Pennsylvanian tetrapod diversification in Euramerica. Geology 38: 1079-1082.

[13] Seufert, G., Kotzias, D., Sparta, C. and Versino, B. (1995). Volatile organics in Mediterranean shrubs and their potential role in a changing en-vironment. Global change and Mediterranean type ecosystems pp.343-370. Springer-Verlag, New York.

[14] Cook, B.I., Anchukaitis, K.J., Touchan, R., Meko, D.M. and Cook, E.R. (2016). Spatiotemporal drought variability in the Mediterranean over the last 900 years. J. Geophys. Res. Atmos. 121 (5): 2060-2074..

[15] IPCC (2014). Summary for Policymakers. In: Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the IPCC [Edenhofer, O., R. Pichs-Madruga, Y. Sokona, E. Farahani, S. Kadner, K. Seyboth, A. Adler, I. Baum, S. Brunner, P. Eickemeier, B. Kriemann, J. Savolainen, S. Schlömer, C. von Stechow, T. Zwickel and J.C. Minx (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

[16] Gao, X. &Giorgi, F. (2008). Increased aridity in the Mediterranean region under greenhouse gas forcing estimated from high resolution simulations with a regional climate model. Global and Planetary Change 62: 195–209.

[17] Hoerling, M., Eischeid, J., Perlwitz, J., Quan, X., Zhang, T. and Pegion, P. (2011). On the increased frequency of Mediterranean drought. Journal of Climate 25, 2146–2161.

[18] Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. and Miller, H.L. (2007). Climate Change 2007: The Physical Science Basis. Working Group I Contribution to the Fourth Assessment Report of the IPCC. Cambridge University Press, Cambridge, 996 pp.

[19] Hatfield, J.L. &Prueger, J.H. (2015). Temperature extremes: Effect on plant growth and development. Weather Clim. Extremes 10:4–10.

[20] Giorgi, F. &Lionello, P. (2008). Climate Change Projections for the Mediterranean Region Global and Planetary Change 63:90-104.

[21] Petit, S., Firbank, L., Wyatt B. and Howard, D. (2001). MIRABEL: Models for Integrated Review and Assessment of Biodiversity in European Landscapes. A Journal of the Human Environment 30: 81-88.

[22] Tinner, W., van Leeuwen, J.F.N., Colombaroli, D., Vescovi, E., van der Knaap, W.O., Henne, P., Pasta, S., D'Angelo, S. and La Mantiaf, T. (2009). Holocene environmental and climatic changes at Gorgo Basso, a coastal lake in southern Sicily, Italy. QuatSci Rev 28(15–16):1498–1510.

[23] Henne, P.D., Elkin, C., Colombaroli, D., Samartin, S., Bugmann, H., Heiri, O. and Tinner, W. (2013). Impacts of changing climate and land use on vegetation dynamics in a Mediterranean ecosystem: insights from paleoecology and dynamic modeling. Landscape Ecol. 28: 819–833.

[24] Sala, O.E., Chapin III, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M. and Wall, D.H. (2000). Global biodiversity scenarios for the year 2100. Science, 287 (5459), 1770–1774.

[25] Christensen, J.H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli,R.K., Kwon, W.-T., Laprise, R., Maga˜na Rueda, V., Mearns, L., Menéndez, C.G.,Räisänen, J., Rinke, A., Sarr, A. and Whetton, P. (2007). Regional climate projections. In: Solomon, S., Quin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. and Miller, H.L. (Eds.), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report on IPCC. Cambridge University Press, UK and New York,USA.

[26] Bussotti, F., Ferrini, F., Pollastrini, M. and Fini, A. (2014). The challenge of Mediterranean sclerophyllous vegetation under climate change: From acclimation to adaptation. Environmental and Experimental Botany 103: 80–98.

[27] Steffen, W. &Canadell, P. (2005). Carbon Dioxide Fertilisation and Climate Change Policy. Australian Greenhouse Office, Department of Environment and Heritage: Canberra. pp. 33.

[28] Ainsworth, E.A. & Long, S.P. (2005). What we have learned from 15 years of free-air CO_2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. New Phytol. 165: 351–372.

[29] Asshoff, R., Zotz, G. and Korner, C. (2006). Growth and phenology of mature temperateforest trees in elevated CO. Global Change Biology 12: 848–861.

[30] Hungate, B.A., Dukes, J.S., Shaw, M.R., Luo, Y. and Field, C.B. (2003). Nitrogen and Climate Change. Science 302: 1512–1513.

[31] Luo, Y., Su, B., Currie, W.S., Dukes, J.S., Finzi, A., Hartwig, U., Hungate, B., McMurtrie, R.E., Oren, R., Parton, W.J., Pataki, D.E., Shaw, M.R., Zak, D.R. and Field, C.B. (2004). Progressive nitrogen limitation of ecosystem responses to rising atmospheric carbon dioxide. BioScience 54: 731–739.

[32] Norby, R.J., DeLucia, E.H., Gielen, B., Calfapietra, C., Giardina, C.P., King, J.S., Ledford, J., McCarthy, H.R., Moore, D.J.P., Ceulemans, R., de Angelis, P., Finzi, A.C., Karnosky, D.F., Kubiske, M.E., Lukac, M., Pregitzer, K.S., Scarascia-Mugnozza, G.E., Schlesinger, W.H. and Oren, R. (2005). Forest response to elevated CO2 is conserved across a broad range of productivity. Proceedings of the National Academy of Sciences USA 102: 18052–18056.

[33] Nicault, A., Alleaume, S., Brewer, S., Carrer, M., Nola, P. and Guiot, J. (2008). Mediterranean drought fluctuation during the last 500 years based on tree-ring data. Climate Dynamics 31: 227–245

[34] Zhao, M. & Running, S.W. (2010). Drought-induced reduction in global terrestrial net primary production from 2000 through 2009. Science 329: 940–943.

[35] Leonardi, S., Gentilesca, T., Guerrieri, R., Ripullone, F., Magnani, F., Mencuccini, M., Noije, T.V. and Borghetti, M. (2012). Assessing the effects of nitrogen deposition and climate on carbon isotope discrimination and intrinsic water-use efficiency of angiosperm and conifer trees under rising CO_2 conditions. Global Change Biology 18: 2925–2944.

[36] Sarris, D., Christodoulakis, D. and Körner, C. (2007). Recent decline in precipitation and tree growth in the eastern Mediterranean. Global Change Biology 13: 1187–1200.

[37] Vieira, J., Campelo, F. and Nabais, C. (2010). Intra-annual density fluctuations of *Pinuspinaster* are a record of climatic changes in the western Mediterranean region. Canadian Journal of Forest Research 40: 1567-1575.

[38] Kennedy, C. (2015). 2014 State of the Climate: Carbon Dioxide. NOAA-www.climate.gov/

[39] Saxe, H., Ellsworth, D.S. and Heath, J. (1998). Tree and forest functioning in an enriched CO_2 atmosphere. New Phytologist 139: 395–436.

[40] Saxe, H., Cannell, M.G.R., Johnsen, Ø., Ryan, M.G. and Vourlitis, G. (2001). Trees and forest functioning in response to global warming. New Phytologist 149: 369–400.

[41] Wertin, T.M., McGuire, M.A. and Teskey, R.O. (2011). Higher growth temperatures decreased net carbon assimilation and biomass accumulation of northern red oak seedlings near the southern limit of species range. Tree Physiology 31: 1277–1288.

[42] Fini, A., Guidi, L., Ferrini, F., Brunetti, C., Di Ferdinando, M., Biricolti, S., Pollastri, S.,Calamai, L. and Tattini, M. (2012). Drought stress has contrasting effects on antioxidant enzymes activity and phenylpropanoid biosynthesis in Fraxinusornus leaves: an excess light stress affair? Journal of Plant Physiology 169: 929–939.

[43] Scalercio, S. (2009). On top of a Mediterranean Massif: Climate change and conservation of orophilous moths at the southern boundary of their range (Lepidoptera: Macroheterocera). European Journal of Entomology 106: 231–239.

[44] Sánchez-Humanes, B. &Espelta, J.M. (2011). Increased drought reduces acorn production in Quercus ilex coppices: thinning mitigates this effect but only in the short term. Forestry 84: 73–82.

[45] Legakis, A. &Adamopoulou, C. (2005). Temporal responses of soil invertebrate communities to draught stress in two semiarid ecosystems of the Mediterranean. Isr. J. Zool. 51: 331–348.

[46] Valladares, F., Zaragoza-Castells, J., Sánchez-Gómez, D., Matesanz, S., Alonso, B., Portsmuth, A., Delgado, A. and Atkin, O.K. (2008). Is shade beneficial for Mediterranean shrubs experiencing periods of extreme drought and late-winter frosts? Annals of Botany 102: 923–933

[47] Lenoir, J., Gegout, J.C., Marquet, P.A., de Ruffray, P. and Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. Science 320(5884): 1768-1771.

[48] Richebusch, S., Thuiller, W., Hickler, T., Araujo, M.B., Sykes, M.T., Schweiger, O. and Lafourcade, B. (2008). Incorporating the effects of changes in vegetation functioning and CO_2 on water availability in plant habitat models. Biology Letters 4: 556- 559 .

[49] Higgins, S.I. &Scheiter, S. (2012). Atmospheric CO_2 forces abrupt vegetation shifts locally, but not globally. Nature 488: 209 – 212 .

[50] Skov, F. &Svenning, J.C. (2004). Potential impact of climatic change on the distribution of forest herbs in Europe. Ecography 27: 366-380.

[51] Tinner, W. &Lotter, A.F. (2006). Holocene expansions of *Fagussilvatica* and Abiesalba in Central Europe: Where are we after eight decades of debate? Quaternary Science Reviews 25: 526 – 549 .

[52] Loarie, S.R., Duffy, P.B., Hamilton, H., Asner, G.P., Field, C.B. and Ackerly, D.D. (2009). The velocity of climate change. Nature 462: 1052–1055.

[53] Svenning, J.-C. &Sandel, B. (2013). Disequilibrium vegetation dynamics under future climate change. American Journal of Botany 100(7): 1266–1286.

[54] Clark, J.S., Lewis, M. and Horvath, L. (2001). Invasion by extremes: Population spread with variation in dispersal and reproduction. American Naturalist 157: 537 – 554 .

[55] Higgins S.I., Lavorel S. and Revilla E., 2003. Estimating plant migration rates under habitat loss and fragmentation. Oikos101 : 354-366.

[56] Davis, M.B., Shaw, R.G. and Etterson, J.R. (2005). Evolutionary responses to changing climate. Ecology 86: 1704-1714.

[57] Reyer, C.P.O., Leuzinger, S., Rammig, A., Wolf, A., Bartholomeus, R.P., Bonfante, A., DeLorenzi, F., Dury, M., Gloning, P., AbouJaoudé, R., Klein, T., Kuster, T.M., Martins,M., Niedrist, G., Riccardi, M., Wohlfahrt, G., De Angelis, P., De Dato, G., François, L., Menzel, A. and Pereira, M. (2013). A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. Global Change Biology 19: 75–89.

[58] Ogaya, R. &Peñuelas, J. (2003). Comparative field study of *Quercus ilex* and *Phillyrealatifolia*: photosynthetic response to experimental drought conditions. Environ. andExper. Botany 50: 137-148.

[59] Prieto, P., Peñuelas, J., Lluisà, J., Asensio, D. and Estiarte, M. (2009). Effects of long-term experimental night-time warming and drought on photosynthesis, Fv/Fm and stomatal conductance in the dominant species of the Mediterranean shrubland. ActaPhysiologiaPlantarum 31: 729–739.

[60] Ogaya, R., Peñuelas, J., Asensio, D. and Llusià, J. (2011). Chlorophyll fluorescence responses to temperature and water availability in two co-dominant Mediterranean shrub and tree species in a long-term field experiment simulating climate change. Environmental and Experimental Botany 73: 89-93.

[61] Bakkenes, M., Eickhout, B. and Alkemade, R. (2006). Impacts of different climate stabilization scenarios on plant species in Europe. Glob. Environ. Change 16: 19–28.

[62] Ruiz-Labourdette, D., Fe Schmitz, M. and Pineda, F.D. (2013). Changes in tree species composition in Mediterranean mountains under climate change: Indicators for conservation planning. Ecological Indicators 24: 310–323.

[63] Morin, X., Roy, J., Sonié, L. and Chuine, I. (2010). Changes in leaf phenology of three European oak species in response to experimental climate change. New Phytologist 186: 900–910.

[64] Klein, T., Di Matteo, G., Rotenberg, E., Cohen, S. and Yakir, D. (2013). Differential ecophysiological response of a major Mediterranean pine species across a climatic gradient. Tree Physiology 33: 26–36.

[65] Rosenzweig, C., Casassa, G., Karoly, D.J., Imeson, A., Liu, C., Menzel, A., Rawlins, S., Root, T.L., Seguin, B. and Tryjanowski, P. (2007). Assessment of observed changes and responses in natural and managed systems. In: Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J., Hanson, C.E. (Eds.), Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the IPCC. Cambridge UP, Cambridge, UK, pp. 79–131.

[66] Peñuelas, J. &Filella, I. (2001). Phenology: responses to a warming world. Science 294: 793–795.

[67] Parry, M.L., Canziani, O.F., Palutikof, J.P., van der Linden, P.J. and Hanson, C.E. (Eds.) (2007). Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the IPCC. Cambridge UP, Cambridge, UK, 976 pp.

[68] Gordo, O. &Sanz, J.J. (2010). Impact of climate change on plant phenology in Mediterranean ecosystems. Global Change Biol. 16: 1082–1106.

[69] Cleland, E.E., Chiariello, N.R., Loarie, S.R., Mooney, H.A. and Field, C.B. (2006). Diverse responses of phenology to global changes in a grassland ecosystem. Proc. Natl. Acad. Sci. U. S. A. 103: 13740–21374.

[70] Richardson, A.D., Keenan, T.F., Migliavacca, M., Ryu, Y., Sonnentag, O. and Toomey, M. (2013). Climate change, phenology, and phenological control of vegetation feedbacks to the climate system. Agricultural and Forest Meteorology 169: 156– 173.

[71] Matesanz, S., Escudero, A. and Valladares, F. (2009). Impact of three global change drivers on a Mediterranean shrub. Ecology 90: 2609–2621.

[72] Peñuelas, J., Filella, I. and Comas, P. (2003). Changed plant and animal lifecycles from 1952 to 2000 in the Mediterranean region. Glob. Change Biol. 8:531–544.

[73] Ma, S., Baldocchi, D.D., Xu, L. and Hehn, T. (2007). Inter-annual variability in carbon dioxide exchange of an oak/grass savanna and open grassland in California. Agric. Forest Meteorol. 147, 157–171.

[74] Carmo, M., Moreira, F., Casimiro, P. and Vaz, P. (2011). Land use and topography influences on wildfire occurrence in northern Portugal. Landscape and Urban Planning 100: 169–176.

[75] Moriondo, M., Good, P., Durao, R., Bindi, M., Giannakopoulos, C. and Corte-Real, J. (2006). Potential impact of climate change on fire risk in the Mediterranean area. Clim. Res. 31: 85–95.

[76] Podur, J. & Wotton, M. (2010). Will climate change overwhelm fire management capacity? Ecol. Model. 221: 1301-1309.

[77] Lloret, F., Estevan, H., Vayreda, J. and Terradas, J. (2005). Fire regenerative syndromes of forest woody species across fire and climatic gradients. Oecologia 146: 461-468.
[78] Bendel, M., Tinner, W. and Ammann, B. (2006). Forest dynamics in the Pfyn forest in recent centuries (Valais, Switzerland, Central Alps): interaction of pine (*Pinussylvestris*) and oak (*Quercus* sp.) under changing land use and fire frequency. The Holocene 16: 81–89.
[79] Zedler, P.H. (1995). Are some plants born to burn? Trends Ecol. Evol. 10: 393-395.
[80] Valdecantos, A. (2008). Post-fire restoration strategies/interventions to increase forest resilience against large forest fires exacerbated by climate change: The case of Valencia (Spain). In: Compte-renduprésenté los de l'atelier international UICN-WWF «Adaptation au changementclimatiquedans la gestion et la conservation des forêtsméditerranéennes », Athènes.
[81] Sherman, C., Sternberg, M. and Steinberger, Y. (2012). Effects of climate change on soil respiration and carbon processing in Mediterranean and semi-arid regions: An experimental approach. European Journal of Soil Biology 52: 48–58.
[82] Birkeland, P.W. (1999). Soils and geomorphology. Oxford University Press, New York, NY, USA.
[83] Miles, J. (1985). The pedogenic effects of different species and vegetation types and the implications of succession. Journal of Soil Science 36: 571-584.
[84] Kunes, P., Odgaard, B.V. and Gaillard, M.-J. (2011). Soil phosphorus as a control of productivity and openness in temperate interglacial forest ecosystems. Journal of Biogeography 38: 2150-2164 .
[85] Johnstone, J.F. & Chapin, F.S. (2003). Non-equilibrium succession dynamics indicate continued northern migration of lodgepole pine. Global Change Biology 9: 1401-1409 .
[86] Willis, K.J., Braun, M., Sümegi, P. and Töth, A. (1997). Does soil change cause vegetation change or vice versa: A temporal perspective from Hungary. Ecology 78: 740 – 750 .
[87] Webb, III, T., (1986). Is vegetation in equilibrium with climate? How to interpret late-Quaternary pollen data. Vegetatio 67: 75 – 91 .

http://dx.doi.org/10.22161/ijeab/2.1.32 *ISSN: 2456-1878*

Effects of Pruning on Diameter and Height Growth of *Pinus nigra* Arnold subsp. *pallasina* Plantations in Turkey

Fatih Tonguc[1], Sinan Guner[2]

[1]Süleyman Demirel University, Faculty of Forestry, 32100, Isparta, Turkey
[2]Artvin Çoruh University, Faculty of Forestry, Artvin, Turkey

***Abstract*—** *Pruning is a costly silvicultural operation and allows the production of high value timber. Effect of pruning on black pine and especially on Anatolian black pine (Pinus nigra Arnold subsp. pallasina) is not well known. The objective of the study was to evaluate pruning effects on diameter and height growth of Anatolian black pine. Pruned and the control treatments were carried out using 20 year old black pine plantation in Çorum providence of Turkey. Three pruning treatments were applied in 2004, 2009 and 2016 and diameter and height growths of trees were measured. Each pruning was done from the bottom to include one third of the crown. At the beginning of the study (first pruning) and after 5 years of the first pruning no difference was observed for DBH and height growth. However, diameter and height growth became important after 12 years of pruning between pruned and control stands. Results show that pruning could increase diameter growth and height of Anatolian black pine stands. To better understand, further detailed studies must be carried out investigating site effects, plant density and environmental variations.*

***Keywords*—Anatolian black pine, diameter growth, height growth, Pinus nigra, pruning.**

I. INTRODUCTION

Silvicultural treatments are applied from the beginning of the seedling emergence throughout the final harvesting in the forests. Pruning is an application for producing clear wood among the silvicultural managements for forest tree species (Forrester, 2013; Forrester et al. 2013). Clear wood is called as high quality knot-free timber and veneer logs (Pinkard and Beadle, 2010; Moreno-Fernández et al. 2014). In coniferous trees, diameter and the number of the knots present at the tree trunk are among the main factors decreasing the wood quality of timber (Víquez and Pérez, 2005; Erkan et al. 2016). Obtaining clear wood via pruning has the potential benefit to increase the net revenues (Neilsen and Pinkard, 2003). Pruning starts from the surface of the earth and is applied as a cut of living and non-living branches to a certain height of a tree. As the photosynthetic active leaf area is removed from the top crown by pruning, it may adversely affect the diameter increase depending on the tree species and the intensity of the pruning (Forrester, 2013; Långström and Hellqvist, 1991), but the diameter increase may not be adversely affected when the density of pruning light (Forrester, 2013; Uotila and Mustonen, 1994; Amateis and Burkhart, 2011). Moreover, some leaves on the lower branches may respire more than they photosynthesize. Thus they might be removed without negatively affecting diameter growth (Savill et al. 1997). The pruning of such sections of the crown may result in increase in the light usage efficiency and increases photosynthesis rates in the remaining upper section of the canopy (Forrester, 2013).

Pruning has generally limited and temporary effects on the height growth of trees as compared to the diameter increase (Amateis and Burkhart, 2011). However, there is no effect of pruning on the height increase for *Ficus microcarpa* or *F. virens* (Zhang et al. 2007), *Juniperus virginiana* (Schmidt and Wardle, 2002) and *P. pinaster* and *P. radiata* (Hevia et al, 2016). On the other hand, pruning affected height growth slightly for *P. sylvestris* (Långström and Hellqvist, 1991) and had no effect for *P. pinaster* and *P. radiata* (Hevia et al, 2016). Although pruning of forest trees requires high investment costs (Moreno-Fernández et al. 2014), the costs could be reduced to when applied only to selected trees in the forest (Neilsen and Pinkard, 2003). Moreover, pruning provides easiness for other silvicultural applications and making the stands more resistant to the forest fire (Savill et al. 1997).

In Turkey, State Forest Service (SFS) owns 99% of the forested areas of which 67% consisted of pure coniferous and mixed species with broadleaf trees and all the silvicultural treatments and management plans are prepared by SFS including thinning and pruning (URL 1). Having economic and ecological importance, pine species are widely utilized all over the world. Black pine (*P. nigra* Arnold) is broadly distributed in the Mediterranean region and is among the frequently used pine species in large plantations all over the region (Moreno-Fernández et al. 2014). Anatolian black pine (*P. nigra* Arnold subsp. *pallasina* (Lamb.) Holmboe) is one of the most important

tree species, mainly distributed in Western Anatolia and forms as pure or mixed stands (Tonguc et al. 2013). Anatolian Black pine has the third largest distribution area (4.2 million ha) after *P. brutia* and oak species in Turkey (URL 1) and largely used for reforestation and rehabilitation studies. It is also suitable for afforestation of high altitude lands with dry climatic conditions and steppes (Koski and Antola, 1993; Kaya and Temerit, 1994).

Pruning exercises are being implemented in Turkey (Erkan et al. 2016) and in other Mediterranean countries as a precaution against fire risk by reducing the flammable biomass on the ground and opening gap between the surface and tree crown (Bilgili, 2003; Bilgili et al. 2010; Ganteaume et al. 2011). Although black pine is largely planted, the effects of pruning on its diameter and height growth are not well documented (Moreno-Fernández et al. 2014). It is important that the pruning should be feasible before applying in large areas. Therefore, it is necessary to study whether or not pruning is feasible in specific tree species (Schmidt and Wardle, 2002). Although producing clear wood increases the value of the plantation, currently SFS conducts limited and low level pruning activities due to associated costs and the lack of accurate data about the effects of pruning on harvest quality.

The purpose of the present study was to determine the effects of pruning on diameter and height growth of Anatolian black pine plantations growing on dry climatic conditions located in Çorum city.

II. MATERIAL AND METHODS

2.1. Study sites

The study was carried out in Çorum providence of Turkey located on the north of the country (40º 35´ N- 34º 59´ S). The study area has continental dry climate characteristics. Soil of the plantation area is flat with loamy soils. Mean altitudes of the plantation areas are 925 and 920 m for pruning and control sites; respectively. The closest meteorological observation station is located in Çorum (806 m) and the annual mean temperature of the region is 10.8 °C, ranging from -0.3 °C to 21.3 °C in January and July, respectively (URL 2). Mean relative humidity is 68% where July and August are the driest months (58.8% and 60.5%, respectively) and 65 year (1950-2015) average precipitation is 432 mm (Table 1).

Table.1: Long term average meteorological conditions of the study area (1950-2015).

Parameter	Jan.	Feb.	Mar.	Apr.	May.	Jun.	July.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean temperature (°C)	-0,3	1	5	10,6	15	18,6	21,3	21,2	17,2	11,8	5,9	1,8
Relative humidity (%)	77,4	72,2	68,5	66,7	65,2	62,9	58,8	60,5	64,4	70,3	75,6	79,2
Mean precipitation(mm)	38,9	30,2	39,5	49,4	60,9	52,3	18,9	13,7	22,2	28,6	33,4	43,5

2.2. Experimental design and pruning regimes

Previously, Anatolian black pine trees were planted as 1 m x 2 m planting distance (Anonymous, 1976). The current planting distance is 1.5 x 3 m in Turkey (URL 3). The site was established in 1985. Following two years after planting, weed control and replacement of dead saplings were carried out. In 2004, when the stands were 20 years old, two plots were established for pruning and control. Prior to establishing the study sites, homogenous sites were searched and the chosen sites had uniform growth and ground vegetation covers. The first pruning application was set up on the same year. For every tree a unique number was given and then diameters at breast height (DBH) were measured and marked. A total of 100 trees with 5 replications were recorded. At the same time, 100 trees with 5 replications were also measured as the control. Each replication (row) consisted of 20 trees with surrounding unpruned trees around them for buffering in order to eliminate edge effect.

Second pruning application was carried out in 2009 and the third one in 2016. After each pruning applications, diameter growths of pruned trees and the trees in control were recorded. At the same time, for height growth measurements, a total of 10 trees from each replication were randomly chosen and numbered in pruned trees and in control. Height growths of trees were also recorded together with the diameter measurements. In each inventory, diameters of the trees were measured using a caliper at breast height of all trees (cm) and height growth of trees were measured using telescopic height measurement device (m).

For each pruning application, (2004, 2009 and 2016), one-third of the tree height of the crown area was removed including dead branches and live crown starting from ground level to upper canopy because pruning one-third of the crown reduces production very slightly (1%) at the end of rotation (Savill et al. 1997).

2.3. Data analysis

http://dx.doi.org/10.22161/ijeab/2.1.32 *ISSN: 2456-1878*

Data was subjected to one-way analysis of variance (ANOVA) with Minitab statistical program. Means were separated using Tukey's procedure.

III. RESULTS AND DISCUSSIONS

The first measurements and pruning were conducted in 2004 when trees were 20 years old. The ANOVA results revealed that DBH and height growth did not show any significant differences ($p< 0.05$) during the establishments of control (mean DBH 5.1 cm, mean height 4.9 m) and pruning parcels (mean DBH 5.6 cm, mean height 5.1 m) (Table 2).

At the age of 25 (5 years after pruning), mean DBH was measured as 6.6 cm and 5.6 cm for pruning parcels and control, respectively. Although there were small differences between them, the values for pruning and control parcels were not significant ($p< 0.05$). Average height growths of trees were measured as 5.3 m for both pruning and control parcels, hence there was not any statically significance ($p< 0.05$) between the pruning and control parcels for height growth. Pruning treatments did not effected either DBH or height growth of trees after 5 years (Fig. 1, Fig. 2).

The effects of pruning on DBH growth and height growth were found to be statically significant ($p<0.05$) when pine trees were 32 years old (12 years after the first pruning and 7 years after the second pruning). Mean DBH was 18.1 cm and 13.9 cm for pruned parcels and the control; respectively. The height growth of the parcels were also statistically significant between pruned and control trees. While average height was 7.5 m for control trees, average height was 9.6 m for pruned trees (Table 2, Fig 1, Fig. 2).

Table.2: ANOVA results showing DBH and height growth of Anatolian black pine after pruning treatment

	First Pruning			Second Pruning			Third Pruning		
	Age (year)	Mean height (m)	Mean diameter (cm)	Age (year)	Mean height (m)	Mean diameter (cm)	Age (year)	Mean height (m)	Mean diameter (cm)
Pruning	20	4.9 c*	5.7cd	25	5.3 c	6.6c	32	9.6a	18.1a
Control	20	5.1 c	5.1d	25	5.3 c	5.6cd	32	7.5b	13.9b

*Means followed by the same letters is not significantly different from one another. Mean separation within each column by Tukey's test at $p<0.05$.

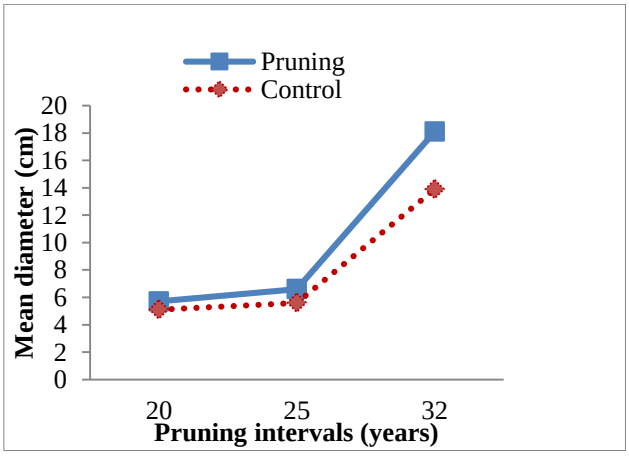


Fig.1: Mean diameter growth and pruning intervals

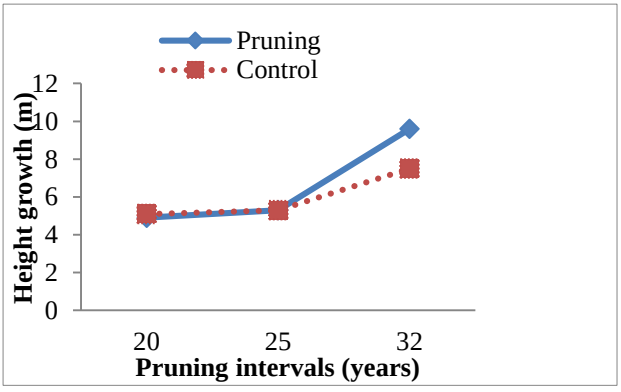


Fig.2: Height growth and pruning intervals

Tree species differ in their response to pruning time and pruning intensity. There are many reports regarding effects of pruning on different conifer and other tree species. While some researchers reported that pruning reduced diameter growth (Långström and Hellqvist, 1991; Uotila and Mustonen, 1994; Hevia et al, 2016) others did not report reduction in diameter growth (Pinkard and Beadle, 2010; Neilsen and Pinkard, 2003; Amateis and Burkhart, 2011). Effect on pruning on height growth is limited and usually is not as important as diameter growth (Amateis and Burkhart, 2011).

Studies dealing with the effects of pruning on black pine is scarce (Moreno-Fernández et al. 2014), and no published results exist for Anatolian black pine studying pruning effects of this subspecies. In the present paper, we report the effects of pruning on DBH growth and height increase for the Anatolian black pine 5 and 12 years after pruning. At the beginning of the study (20 year old plantation), mean height and mean diameter did not differ significantly between control and pruning treatment (Table 2). After 5 years, second pruning was performed mean height of the control and pruned trees were the same (5.3 m). Amateis and Burkhart (2011) reported that height growth is favored than DBH growth for young *P. taeda* to remain competitive in a stand. Our results indicated that pruning did not adversely affected Anatolian black pine's

competitive ability since they stand next to unpruned trees and had the same mean height. However; there was difference between pruned and control trees for mean diameter. Diameter increase was more pronounced for pruned trees, though not statistically significant. After 12 years of first pruning and 7 years of the second pruning, both height and diameter increase became statistically significant compared to control. After the second pruning the average height of the pine trees were 9.6 m compared to 7.5 m to control. Cannell and Dewar, (1994) argued that many tree species allocate more resources to apex than stem to keep dominance of crown position in the stand. Reducing lower branches further lowers the allocated resources to dead branches (Savill et al. 1997) and increase light intensity reaching to lower branches in a pruned stand (Takiya et al. 2010), thus increasing photosynthetic capacity to stimulate growth. DBH growth was also increased for pruned trees (18.1 cm) than control (13.9 cm). Similar results reported by Hevia et al, (2016) that DBH is positively affected by pruning application for *P. radiata* and *P. pinaster*. For black pine, pruning simultaneously applied with thinning had positive effects on DBH growth but only thinning with or without pruning did not produce statistically significant results for DBH growth, showing pruning is important for DBH growth for the black pine (Moreno-Fernández et al. 2014). Contrary to our results, there was not any statistically significant data for height increase with pruning for *P. nigra* subsp. *nigra* and *P. nigra* subsp. *salzmannii* (Moreno-Fernández et al. 2014). It is also noted that soil properties, mean rainfall and summer temperatures had significant effects on DBH growth for black pine (Moreno-Fernández et al. 2014). The present study conducted in an area where rainfall is 432 mm. Effects of pruning on DBH and height growth are more pronounced where soil properties and mean rainfall is lower (Moreno-Fernández et al. 2014; Hevia et al, 2016), which might explain why height growth was important in our study for black pine.

IV. CONCLUSION

The present study is the first report on pruning of Anatolian black pine. Pruning intensity along with stand intensity, their interaction with the environmental variations needs to be studied. Further work is necessary to fully understand pruning effects and to make appropriate recommendations suitable to Turkey's needs.

ACKNOWLEDGEMENTS

We would like to thank Orhan Çatalçam and Selim Umaysız of Çorum State Forestry Service for their help during the study.

REFERENCES

[1] Forrester, D. I. 2013. Growth responses to thinning, pruning and fertilizer application in Eucalyptus plantations: a review of their production ecology and interactions. Forest Ecol. Manag, 310, 336-347.

[2] Forrester, D. I., Collopy, J. J., Beadle, C. L., Baker, T. G. 2013. Effect of thinning, pruning and nitrogen fertilizer application on light interception and light-use efficiency in a young *Eucalyptus nitens* plantation. Forest Ecol. Manag, 288, 21-30.

[3] Pinkard, E. A., Beadle, C. L. 2010. A physiological approach to pruning. The Int. For. Review, 295-305.

[4] Moreno-Fernández, D., Sánchez-González, M., Álvarez-González, J. G., Hevia, A., Majada, J. P., Cañellas, I., Gea-Izquierdo, G. 2014. Response to the interaction of thinning and pruning of pine species in Mediterranean mountains. Eur. J. Forest. Res, 133(5), 833.

[5] Víquez, E., Pérez, D. 2005. Effect of pruning on tree growth, yield, and wood properties of *Tectona grandis* plantations in Costa Rica. Silva Fenn, 39(3), 381.

[6] Erkan, N., Uzun, E., Aydin, A. C., Bas, M. N. 2016. Effect of Pruning on Diameter Growth in *Pinus brutia* Ten. Plantations in Turkey. Croat. J. For. Eng, 37(2), 365-373.

[7] Neilsen, W. A., Pinkard, E. A. 2003. Effects of green pruning on growth of *Pinus radiata*. Can. J. For. Res, 33(11), 2067-2073.

[8] Långström, B., Hellqvist, C. 1991. Effects of different pruning regimes on growth and sapwood area of Scots pine. Forest Ecol. Manag, 44(2), 239-254.

[9] Uotila, A., Mustonen, S. 1994. The effect of different levels of green pruning on the diameter growth of *Pinus sylvestris* L. Scand. J. For. Res, 9(1-4), 226-232.

[10] Amateis, R. L., Burkhart, H. E. 2011. Growth of young loblolly pine trees following pruning. Forest Ecol. Manag, 262(12), 2338-2343.

[11] P. Savill, D. Evans J., Auclair, D. and J. Falck, Plantation Silviculture in Europe, 1st. ed., Oxford University Press Inc, New York, 1997.

[12] Zhang, X., Zeng, B., Zhong, Z. 2007. Differential responsiveness in stem height and diameter growth of two Ficus tree species in the Three Gorges reservoir region of China as affected by branch removal. Can. J. For. Res, 37(9), 1748-1754.

[13] Schmidt, T. L., Wardle, T. D. 2002. Impact of pruning eastern redcedar (*Juniperus virginiana*). West. J. Appl. For, 17(4), 189-193.

[14] Hevia, A., Álvarez-González, J. G., Majada, J. 2016. Comparison of pruning effects on tree growth,

productivity and dominance of two major timber conifer species. Forest Ecol. Manag, 374, 82-92.

[15] Långström, B., Hellqvist, C. 1991. Effects of different pruning regimes on growth and sapwood area of Scots pine. Forest Ecol. Manag, 44(2), 239-254.

[16] URL1. https://www.ogm.gov.tr/ekutuphane/Yayinlar/T%C3%BCrkiye%20Orman%20Varl%C4%B1%C4%9F%C4%B1-2015.pdf

[17] Tonguc, F., Keles, H., Tasdemyr, C. 2013. Variation in cone and seed characteristics in three populations of Anatolian Black Pine (*Pinus nigra* subsp. *pallasiana*) in Turkey. Research Journal of Forestry, 7(1), 34-40.

[18] Koski, V., Antola, J. 1993. Turkish national tree breeding and seed production program for Turkey (1994-2003), Cooperated with ENSO Forest Development Inc. and Forest Tree Seeds and Tree Breeding Institute.

[19] Kaya, Z., Temerit, A. 1994. Genetic structure of marginally located *Pinus nigra* var. *pallasiana* populations in central Turkey. Silvae Genet, 43(5-6), 272-277.

[20] Bilgili, E. 2003. Stand development and fire behavior. Forest Ecol. Manag, 179(1), 333-339.

[21] E. Bilgili, B. D. Durmaz, M. E. Alexander, B. Saglam, O. Kucuk, I. Baysal, "The effect of pruning on crown fire behavior in a Calabrian pine plantation, northeastern Turkey" in Proc. VI International Conference on Forest Fire Research. pp. 15-18, 2010.

[22] Ganteaume, A., Marielle, J., Corinne, L. M., Thomas, C., Laurent, B. 2011. Effects of vegetation type and fire regime on flammability of undisturbed litter in Southeastern France. Forest Ecol. Manag, 261(12), 2223-2231.

[23] URL 2. https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?m=CORUM

[24] Anomymous. 1976. Turkish Republic, Ministry of Forestry, General Directorate of Afforestation and erosion control, 20.10.1976 dated Circular #136. Ankara. [In Turkish].

[25] URL3.https://www.ogm.gov.tr/ekutuphane/Tamimler/18%20Nolu%20Tamim.pdf.

[26] Cannell, M. G. R., Dewar, R. C. 1994. Carbon allocation in trees: a review of concepts for modelling. Adv. Ecol. Res, 25, 59-104.

[27] Takiya, M., Koyama, H., Umeki, K., Yasaka, M., Ohno, Y., Watanabe, I., Terazawa, K. 2010. The effects of early and intense pruning on light penetration, tree growth, and epicormic shoot dynamics in a young hybrid larch stand. J. For. Res, 15(3), 149-160.

http://dx.doi.org/10.22161/ijeab/2.1.33 *ISSN: 2456-1878*

Quantitative structure activity relationship studies of anti-proliferative activity of some indole derivatives combining DFT calculations and statistical results

Halima Hazhazi[1], Hanane Zaki[2,3*], Mohamed Benlyas[2], Youness Filali -Zegzouti[2], Youcef Boumedjane[1], Mohammed Bouachrine[3*]

[1]Research team computatinal & pharmaceutical chemistry,LMCE laboratory, department of chemistry, Faculty of sciences, Biskra University, Biskra , Algeria.
[2]Research team Biologiy, Environnement & health. Department of biology Faculty of Science and Technics. My Ismail University. Meknes. Morocco
[3]Research team Matériaux. Environnement & Modélisation. ESTM. My Ismail University. Meknes. Morocco

Abstract— *Many studies have focused on indole derivatives mainly their antiproliferative effect. The therapeutic effect of this group of molecule is very important. Quantitative structure–activity relationships (QSAR) have been applied for development relationships between physicochemical properties and their biological activities.*

A series of 30 molecules derived from indole is based on the quantitative structure-activity relationship (QSAR). This study was carried out using the principal component analysis (PCA) method, the multiple linear regression method (MLR), non-linear regression (RNLM), the artificial neural network (ANN) and it was validated using cross validation analysis (CV). We accordingly propose a quantitative model and we try to interpret the activity of the compounds relying on the multivariate statistical analyses. A theoretical study of series was studied using density functional theory (DFT) calculations at B3LYP/6-31G(d) level of theory for employing to calculate electronic descriptors when, the topological descriptors were computed with ACD/ChemSketch and ChemDraw 8.0 programs. The best QSAR model was found in agreement with the experimental by ANN (R = 0,99).

Keywords— Breast cancer, anti-proliferative, indole derivatives, QSAR, MLR, MNLR, ANN, CV.

I. INTRODUCTION

Breast cancer is considered as one of the major and widespread reasons that cause death among women all over the world, in case of the late diagnosis [1]. Despite the improvements and the efficiency in early detection, chemotherapy and radiotherapy breast cancer is still at high risks [2-4]. Therefore, it is necessary to find a cure for this disease, a lot of scientific researches were carried out to determine a particular molecule to this treatment [5]. Among the great number of compounds that occur in nature, Indole is the main component. Moreover, indole derivatives have many applications, in the pharmaceutical, industry in the treatment of various diseases [6]. Indole derivatives are one of the most promising heterocyclic, which have active sites in treating various diseases [7]. In addition, these compounds have broad spectrum of biological activities involving anticancer, antioxidant, antimicrobial, anticonvulsant, anti-leishmanial, antidepressants, anti-inflammatory activities and they were found to have capabilities of anti-proliferative activity on cancer cells lines [8-14].

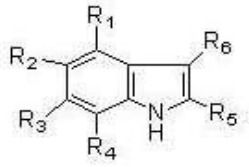


Fig.1: Studied compounds (indole)

On the other hand, Quantitative structure-activity relationship (QSAR) seeks to inquire into the relationship between molecular descriptors which describe the physicochemical properties correlated with biological activity of the set of compounds [15, 16]. The QSAR study is an important step in the development of new drugs. In this paper we have studied a quantitative structure- activity relationship (QSAR) of indole against human breast cancer cells (MCF-7) based on 30 indole derivatives taken from the literature [17-20]. Therefore,

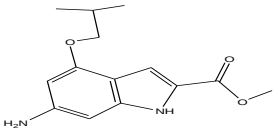
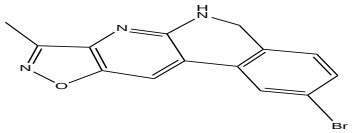
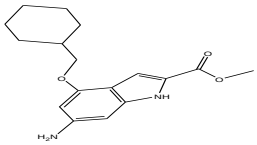
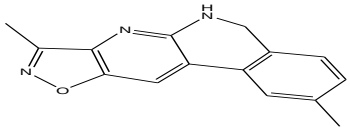
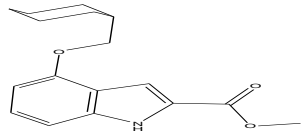
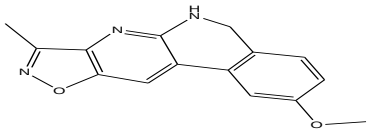
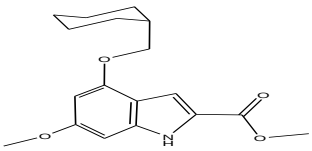
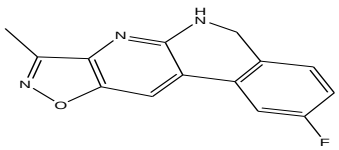
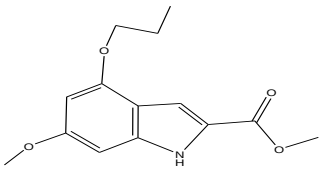
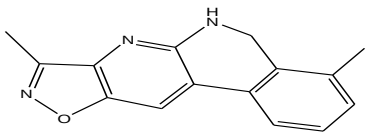
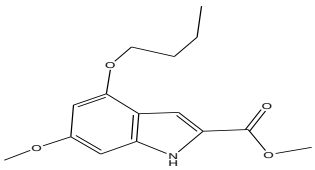
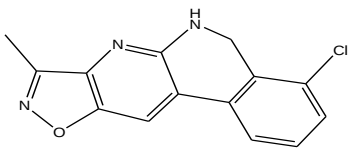
we propose to develop a quantitative model, and we try to predict the activity of these compounds based on the several statistical methods: Principal Component Analysis (PCA), Multiple Non-Linear Regression (MNLR) and Multiple Linear Regression (MLR), Artificial Neural Network (ANN) and Cross Validation analyses (CV). The development of a performant model will help to explain the role of indole derivatives in chemotherapy against breast cancer and also propose other molecules, then predict their anti-cancer activity.

2.1 Chemical data

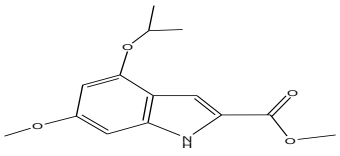
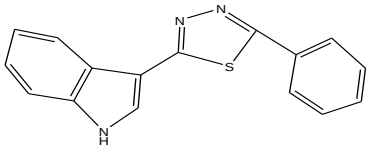
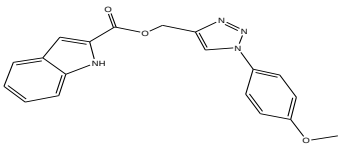
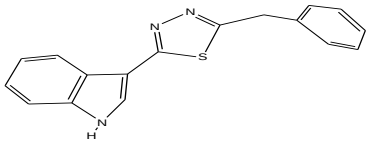
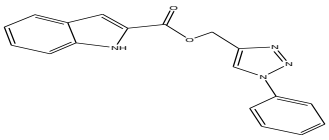
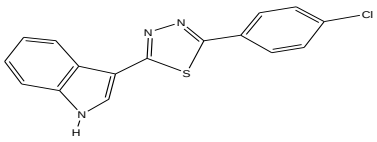
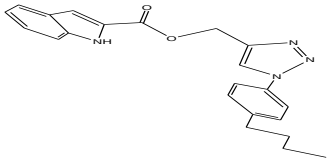
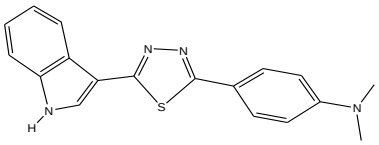
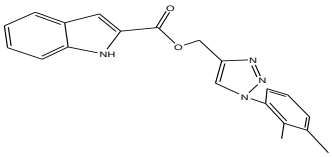
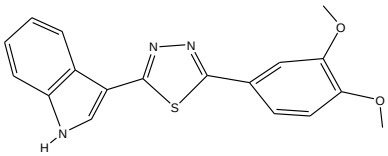
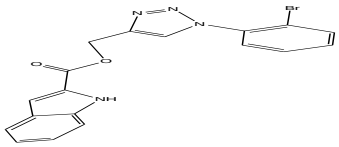
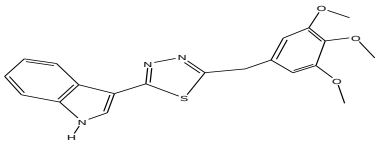
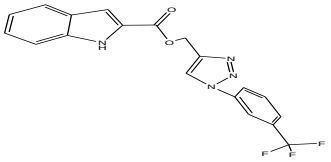
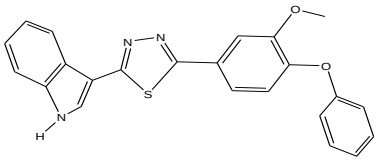
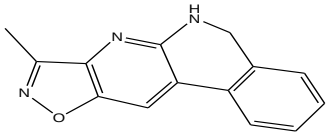
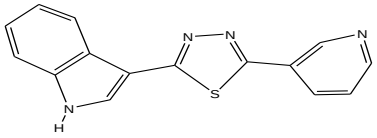
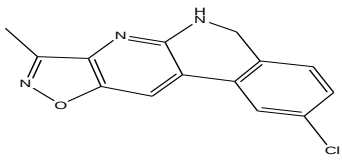
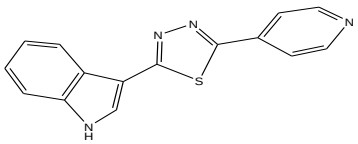
A dataset of the series of indole Compounds collected from literature [17-20], are listed in table 1. A total of 30 derivatives of indole were studied and analyzed in order to find quantitative structure activity relationship between the anti-proliferative activity and the structure of these molecules. The IC50 values in µM units exhibiting 50% inhibition of cell growth for human breast cancer (MCF 7) were converted in pIC50 by taking logarithm (pIC50 = log10 IC50) for QSAR stady.

II. MATERIALS AND METHODS

Table.1: Observed IC50 of the indole derivatives anti-proliferative agents

S.N	Structure	pIC50	S.N	Structure	pIC50
M1		1.988	M16		1.690
M2		0.577	M17		1.781
M3		1.953	M18		1.274
M4		1.273	M19		1.684
M5		1.908	M20		1.703
M6		1.273	M21		1.674

http://dx.doi.org/10.22161/ijeab/2.1.33 *ISSN: 2456-1878*

M7		1.621	M22		1.745
M8		1.105	M23		2.447
M9		1.209	M24		1.130
M10		1.484	M25		2.174
M11		1.459	M26		1.089
M12		1.233	M27		0.832
M13		1.588	M28		2.161
M14		1.218	M29		0.812
M15		1.727	M30		1.961

2.2. Molecular descriptor

The present work is necessary for us to determine several different descriptors to estimate in the QSAR model. The quantum chemical calculations are performed at the B3LYP/6-31G(d) level of theory using GAUSSIAN 03 of programs [21] to calculate some electronic descriptors such as: Frontier molecular orbital's highest occupied molecular orbital: EHOMO (eV); lowest unoccupied molecular orbital energy :ELUMO (eV) ; The Gap energetic (Gap) (eV), (the difference between EHOMO and ELUMO); Total Energy TE (ua); The absolute electronegativity χ (eV), χ = (EHOMO + ELUMO)/2; the absolute hardness η (eV): η = (EHOMO - ELUMO)/2; The Softness S (eV), it is the reactivity index and defined reciprocal of hardness S= 1/η; The electrophilicity index ω (eV), ω = 2χ/2 η [22] and The dipole moment μ (Debye). On the other side, we have chosen some physico-chemical descriptors, which were computed with Advanced chemistry development's ACD/ Chem Sketch [23] and ChemDraw Ultra8.0 [24] programs was employed to calculate: Molecular Weight (MW), Torsion energy (TE), Repulsion energy (RE), electronic energy (EE), the octanol/water partition coefficient (log P), Parachor (Pc) and Density (D) Thus 12 descriptors. Data was presented in **Table 2**.

2. 3- Statistical methods

To explain the structure-activity relationship, The 12 quantitative descriptors of the compounds of indole (1 to 30) are studied using different statistical methods:

The principal component analysis (PCA) [25] using the software XLSTAT version 2013 [26]. This is an essentially descriptive statistical method which aims to present, in graphic form. The large information contained in a data, as shown in **table 1**. PCA is a helpful statistical technique for summarizing the maximum of information encoded in the structures of compounds. This method is very useful for understanding the distribution of the compounds. The Multiple Linear Regression (MLR) statistical technique is used to study the relation between one dependent variable and several independent variables. It is a mathematic technique that minimizes the differences between actual and predicted values. The multiple linear regression model (MLR) was performed to predict pIC50. and it served to select the used descriptors as the input parameters for (NLMR). MLR and MNLR were generated using the software XLSTAT version 2014. The obtained equations were justified by the determination coefficient (R^2) correlation coefficient (R) , mean squared error (MSE), Mean Absolute Error (MAE) and Fisher's criterion (F). [27,28].

The ANN analysis was performed with the use of Matlab software version 2009. A Neural Fitting tool (nftool) toolbox on a data set of the indole compounds [29]. Three components constitute a neural network: the topology of the connections between the nodes, the processing elements or nodes and the learning rule by which new information is encoded in the network. However, there are a number of different ANN models; the most frequent type of ANN in QSAR is the three-layered feed-forward network [30]. In this kind of networks, the neurons are arranged in layers (an input layer, one hidden layer and an output layer). the neurons in any layer is fully connected with the neurons of a succeeding layer and no connections are between neurons belonging to the same layer.

Cross-validation (CV) is a popular technique used to explore the reliability of statistical models. Based on this technique, a number of modified data sets are created by deleting in each case one or a small group of molecules. These procedures are named respectively "leave-one-out" and "leave-some-out" [31-33]. For each data set, an input-output model is developed. In this study we used, the Leave-One-Out (LOO) procedure.

III. RESULTS AND DISCUSSION

3.1. Data set for analysis

A QSAR study was performed on 30 indole derivatives as reported previously, in order to identify a quantitative relationship between the structure and anti-proliferative activity against breast cancer cells lines (MCF7). The values of the 12 descriptors (2D and 3D descriptors) are shown in **Table 2**.

Table.2: Dataset used for QSAR analysis of series of indole derivatives

molecules	E_{HOMO}	E_{LUMO}	ΔE	μ	χ	TotE	*Log P*	*RE*	*TE*	*Kow*	MW	*D*
M1	-4.907	-0.846	4.061	2.855	2.030	-879.519	1.744	16622.8	7.5280	2.860	262.31	1.214
M2	-4.905	-0.064	4.841	2.796	2.484	-996.254	2.498	20936.1	14.036	4.053	302.37	1.216
M3	-5.395	-1.403	3.992	2.423	3.399	-940.636	3.300	17066.4	16.621	5.171	287.36	1.166
M4	-5.229	-1.297	3.932	1.239	3.263	-1055.11	3.174	20431.2	0.9840	5.137	317.38	1.174
M5	-5.171	-1.158	4.013	3.782	3.164	-899.114	2.022	15064.2	24.456	3.545	263.29	1.192

M6	-5.013	-0.882	4.131	3.402	2.947	-938.687	2.439	16703.9	24.443	4.074	277.32	1.168
M7	-5.032	-0.991	4.041	1.192	3.011	-899.376	1.854	15479.6	23.082	3.325	263.29	1.190
M8	-0.218	-0.160	0.058	1.964	0.189	-1175.25	3.086	21693.7	17.771	4.112	348.36	1.340
M9	-0.220	-0.141	0.079	1.242	0.180	-1138.07	3.213	18040.2	5.7020	3.884	318.33	1.340
M10	-0.210	-0.044	0.166	5.920	0.127	-1221.04	4.952	24448.4	19.596	5.970	374.44	1.240
M11	-0.213	-0.048	0.165	1.881	0.130	-1142.41	4.187	22242.3	19.484	4.832	346.39	1.290
M12	-5.820	-1.331	4.489	2.474	3.575	-3634.88	4.042	20755.0	22.713	4.918	397.23	1.590
M13	-0.217	-0.054	0.163	3.348	0.135	-1400.82	4.134	25558.2	8.8150	5.067	386.33	1.440
M14	-5.564	-1.687	3.877	4.393	3.625	-779.571	2.636	13236.6	11.444	3.202	237.26	1.305
M15	-5.748	-1.905	3.843	4.656	3.826	-1239.16	3.195	14469.8	11.896	3.918	271.70	1.402
M16	-5.741	-1.900	3.840	4.592	3.820	-3350.67	3.465	14425.7	11.537	4.068	316.15	1.597
M17	-5.509	-1.646	3.863	4.521	3.577	-818.890	3.123	14613.0	11.395	3.701	521.28	1.269
M18	-5.485	-1.633	3.852	5.655	3.559	-894.094	2.510	16064.6	10.890	3.213	267.28	1.299
M19	-5.709	-2.029	3.680	5.824	3.869	-878.558	2.794	14600.0	11.608	3.348	255.25	1.372
M20	-5.508	-1.628	3.880	4.839	3.568	-818.888	3.123	14634.9	14.399	3.701	251.28	1.269
M21	-5.696	-2.044	3.652	3.172	3.870	-1238.92	3.195	14502.5	11.543	3.918	271.70	1.402
M22	-5.489	-1.612	3.877	4.567	3.550	-1178.76	4.175	14005.1	28.720	3.652	277.35	1.331
M23	-5.509	-1.181	4.328	4.793	3.345	-1218.06	4.554	14998.9	11.231	3.621	291.37	1.309
M24	-5.593	-1.808	3.785	5.518	3.7005	-1638.35	4.734	15919.8	13.132	4.368	311.79	1.415
M25	-5.463	-1.579	3.884	3.715	3.521	-1312.71	4.46	18832.6	12.738	3.984	320.42	1.300
M26	-5.506	-1.659	3.847	3.898	3.5825	-1407.78	3.923	19998.1	10.607	3.433	337.4	1.316
M27	-5.429	-1.57	3.859	4.217	3.4995	-1522.31	4.175	25455.7	53.488	2.921	381.45	1.295
M28	-5.468	-1.633	3.835	3.813	3.5505	-1599.54	5.586	26277.4	11.423	5.458	399.47	1.318
M29	-5.627	-1.849	3.778	6.858	3.738	-1194.79	2.838	14662.8	26.266	2.255	278.33	1.380
M30	-5.718	-2.101	3.617	6.78	3.9095	-1194.72	2.838	14721.1	28.986	2.255	278.33	1.380

3.2. Data Modeling.

3.2.1 Principal component analysis

The 12 descriptors (variables) describing the 30 molecules were submitted to Principal Components Analysis (PCA). The first two principal axes are sufficient to describe the information provided by the data matrix.

Figure.2 presents the percentages of variance: F1= 40, 50 %. F2= 21,23% and the total information is estimated on 61,74 %.

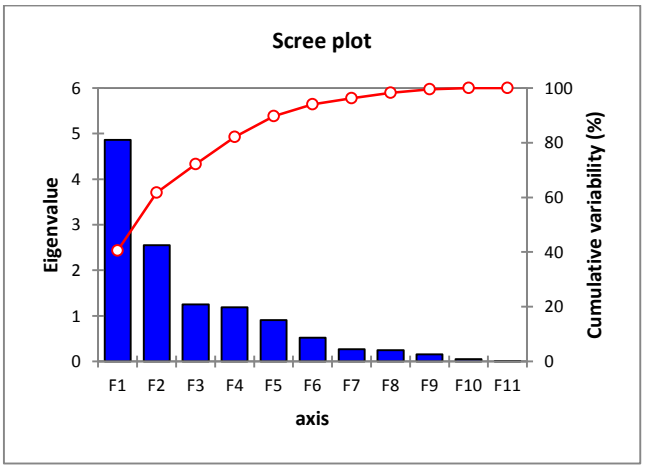


Fig. 2. The principal components and their variances

The principal component analysis (PCA) was carried out to have an idea about the link between the different variables.

The obtained matrix (Table3) summarizes the correlations between the 12 descriptors and provides information on the negative or positive correlation between variables. Figure 3 shows these descriptors in a correlation circle. In general the correlation matrix shows a low interrelationship between most of the descriptors, Good co-linearity (r>0.5) was observed between some of the variables. Hight interrelationship was observed between E_{HOMO} and χ (r = -0.979), E_{HOMO} and ΔE (R= -0 .96), E_{LUMO} and χ (R=-0.91) and ΔE and χ (R= 0.90), the variables ΔE and χ are removed to decrease the correlations.

Table.3: The correlation matrix (pearson (n)) between different obtained descriptors

Variables	E_{HOMO}	E_{LUMO}	ΔE	μ	χ	TotE	Log P	RE	TE	Kow	MW	D
E_{HOMO}	**1**											
E_{LUMO}	**0.845**	**1**										
ΔE	**-0.969**	**-0.688**	**1**									
μ	-0.347	**-0.536**	0.224	**1**								
χ	**-0.979**	**-0.918**	**0.907**	**0.419**	**1**							
TotE	0.106	0.142	-0.078	0.020	-0.132	**1**						
Log P	0.194	0.018	-0.255	0.149	-0.114	**-0.378**	**1**					
RE	**0.538**	**0.559**	**-0.474**	-0.317	**-0.549**	-0.186	**0.541**	**1**				
TE	-0.106	-0.093	0.101	0.219	0.125	-0.090	0.042	0.142	**1**			
Kow	**0.428**	**0.436**	**-0.381**	**-0.380**	**-0.413**	-0.257	**0.564**	**0.583**	-0.312	**1**		
MW	0.288	0.225	-0.288	-0.119	-0.259	-0.312	**0.509**	**0.576**	0.049	**0.428**	**1**	
D	-0.040	-0.293	-0.080	0.267	0.128	**-0.790**	0.326	-0.042	-0.028	-0.009	0.178	**1**

The correlation circle (Figure 3**)** which shows that the F1 axis (40.50 % of the variance) appears to represent the Density (D) and the Total energy (TE). The F2 axis (21.24% of the variance) seems to represent the E_{HOMO} and gap Energy (ΔE).

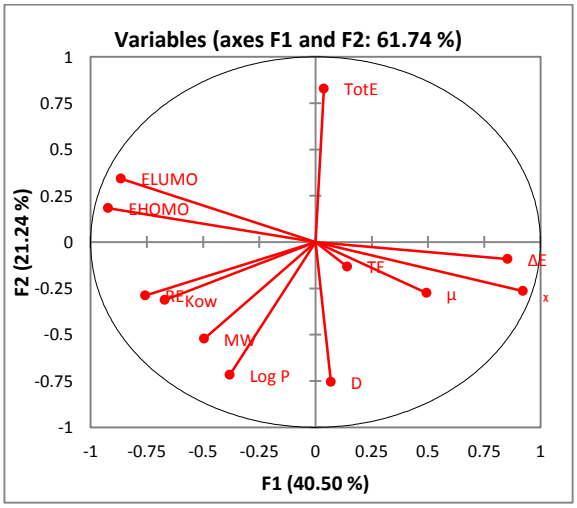


Fig. 3: Correlation circle between descriptors

From other side the analysis of diagrams according to the planes F1 and F2 (of the total variance) of the studied series are presented in Figure 4 we can discern three groups of molecules:

- Group 1: contains the molecules: M8, M9, M11, M10, and M13. (Green color)
- Group 2: contains the molecules M27, M24, M16, M28 and M12. (Red color)
- Group 3: contains the rest of the molecules. (Blue color)

When we return to the structures of molecules M8, M9, M10 and M11 (group 1) , we note that all these molecules are alike in their structures, and have as basic structure compound 1- Aryl- 1H- 1,2,3- Triazol-4-yl methyl 1H indole-2-carboxylate. The molecules M24, M27 and M28 (group 2) have the same basic derivative which is 5- (3-indolyl) -2-Substituted-1,3,4-thiadiazoles. Group 3 is the most important of the groups because it contains a large number of molecules (20 molecules) which have the same behavior.

http://dx.doi.org/10.22161/ijeab/2.1.33 *ISSN: 2456-1878*

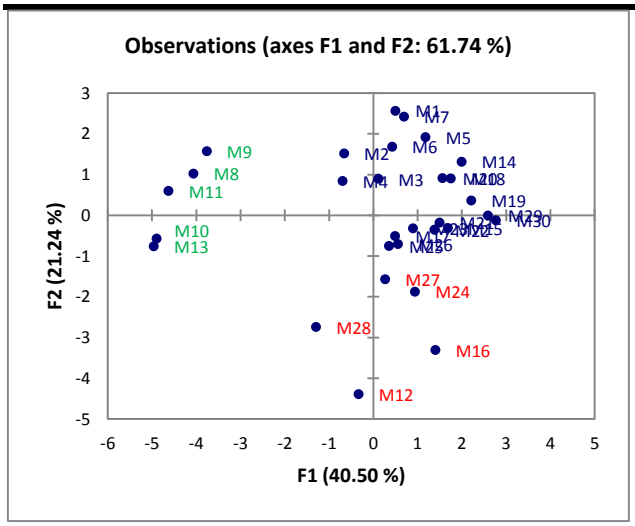


Fig.4: Correlation plot between the different molecules

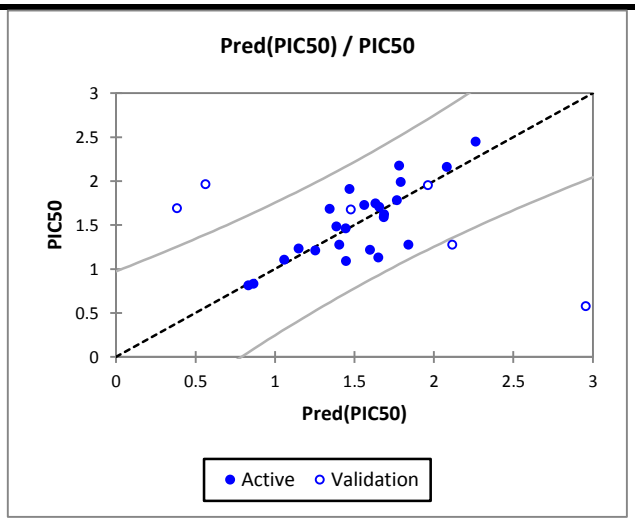


Fig. 5: Graphical representation of calculated and observed pIC50 by MLR

3.2.2 Multiple linear regressions MLR

Our work is based on the development of the best QSAR model to clarify the correlation between the different descriptors and the biological activities pI50 values of the indole derivatives. This method utilised several coefficients: R is the correlation coefficient, R^2 is the coefficient of determination, MSE mean squared error , MAE Mean Absolute Error and F is the Fisher F-statistic those coefficients adopt the best regression performance.

The obtained relationship in this model by the linear combination of the essential descriptors: E_{HOMO}, E_{LUMO}, μ, TE, Log P, RE, TE, Kow, MW, D.

The QSAR models using multiple linear regressions method is represented by the following equations:

pIC50 = -2.91 - 0.45* $\mathbf{E_{HOMO}}$ + 1.15 * $\mathbf{E_{LUMO}}$ - 6.44 E-02 * **μ** + 8.74 E-04 * **TE** + 0.20 * **LogP** - 4.12 E-06 * **RE** - 9.31 E-03 * **TE** + 0.22 * **Kow** + 3.03 E-04* **MW** + 2.84 * **D**.

N= 24 N_{test}= 6 R= 0.80 R^2= 0.641
MSE = 0.48 MAE = 0.44 F = 2.326

The model shows a good correlation coefficient (R =0.800) between ten descriptors and the anti-proliferative activity. This equation shows that the anti-proliferative activity of the indole derivatives depends on the electronic and the topological side of the molecule. Anti-proliferative activity increases by increasing the topological properties, Log P, RE, Kow, MW, D and by diminishing the electronic properties E_{HOMO}, μ, TE, RE, TE. Figure 5 presents the graphical representations of graphical calculated and observed pIC50 by MLR.

As illustrated in Figure 5, the correlation between calculated and experimental activities is very remarkable.

3.2.3 Multiple nonlinear regressions MNLR

We have utilized the technique of nonlinear regression model to improve the structure activity relationship in a quantitative way, the selected descriptors from the MLR model are used like data base matrix for the MNLR. The resulting equation is:

PIC50 = 120.18 + 2.44 * $\mathbf{E_{HOMO}}$ + 1.01 * $\mathbf{E_{LUMO}}$ + 0.75 * **μ** + 6.63 E-03 * **TE** - 4.56 * **LogP** - 5.06 E-05 * **RE** - 4.73 E-02 * **TE** - 1.44 * **Kow** + 0.16 * **MW** - 203.25 * **D** + 0.44 * $\mathbf{E_{HOMO}}^2$ + 0.43 * $\mathbf{E_{LUMO}}^2$ - 0.11 * $\boldsymbol{\mu}^2$ + 6.27 E07 * $\mathbf{TE}^2$ + 0.74 * $\mathbf{LogP}^2$ - 5.9 E-09 * $\mathbf{RE}^2$ + 1.13 E-03 * $\mathbf{TE}^2$ + 0.12 * $\mathbf{Kow}^2$ - 2.15 E-04 * $\mathbf{MW}^2$ + 79.02 *$\mathbf{D}^2$

N= 24 N_{test} = 6 R= 0.95 R^2= 0.90 MSE = 0.13
MAE= 0.24

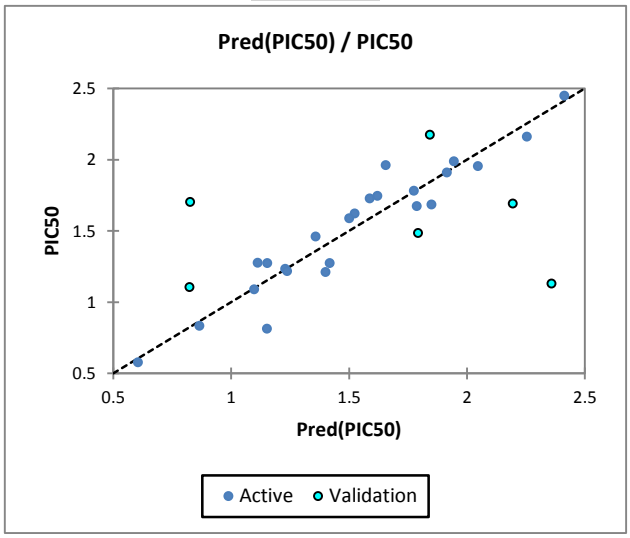


Fig. 6: Graphical representation of calculated and observed pIC50 by MNLR

http://dx.doi.org/10.22161/ijeab/2.1.33 *ISSN: 2456-1878*

The obtained correlation coefficient was significant R = 0,95. **Figure 6** shows a regular distribution of the PIC50 observed values depend on the experimental values.

3.2.3 Artificial Neural Networks (ANN)

In order to increase the probability of good characterization of studied compounds, artificial neural networks (ANN) can be used to generate predictive models of quantitative structure-activity relationships (QSAR) between a set of molecular descriptors obtained from the MLR, and the observed activity. The calculated activities model was developed using the properties of several studied compounds. Some authors **[34, 35]** have proposed a parameter ρ, leading to determine the number of hidden neurons, which play a major role in determining the best ANN architecture. These are defined as follows:

ρ = (Number of data points in the training set /Sum of the number of connections in the ANN)

The values of predicted activities (pIC50) using ANN and the observed values are given in **Table 4**. The correlation between calculated ANN and experimental anti-proliferative values is very significant as indicated by R and R^2 values illustrated in **figure 8.**

Table.4: The observed and ANN predicted activities

Molecules	pIC50	Pred (pIC50)
M1	1,988	2.0078
M2	0,577	0.5947
M3	1,953	2.0063
M4	1,273	1.2653
M5	1,908	1.7715
M6	1,273	1.4139
M7	1,621	1.5753
M8	1,105	1.0249
M9	1,209	1.2162
M10	1,484	1.4810
M11	1,459	1.5133
M12	1,233	1.2304
M13	1,588	1.5925
M14	1,218	1.3461
M15	1,727	1.7242
M16	1,690	1.7099
M17	1,781	1.7874
M18	1,274	1.2230
M19	1,684	1.7271
M20	1,703	1.5881
M21	1,674	1.6810
M22	1,745	1.7352
M23	2,447	2.4466
M24	1,130	1.0820
M25	2,174	2.1477
M26	1,089	1.1153
M27	0,832	0.8418
M28	2,161	2.1485
M29	0,812	0.8772
M30	1,961	1.9345

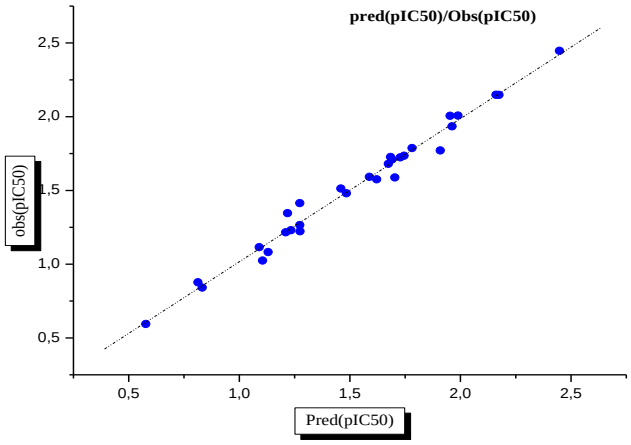


Fig. 8: Correlations of observed and predicted activities

$TC_{50\ (2)}$ calculated using ANN

N=30 R= 0.99 R^2 = 0.98 MSE=0.003 MAE= 0.03

The obtained correlation coefficient R value confirms that the artificial neural network result was the best to build the quantitative structure activity relationship models.

A comparison of the quality of MLR, MNLR and ANN models **table 5** shows that the ANN models have substantially better predictive capability because the ANN approach gives better results than MLR and MNLR. ANN was able to establish a satisfactory relationship between the molecular descriptors and the activity of the studied compounds.

Table.5: observed, predicted activities according to different used methods

Molecules	Obs (pIC50)	(pIC50) RLM	(pIC50) RNLM	(pIC50) ANN	(pIC50) CV
M1	1,989	1,791	1,944	2,0078	1,78
M2	1,909	1,470	0,605	0,5947	0,61

M3	1,273	1,840	2,046	2,0063	1,39	
M4	1,621	1,688	1,154	1,2653	1,2	
M5	1,106	1,058	1,915	1,7715	1,79	
M6	1,210	1,254	1,418	1,4139	1,34	
M7	1,484	1,386	1,524	1,5753	1,63	
M8	1,459	1,446	1,400	1,0249	1,21	
M9	1,233	1,149	1,358	1,2162	1,37	
M10	1,588	1,686	1,230	1,481	1,43	
M11	1,218	1,598	1,500	1,5133	1,46	
M12	1,728	1,563	1,237	1,2304	1,22	
M13	1,781	1,768	1,588	1,5925	1,57	
M14	1,275	1,405	1,774	1,3461	1,32	
M15	1,684	1,344	1,113	1,7242	1,68	
M16	1,703	1,658	1,850	1,7099	1,72	
M17	1,745	1,632	1,787	1,7874	1,61	
M18	2,448	2,263	1,620	1,223	1,39	
M19	1,130	1,651	2,413	1,7271	1,67	
M20	2,175	1,782	1,097	1,5881	1,63	
M21	1,090	1,447	0,865	1,681	1,58	
M22	0,833	0,865	2,254	1,7352	1,64	
M23	2,161	2,081	1,151	2,4466	1,99	
M24	0,813	0,833	1,656	1,082	1,23	
M25	0,577	2,957	0,823	2,1477	1,95	
M26	1,953	1,964	1,793	1,1153	1,42	
M27	1,273	2,116	2,195	0,8418	1,71	
M28	1,691	0,383	0,827	2,1485		1,93
M29	1,674	1,477	2,358	0,8772	1,71	
M30	1,962	0,562	1,843	1,9345	1,82	

3.2.4 Cross Validation

It is important to be able to use ANN to predict the activity of new compounds. To evaluate the predictive ability of the ANN models, 'Leave-one-out' is an approach which is well adapted to the estimation of that ability. A good correlation was obtained with cross validation RCV = 0,74. So, the predictive power of this model is very significant. The results obtained showed that models MLR, MNLR and ANN are validated, which means that the prediction of the new compounds is feasible

In this study, three different modelling methods, MLR, MNLR and ANN were used in the construction of a QSAR model for 30 derivatives of indole and the resulting models were compared (**table 5 - table 6)**. It was shown that the artificial neural network ANN results have better predictive capability than the MLR and MNLR. we established a relationship between several descriptors and the anti-proliferative activity p**IC**$_{50}$ in satisfactory manners. The good results obtained with the cross validation (CV) shows that the model proposed in this paper are able to predict activity with a good performance, and that the selected descriptors are pertinent.

Table.6: Statistical values obtained by different methods

	RLM	**RNLM**	**ANN**	**CV**
R	0.80	0.95	0.99	0.74
MSE	0.48	0.13	0.003	0.08
MAE	0.44	0.24	0.03	0.18

Correlation coefficient (R), Mean squared error (MSE), Mean Absolute Error (MAE)

IV. CONCLUSION

In this paper we have used different statistical methods: MLR, MNLR, ANN, cross validation CV and various electronic and topologic descriptors for construction of QSAR model for the anti-proliferative activity of indole derivatives, also, were compared the statistical terms R, R2 , MAE, MSE Resulting models. Moreover, the neural network ANN results (R= 99, MAE= 0.03 MSE= 0.003) have better predictive capability than the MLR and MNLR. A good correlation was obtained with cross validation RCV = 0,74 that confirms the great ability of our model to predict the activity. we established a relationship between several descriptors and inhibition values pIC50 of several organic compounds based on substituted indole in satisfactory manners. That studied model which is sufficiently rich in chemical, electronic and topological information may be utilized for predicting and developing new molecules with better effect. Thus, thanks to QSAR studies, especially with the ANN that allowed us to improve the correlation between the observed biological activity and that predicted, we can enjoy the performance of the predictive power of this model to explore and propose new molecules that could be active in experiment.

REFERENCES

[1] Siegel R, Naishadham D and Jemal A: Cancer statistics, 2013. CA cancer J clin 63: 11-30, 2013.

[2] Aliabadi HM, Maranchuk R, Kucharaki C, Mahdipoor P, Hugh J, Uludag H. Effective response of doxorubicin- sensitive and resistant breast cancer cells to combinational siRNA therapy. J Contr Release Official: J Contr Release Soc 2013; 172(1): 219-228.

[3] Hong SE, Kim EK, Jin HO, Kim HA, Lee JK, Koh JS, et al S6K1 inhibition enhances tamoxifen-induced cell death in MCF-7 cells through translational inhibition o Mcl-1 and survinn. Cell Biol Toxicol 2013: 29(4): 273-282.

[4] Choi HN, Jin HO, Kim JH, Hong SE, Kim HA, Kim EK, et al. Inhibition of S6K1 enhances glucose deprivation-induced cell death via downregulation of anti-apoptotic proteins in MCF-7 breast cancer

[5] celles Biochem Biophys Res Comm 2013; 432(1): 123-128

[6] Hao Wang, Yi-Feng Ye, Effect of survivin siRNA on biological behaviour of breast cancer MCF7 cells. J Tropical Medicine (2015)225-228.

[7] T.M.T. Carvalho, L.M.P. Amaral, V.M.F. Morais, M.D.M. Ribeiro da Silva, Thermodynamic properties of alkyl 1*H*-indole carboxylate derivatives: a combined experimental and computational study, *J. Chem. Thermodynamics* (2016).

[8] Sirassu Narsimha, Nukala Satheesh Kumar, Kumara Swamy Battula, Vasudeva Reddy Nagavelli ,S. K. Althaf Hussain , M. Srinivasa Rao. Indole-2-carboxylic acid derived mono and bis 1,4 disubstituted 1,2,3-triazoles: Synthesis, characterization and evaluation of anticancer, antibacterial, and DNA-cleavage activities. Bioorg. Med.Chem. Lett 26 (2016) 1639–1644

[9] Daly, S; Hayden, K.; Malik, I.; Porch, N.; Tang, H.; Rogelj, S.; Frolova, L. V.; Lepthien, K.; Kornienko, A.; Magedov, I.V. Bioorg Med. Chem. Lett. 2011, 21, 4720

[10] Holla, B. S.; Mahalinga, M.; Karthikeyan, M. S.; Poojary, B.; Akberali, P. M.; Kumari, N. S. Eur. J. Med. Chem. 2005, 40, 1173.

[11] . Mady, M. F.; Awad, G. E. A.; Jørgensen, K. B. Eur. J. Med. Chem. 2014, 84, 433.

[12] Rao, P. S.; Kurumurthy, C.; Veeraswamy, B.; Kumar, G. S.; Poornachandra, Y.; Kumar, C. G.; Babu, V. S.; Kotamraju, S.; Narsaiah, B. Eur. J. Med. Chem. 2014, 80, 184.

[13] H.N. Dogan, A. Duran, S. Rollas, G. Sener, M.K. Uysal, D. Gülen, Bioorg. Med. Chem. 10 (2002) 2893e2898.

[14] F. Poorrajab, S.K. Ardestani, S. Emami, M. Behrouzi-Fardmoghadam, A. Shafiee, A. Foroumadi, Eur. J. Med. Chem. 44 (2009) 1758e1762.

[15] M. Yusuf, R.A. Khan, B. Ahmed, Bioorg. Med. Chem. 17 (2008) 8029e8034.

[16] Hansch C., Muir R.M., Fujita T., Maloney P.P, Geiger F., Streich M., J. Am. Chem. Soc. 85 (1963) 2817–2825.

[17] Bodor N., Current Medicinal Chemistry 5 (1988) 353-380. From book: Biochemistry of Redox Reactions, by Bernard Testa, editor: London [u, a], Acad. Press (1995).

[18] Xing-yue Ji , Si-tu Xue, Yue-chen Zhan, Jia-jia Shen, Lin-tao Wu, Jie Jin, Zhen Wang, Zhuo-rong Li , J. Med. Chem. 83 (2014) 409-418.

[19] Sirassu Narsimha, Nukala Satheesh Kumar, Kumara Swamy Battula , Vasudeva Reddy Nagavelli, S. K. Althaf Hussain, M. Srinivasa Rao. Bioorg. Med. Chem , 26 (2016) 1639–1644.

[20] Dalip Kumar , N. Maruthi Kumar , Kuei-Hua Chang , Kavita Shah, Eur. J. Med. Chem. 45 (2010) 4664-4668.

[21] E. Rajanarendar, K. Govardhan Reddy, S. Ramakrishna, M. Nagi Reddy, B. Shireesha, G. Durgaiah, Y. N. Reddy. Bioorg . Med. Chem . 22 (2012) 6677–6680.

[22] M.J. Frisch & al, Gaussian 03, Revision B.01, Gaussian, Inc., Pittsburgh, PA, (2003).
[23] U. Sakar, R. Parthasarathi, V. Subramanian & P.K. Chattaraji, Toxicity analysis of polychlorinated dibenzofurans through global, J. Mol. Des. IECMD, (2004) 1-24.
[24] Advanced Chemistry Development Inc., Toronto, Canada, (2009), (www.acdlabs.com/resources/freeware/chemsketch/) .
[25] ACD/Labs Extension for ChemDraw Version 8.0 for Microsoft Windows User's Guide.
[26] M Larif, A Adad , R Hmamouchi, A I Taghki, A Soulaymani , A Elmidaoui, M Bouachrine, T Lakhlifi article in press in *Arabian Journal of Chemistry* **2013**.
[27] SYSTAT 13 Add-in software (SYSTAT Company). www.systat.com.
[28] Y. Boukarai, F. Khalil, M. Bouachrine, QSAR study of isatin (1H-indole-2-3-dione) analogues as in vitro anticancer agents using the statistical analysis methods, *International Journal of Scientific & Engineering Research* (**2015**)
[29] Youness Boukarai, Fouad Khalil and Mohamed Bouachrine, QSAR study of 5,6-bicyclic heterocycles analogues as anti-Alzheimer's agents using the statistical analysis methods. J. Chem. Pharm. Res., 2016, 8(3):1000-1013
[30] Adad A., Hmamouchi R., Taghki A. I., Abdellaoui A., Bouachrine M., Lakhlifi T., Atmospheric half-lives of persistent organic pollutants (POPs) study combining DFT and QSPR results, J. of Chemical and Pharmaceutical Research, Vol. 5, Issue 7 (2013) 28-41 .
[31] V.J. Zupan, J. Gasteiger, Neural Networks for Chemists - An Introduction, VCH Verlagsgesellschaft, Weinheim/VCH Publishers, New York. 106(12) (**1993**) 1367-1368.
[32] B. Efron, Estimating the error rates of a predictive rule: improvement on crossvalidation, J. Am.Stat. Assoc. 78 (1983) 316-331
[33] M.A. Efroymson, Multiple regression analysis, In Mathematical Methods for DigitalComputers, Ralston, A., Wilf, H.S., Eds,WileyNewYork, (1960).
[34] D.W. Osten, Selection of optimal regression models via cross-validation, J. Chemom. 2(1998) 39-48.
[35] S-S. So & W.G. Richards, Application of neural networks: quantitative structure-activity relationships of the derivatives of 2, 4 diamino (substituted-benzyl) pyrimidines as DHFR inhibitors, J. Med. Chem. 35 (1992) 3201-3207.
[36] T.A. Andrea & H. Kalayeh, Applications of neural networks in quantitative structure-activity relationships of dihydrofolate reductase inhibitors, J. Med. Chem. 34 (1991) 2824–2836.

http://dx.doi.org/10.22161/ijeab/2.1.34 *ISSN: 2456-1878*

Biodiversity of Medicinal Plants in Thudaripettai Village, Nagapattinam District, Tamil Nadu, India

Kathirvel Murugan, A., T. Vaithiyanathan, P. Sundaramoorthy*

Department of Botany, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India.

Abstract— *The medicinal plants have received more attention among researchers to treat various diseases and disorders. This study was aimed to record the various medicinal plants present in Thudaripettai Village situated in Tharangambadi Taluk, Nagapattinam district of Tamil Nadu. A total of 60 plant species belonging to 35 families were reported with their medicinal values. These results will provide information about medicinal plants and methods of utilization of these plants to cure various diseases of mankind. Survey of the information of medicinal plants used by the villagers were collected and arranged alphabetically followed by common name, vernacular name, family name, parts of use, methods of uses, medicinal uses and their habit. The information is very much useful for further research which will lead to the discovery of new bioactive compounds from the above medicinal plants.*

Keywords— Medicinal plants, diseases, survey, Thudaripettai village.

I. INTRODUCTION

Medicinal plants play an important role in supporting healthcare system in world. According to the World Health Organization (WHO, 2001), 80% of the rural population in developing countries utilizes locally available medicinal plants for their primary healthcare needs. About 90% of the country's medicinal plants are found in forest habitats. Only 10% of the medicinal plants are distributed among other landscape sources like open grasslands, agricultural pastures and in and around fresh water bodies, etc. It may be noted that India is one amongst those nations which possess a historical track record of having made a significant global contribution by virtue of its traditional knowledge of the medicinal plants. India has rich plant diversity and is one among the mega biodiversity countries of the world. The most of the medicinal drugs used to cure human diseases are obtained from plants and their derivatives (Principle, 2005). The crude drugs are usually obtained from the dried parts of medicinal plants (roots, stem wood, bark, leaves. flowers seeds, fruits, and whole plants etc.). They form the essential raw materials for the production of traditional remedies of Ayurveda, Siddha, Unani, Homeopathy, Tibetan and other systems of medicine including the folk, ethno or tribal medicines (Fabricant and Farnswirth, 2001).

The Indian systems of medicine still continue to provide medical care to majority of the people on account of their cheaper cost, easily available with no side effects (Kokate *et al.*, 2002). Herbal drugs are safer in the treatment of various diseases (Ayyanar and Ignacimuthu, 2005, Sathyavathi and Janardhan, 2011). The Indian systems of medicine use around 8000 species of plants which include trees (33%), herbs (32%), shrubs (20%), climbers (12%) and epiphytes, grasses, lichens, ferns and algae put together (3%). Among 2000 drugs being used in curing humane ailments in India, only 200 are extracted from various plants (Agarwal and Ghoseh, 1985). India is also home to many language, culture and beliefs which have in turn contributed to the high diversity of traditional knowledge. Large populations in India still rely on traditional herbal medicine (Dubey *et al.*, 2004; Kumar *et al.*, 2015). The village folks developed their own therapeutic knowledge for the treatment of diseases with herbs and this knowledge is only stored in their memory. Therefore, the study was conducted with a view to record the scattered list of the available plants with Knowledge and to point out the potentials of medicinal plants of the area.

II. MATERIALS AND METHODS

A survey was conducted in Thudaripettai Village to record the list of medicinal plants and their importance in medicinal world. The study area Thudaripettai Village is situated in Tharangambadi Taluk in Nagapattinam District of Tamil Nadu which lies between $11^0 1'45^0$ N and $75^0 50'58^0$ E. The plants were collected from different sites of the village area, identified by their local names with the help of old aged people and villagers. The data on medicinal uses of plants was collected through general conversation and questionnaire with people of the area. The Botanical name of the plants was verified with the

http://dx.doi.org/10.22161/ijeab/2.1.34 *ISSN: 2456-1878*

specimens kept in Botany Department, Annamalai University, Tamil Nadu.

III. RESULTS AND DISCUSSION

In most cases, the active molecules of the medicinal plant reported here are unknown. Studying the biological and pharmacological properties of medicinal plant extracts is a rational approach in the quest for new drugs. Phytochemical and pharmacological studies can lead to evidence of potential therapeutic use of medicinal plants and the development of new medicines. The traditional information accumulated by local people has an important role to play in this effort (Kumar *et al.*, 2015). In the Present study, gives documentations of the medicinal plants used by the people of Thudaripettai village were collected and recorded. The list out 60 plant species were belonging to 35 families are reported (Table-1). Among all families, Solanaceae (6 species) is most dominant family followed by Euphorbiaceae (5 species), Apocynaceae and Fabaceae (4 species), Lamiaceae and Moraceae (3 species), Rutaceae, Poaceae, Myrtaceae, Cucurbitaceae, Apiaceae,

S.No	Botanical name	Common name	Vernacular name	Family	Parts of use	Methods	Uses	Habit
1	*Acalypha indica*	Indian acalypha	Kuppaimeni	Euphorbiaceae	Whole plant	Extract	Fire wounds, snake bites, scabies, and eczema.	Herb
2	*Achyranthes aspera*	Spear grass	Nayuruvi	Amaranthaceae	Whole plant	Extract	Skin diseases, constipation, lack of appetite, acidity, cough, ear pain, leprosy and rabies.	Herb
3	*Acorus calamus*	Sweet-flag	Vasambu	Araceae	Rhizomes	Paste	Eczema.	Shrub
4	*Adhatoda vasica*	Malabar nut	Adhatoda	Acanthaceae	Leaves, roots, flowers, barks	Extract, dry leaf	Asthma, cough, fever, vomiting, stomach problems, rheumatism, piles, anti inflammatory.	Shrub
5	*Aegle marmelos*	Wood apple	Vilvam	Rutaceae	Fruits	Paste	Scabies.	Tree
6	*Aloe vera*	Indian aloe	Sotrukatrazha	Liliaceae	Whole plant	Extract, juice	Wounds, diabetics, antibacterial, cooling purposes.	Herb
7	*Andrographis panniculata*	Creyat root	Nilavembu	Acanthaceae	Leaves	Extract, juice	Tines curies.	Tree
8	*Artocarpus heterophillus*	Jackfruit	Palamaram	Moraceae	Leaves	Ash	Skin diseases.	Tree
9	*Azadirachta indica*	Neem tree	Vepamaram	Meliaceae	Whole plant	Extract, oil, powder	Skin diseases, eczema, psoriasis, healthy hair, liver function, detoxify blood,	Tree

http://dx.doi.org/10.22161/ijeab/2.1.34 *ISSN: 2456-1878*

							and balance blood sugar, antifungal, antibiotic, antibacterial and antiviral.	
10	*Bambusa bambos*	Bamboo	Moongil	Poaceae	Leaves, stems	Decoction, juice	Intestinal warms ulcers.	Tree
11	*Bassis longifolia*	Mahaal mow tree	Illupai	Sapotaceae	Fruits	Salads	Piles, constipation, improving appetite.	Tree
12	*Borassus flabellifer*	Palmyra palm	Panai	Palmaceae	Leaves, flowers, fruits	Juice, extract	Wounds, skin diseases, sugar antidote for poisoning.	Tree
13	*Cynodon dactylon*	Bermuda grass	Arugampul	Poaceae	Leaves	Juice	Cold, blood purification, itches and skin diseases.	Grass
14	*Calotropis gigantea*	Crown flower	Erukku	Apocynaceae	Latex, seeds, leaves, root	Powder, latex, fresh root	Toothache, skin care, wounds, boils coughs, improving appetite, scabies, and toothbrush.	Shrub
15	*Cardiospermum helicacabum*	Balloon vine	Mutakuatran	Sapindaceae	Leaves, roots, seeds, extract	Paste	Rheumatism, anti-diarrheal, nervous problems, snake bites.	Climber
16	*Carica papaya*	Papaya tree	Papali	Caricaceae	Fruits, latex	Salads, latex.	Malaria, non-fertility, antiviral, antibacterial, kidney failure.	Tree
17	*Catharanthus roseus*	Madagascar periwinkle	Nithyakalyani	Apocynaceae	Roots, shoots	Extract	Numerous diseases, diabetes, malaria, Hodgkin's lymphoma.	Sub shrub
18	*Centella asiatica*	Indian penny word	Vallarai	Apiaceae	Leaves	Extract, salads, thuvaial	Varicose veins, chronic venous insufficiency, psoriasis, fever, cold.	Herb
19	*Clioria ternatea*	Butterfly pea	Sangupoo	Fabaceae	Leaves	Juice	Scabies.	Climber
20	*Coccinia indica*	Coccinia	Kovai	Cucurbitaceae	Leaves,	Extract	Ulcers, diabetes,	Climbe

http://dx.doi.org/10.22161/ijeab/2.1.34 *ISSN: 2456-1878*

					fruits		fever.	r
21	*Curcuma longa*	Turmeric	Manjal	Zingiberaceae	Rhizomes	Powder	Skin disease, antimicrobial and stomach problems.	Herb
22	*Cyperus rotundus*	Nut grass	Koraikizhangu	Cyperaceae	Whole plant	Juice, paste, bulb.	Fever, digestive system disorders, wounds, stomach pain, toothache.	Grass
23	*Datura metal*	Devil's trumpet	Ummathai	Solanaceae	Leaves	Extract	Swelling, headache, asthma, coughs.	Shrub
24	*Eclipta alba*	Bhringraj	Vellai karisalan gani	Asteraceae	Whole plant	Extract	Jaundice, urinary problems, swelling, cold, ulcer, wounds, eye drops.	Herb
25	*Eichornia crassipes*	Water hyacinth	Akaya thamarai	Pontederiaceae	Flower	Oil	Skin diseases.	Weed
26	*Emblica officinalis*	Indian gooseberry	Nelli	Euphorbiaceae	Leaves, bark flowers, nuts	Extract	Tuberculosis, asthma, cancer, jaundice, liver tonic.	Tree
27	*Ficus benghalensis*	Baniyan	Aalamaram	Moraceae	Leaves, fruits, latex	Extract	Bleeding, piles, joint, muscular pain, skin diseases.	Tree
28	*Ficus religiosa*	Ashwatth a tree	Arasamaram	Moraceae	Whole plant	Extract	50 types of disorders including asthma, diabetes, diarrhea, epilepsy, gastric problems, inflammation disorders, infectious and sexual disorders.	Tree
29	*Helitropium indicum*	Indian heliotrope	Thelkodukku	Boraginaceae	Leaves	Extract	Skin diseases, ulcers.	Herb
30	*Hibiscusrosa-sinensis*	Shoe flower	Semparuthi	Malvaceae	Leaves, roots, flowers, young stem	Paste, decoction, powder, toothbrush	Mouth wound, ulcers, urinary disease, regularizes periods and hair fall.	Shrub

http://dx.doi.org/10.22161/ijeab/2.1.34 *ISSN: 2456-1878*

31	*Jatropha curcas*	Puriging nut	Kattamanakku	Euphorbiaceae	Seeds, leaves	Extract	Jaundice, piles, wounds.	Shrub
32	*Lagenaria vulgaris*	Bottle gourd	Suraika	Cucurbitaceae	Vegetable	Kootu	Digestive problems.	Climber
33	*Lawsonia inermis*	Henna	Maruthani	Lythraceae	Seeds, leaves	Extract	Wounds, skin ulcers, eye drop.	Shrub
34	*Leucas aspera*	Leucas	Thumbai	Labiataceae	Leaves	Constant rubbing	Scorpion bites.	Herb
35	*Mangifera indica*	Mango tree	Mamaram	Anacarddiaceae	Gum, stem, leaves, fruits, bark	Gum, paste, decoction	Skin diseases, sugar and menstrual disorder.	Tree
36	*Manihot esculenta*	Bitter cassava	Maravalli kizhangu	Euphorbiaceae	Rhizomes	Powder	Skin diseases.	Shrub
37	*Mentha spicata*	Mint	Pudina	Lamiaceae	Leaves	Thuvaial	Stomach pain and indigestion problems.	Herb
38	*Mimosa pudica*	Sensitive plant	Thottal surungi	Fabaceae	Roots, leaves	Decoction, infusion	Tuberculosis, wound.	Climber
39	*Morinda tinctoria*	Indian mulberry	Nuna	Rubiaceae	Fruits	Salads	Antibiotic.	Small tree
40	*Murraya koenigii*	Curry tree	Karuveppilai	Rutaceae	Leaves	Thuvaial, rasam, vada.	Lack of appetite, trough infection, digresses of sugar level.	Tree
41	*Musa paradisiaca*	Plantain	Vaalai	Musaceae	Whole plant	Extract, juice	Dysentery, stomach ache, piles, ulcers, kidney stones.	Tree
42	*Nerium indicum*	Rose laural	Arali	Apocynaceae	Leaves, seeds	Extract	Leprosy, skin disease, snake bites.	Shrub
43	*Ocimum sanctum*	Holy basil	Tulasi	Solanaceae	Whole plant	Paste and extract	Asthma, cold, cough, kidney problems, stimulate in fertility and improving appetite.	Herb
44	*Phyllanthus amarus*	Seed under leaf	Keezhanelli	Phyllanthaceae	Whole plant	Paste	Jaundice, hepatitis, kidney and liver related disease.	Herb
45	*Plectranthus*	Aromatic	Karpuravalli	Lamiaceae	Leaves	Extract	Fever, Acidity,	Herb

http://dx.doi.org/10.22161/ijeab/2.1.34 *ISSN: 2456-1878*

	amboinicus	us					cough.	
46	*Polygala crotalariodes*	Green chirayta	Siriyanangai	Polygalaceae	Leaves	Extract	Snake bites, antiviral.	Herb
47	*Pongamia pinnata*	Indian beach	Punkai maram	Fabaceae	Bark, leaves, flowers, seed	Paste, extract	Piles, ulcers, anti-septic, and parasitic rashes.	Tree
48	*Psidium guajava*	Guava	Goya	Myrtaceae	Fruits, bark, leaf	Salads, powder	Diabetes, hypertension, caries, wounds, pain relief, fever, diarrhea, rheumatism, lung disease, ulcers, bleeding, mouthwash.	Small-tree
49	*Ricinus communis*	Castor oil plant	Amanakku	Euphorbiaceae	Seed	Extract	Ulcer, eye irritation.	Shrub
50	*Sesamum indicum*	Gingily	Ellu	Pedaliaceae	Leaves, peel, flowers, seeds	Paste, oil, extract, powder	Piles, eye problems, wound, eczema, scabies, body heat and hair fall problems.	Shrub
51	*Solanum nigrum*	Blacknight-shade	Manathakkali	Solanaceae	Leaves	Rasam, kootu, juice	Heart, lungs, liver diseases, itches, ulcer and stomach problems.	Herb
52	*Solanum torvum*	Night shade plant	Sundaikai	Solanaceae	Fruits	Extract	Asthma, tuberculosis.	Shrub
53	*Solanum trilobatum*	Heliotrope	Thutuvalai	Solanaceae	Whole plant	Paste	Asthma.	Climber
54	*Solanum xanthocarpum*	Wild eggplant	Kadangkatari	Solanaceae	Leaves	Decoction	Asthma, cold, cough, polio.	Herb
55	*Syzygium cumini*	Indian black plum	Naval	Myrtaceae	Seeds, fruits	Extract	Dysentery, reduce sugar, mouthwash.	Tree
56	*Tabernaemontana divaricata*	Crape jasmine	Nandiarvattai	Apocynaceae	Roots, stems, latex, flowers	Latex, decoction	Scabies, dental caries, cough, eye disease, ulcers, anti-diarrheal and kidney stone, skin disease, intestinal worms.	Shrub
57	*Tamarindus indica*	Tamarin	Puliyamaram	Fabaceae	Fruits,	Spice	Laxative,	Tree

		d tree			young stem, bark	Condiment, syrups, decoctions, toothbrush, different pharmaceutical products	digestive, remedy for biliousness, bile disorders, Control of heart rate and blood pressure.	
58	*Trachyspermum ammi*	Capticum	Omum	Apiaceae	Leaves	Extract	Stomach pain, body pain, running nose.	Herb
59	*Vitex negundo*	Chinese chaste tree	Notchi	Lamiaceae	Leaves, roots, seeds	Extract, paste	Eczema, ringworm, other skin diseases, liver disorders, spleen enlargement, rheumatic pain, gout, abscess, backache, vermicide, it is also used to control population of mosquitoes.	Small tree
60	*Zizyphus mauritiana*	Indian plam	illandai	Rhamnaceae	Fruits	Juice, extract	Ulcers, liver trouble, asthma and fever.	Small tree

Acanthaceae (2 species) and 23 families like Amaranthaceae, Anacarddiaceae, Araceae, Asteraceae, Boranginaceae, Caricaceae, Cyperaceae, Labitaceae, Liliaceae, Lythraceae, Malvaceae, Meliaceae, Musaceae, Palmaceae, Pedaliaceae, Phyllanthaceae, Polygalaceae, Pontederiaceae, Phamaceae, Rubiaceae, Sapindaceae, Sapotaceae, Zingiberaceae were represented by single species (Table-2).

S.NO	FAMILY	NUMBER OF SPECIES
1	Acanthaceae	2
2	Amaranthaceae	1
3	Anacarddiaceae	1
4	Apiaceae	2
5	Apocynaceae	4
6	Araceae	1
7	Asteraceae	1
8	Boraginaceae	1
9	Caricaceae	1
10	Cucurbitaceae	2

11	Cyperaceae	1
12	Euphorbiaceae	5
13	Fabaceae	4
14	Labiataceae	1
15	Lamiaceae	3
16	Liliaceae	1
17	Lythraceae	1
18	Malvaceae	1
19	Meliaceae	1
20	Moraceae	3
21	Musaceae	1
22	Myrtaceae	2
23	Palmaceae	1
24	Pedaliaceae	1
25	Phyllanthaceae	1
26	Poaceae	2
27	Polygalaceae	1
28	Pontederiaceae	1
29	Rhamnaceae	1
30	Rubiaceae	1
31	Rutaceae	2
32	Sapindaceae	1
33	Sapotaceae	1
34	Solanaceae	6
35	Zingiberaceae	1

The different parts of the plants were used for the treatment of various diseases or disorders such as cold, itches, eczema, fever, digestive system disorders, diarrhea and dysentery, stomach-ache, asthma, jaundice, polio, joint-ache, headache, toothache, swelling, scabies, malaria, kidney problems etc. Most of the species were used for curing more than one disease. These were administrated mostly orally and a range of preparations such as decoction, paste and powder were adopted. Most of these preparations were made from the freshly collected plants just before the use; however, some are also used in dry form.

IV. CONCLUSION

From this study, many medicinal plants have been documented and this information was obtained from the local people and they are very much useful for further researchers in the field of ethno-medico-botany, taxonomy and pharmacological studies. The conservation and use of medicinal plants should be enhanced for the betterment of our lives. Further research on these medicinal plants will lead to the discovery of new bioactive compounds.

ACKNOWLEDGEMENT

The authors are thankful to the local village people for sharing their knowledge regarding plants and their medicinal uses in their traditional healthcare practices. They are also thankful to The Professor and Head, Department of Botany, Annamalai University for this encouragement to carry out this type of survey study.

REFERENCES

[1] Agarwal, R. and Ghoseh, S., 1985. Antimicrobial activity of certain Indian medicinal plants used in filkoric medicine. *Journal of Ethno pharmacology*, 74(3): 217-220.

[2] Ayyanar, M. and Ignacimuthu, S., 2005. Medicinal plants used by the tribals of Tirunelveli hills, Tamil Nadu to treat poisonous bites and skin diseases. *I. J. Trad. Knowl*., **4**(3): 229-236.

[3] Dubey, N.K., Kumar, R. and Tripati, P., 2004. Global promotion of herbal medicine India's opportunity. *Curr Sci.*, 86(1): 37-41.

http://dx.doi.org/10.22161/ijeab/2.1.34 *ISSN: 2456-1878*

[4] Fabricant, D. and Farnswirth, S., 2001. The value of plants used in traditional medicine for drug discovery. *Environ. Heaith Perspect.*, 109(1): 69-75.
[5] Kokate, C.K., Purohit, A.P. and Gokhale, S.B., 2002. Pharmacognosy, (Nirali Publication, Pune), 1-6.
[6] Kumar, S., Sharma, S.D. and Kumar, N., 2015. Ethnobatanical study of some common plants from district Hamirpur of Himachal Pradesh (India). *Int J Adv Res.*, 3(2):492- 496.
[7] Principle, P., 2005. Monetizing the Pharmacological Benefits of plants. US Environmental Protection Agency., Washington DC.
[8] Sathyavathi, R. and Janardhan. K.J, 2011. Folklore medicinal practices of badaga community in Nilgiri biosphere reserve, Tamilnadu, India. *International Journal of pharma research and Develoment*, 3(2): 50-63.
[9] World Health Organization (WHO), 2001: General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva: WHO Switzerland.

http://dx.doi.org/10.22161/ijeab/2.1.35 *ISSN: 2456-1878*

Effect of ethanolic extract of the leaves of plant *Annona squamosa* on hematological and biochemical parameters in normal rats

Preeti Singh, Veena Batra Kushwaha

Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur, India

Abstract*— *The present study was designed to elucidate the effects of ethanolic extract of leaves of Annona squamosa L. (Annonaceae) on hematological and biochemical indices in normal rat. The male rats were gavaged ethanolic extract of Annona squamosa leaves at the dose level of 200 and 300 mg/Kg body weight/rat/day for 28 days. A significant decrease in hematological indices like hemoglobin, RBC count and increase in WBC count, serum parameter like serum cholesterol, serum GPT and GOT was observed.

Keywords*— Annona squamosa, rat, leaves, Hematological parameters, serum GOT, serum GPT, serum cholesterol.

I. INTRODUCTION

Annona squamosa Linn.belonging to family Annonaceae is commonly known as "Custard apple" and is a native of West Indies and South America and is cultivated throughout India, mainly for its edible fruits (Morton, 1987). The leaves of this plant have been used as insecticide, styptic, suppurant and dried fruit works as antidysentric and vermifuge. This plant also possesses anti-fertility, anti-tumour and anti-diabetic activities in mice and rats. (Gajalakshmi *et. al*.; 2011, Gupta *et.al*.; 2005 ; Jain and Dixit (1982);). The present study was undertaken to evaluate the effect ethanolic extract of the leaves of *Annona squamosa* in normal rats.

II. MATERIALS AND METHODS

Plant material and extract preparation: The fresh fruits of *Annona squamosa* were collected from Gorakhpur district of U.P. (India) and were identified in the department of Botany, University of Gorakhpur. The leaves were removed from stem and were shade dried. The leaves were then powdered and mixed with 100% ethanol. The extract was then filtered and oven-dried.

Animal model and experimental procedure: Healthy colony bred male albino rats weighting 120-150 grams were used for the experiments. The animals were housed in poly-propylene cages and maintained under standard conditions (12h light / 12h dark cycles). Rats were fed with standard diet and water was provided *ad libitum*. The animals were divided into three groups of one control and two treated, each with 16 animals. In treated groups one group received the extract at the dose level of 200mg/Kg body weight/rat/day and another group received the extract at the dose level of 300mg/Kg body weight/rat/day while the control group was received same amount of vehicle (ethanol). The plant extract was dissolved in ethanol and administered orally to animals every morning for 7, 14, 21 and 28 days respectively. After every 7^{th}, 14^{th}, 21^{st} and 28^{th} days both control and treated groups were autopsied under light chloroform anesthesia.

Autopsy schedule: Four animals of each three groups were autopsied for every 7^{th}, 14^{th}, 21^{st} and 28^{th} day. Blood was being collected through cardiac puncture.

Blood and serum analysis: The blood of the experimental rats was analyzed for R.B.C and W.B.C count, hemoglobin, serum cholesterol, serum GPT, serum GOT.

Statistical analysis: The results are expressed as mean± SE followed by student's t-test.

III. RESULTS

A non-significant decrease in hemoglobin concentration after 7 and 14 day, and a significant ($p<0.05$) decrease after 21 and 28 day in rats on exposure to 200 mg/kg body weight of extract and a significant ($p<0.05$) decrease in hemoglobin concentration was observed on exposure to 300 mg/kg body weight was observed in comparison to control rats.

A non-significant decrease in RBC count of treated rats was observed on exposure to 200 mg/kg body weight of extract and while a non significant decrease after 7 days and a significant ($p<0.05$) decrease in was observed after 14, 21 and 28 days on exposure to 300 mg/kg body weight of extract in rats in comparison to control.

A significant ($p<0.05$) increase in WBC count of rats in comparison to control was observed on exposure to both the doses of extract.

Two-way ANOVA test indicates that variation in the strength of dose and exposure time, significantly influence the hemoglobin concentration, RBC count and WBC count of rats (Table 1).

Blood biochemistry

A significant ($p<0.05$) decrease in blood glucose, increase in serum cholesterol, serum GOT and serum GPT in rats exposed to both the doses of extract was recorded in comparison to control rats.

Two-way ANOVA test indicates that variation in the strength of dose and exposure time, significantly ($p<0.01$) influence the blood glucose, serum cholesterol, serum GOT and serum GPT of rats exposed to the extract (Table 2).

Table.1: Effect of ethanolic extract of the leaves of plant Annona squamosa on hematological parameters in normal rats

Parameters	Days	Control Rats Mean±SE	Treated rats		Change in %	
			200mg/kg Body Weight Mean±SE	300mg/kg Body Weight Mean±SE	200mg/kg Body Weight Mean	300mg/kg Body Weight Mean
Hemoglobin (gm%)	**7**	14.46 ±0.17	11.97* ±0.12	11.78* ±0.014	17.21↓	18.53↓
	14	14.75 ±0.08	11.70* ±0.13	11.63* ±0.18	20.68↓	21.15↓
	21	14.83 ±0.06	11.54* ±0.08	10.86* ±0.20	22.18↓	26.77↓
	28	14.88 ±0.04	11.37** ±0.08	10.34* ±0.18	23.59↓	31.66↓
RBC count (million/cumm)	7	8.68 ±0.09	8.52* ±0.12	8.35* ±0.09	1.84↓	3.80↓
	14	8.83 ±0.06	8.47* ±0.12	8.27* ±0.07	4.08↓	6.34↓
	21	9.20 ±0.19	8.31* ±0.11	7.68* ±0.08	9.67↓	16.52↓
	28	9.60 ±0.19	8.14* ±0.09	7.46* ±0.2	15.20↓	22.29↓
WBC count (thousand/cumm)	7	8.61 ±0.04	8.8* ±0.06	10.68* ±0.14	2.2↑	24.04↑
	14	8.52 ±0.07	9.5* ±0.10	11.38* ±0.34	11.50↑	33.57↑
	21	8.87 ±0.01	10.01* ±0.20	11.97* ±0.17	12.85↑	34.94↑
	28	8.59 ±0.04	10.49* ±0.30	12.81* ±0.08	22.11↑	49.12↑

*Indicates significant ($p<0.05$) and ** indicates significant ($p<0.01$).

Table.2: Effect of ethanolic extract of the leaves of plant Annona squamosa on blood biochemical parameters in normal rats.

Parameters	Days	Control Rats Mean±SE	Treated rats		Change in %	
			200mg/kg Body Weight Mean±SE	300mg/kg Body Weight Mean±SE	200mg/kg Body Weight Mean	300mg/kg Body Weight Mean
Blood Glucose	**7**	118.69 ±0.52	111.72* ±0.31	103.99 ±0.63	94.13↓	87.62↓
	14	120.91 ±0.35	111.03* ±0.52	99.82* ±0.35	91.83↓	82.56↓
	21	121.72 ±0.43	108.53* ±0.49	96.92* ±0.87	89.17↓	79.63↓
	28	124.11 ±0.66	106.88* ±0.13	94.24* ±0.48	86.12↓	75.94↓
Serum cholesterol	**7**	58.47 ±0.16	62.89* ±0.38	65.35* ±0.16	7.56↑	11.76↑
	14	59.37 ±0.07	63.92* ±0.14	67.10* ±0.26	7.66↑	13.02↑
	21	60.16 ±0.31	64.63* ±0.29	69.51 ±0.14*	7.43↑	15.54↑
	28	61.60 ±0.36	65.99* ±0.32	72.55** ±0.12	7.12↑	17.77↑
Serum GOT	7	84.03 ±0.19	90.25** ±0.07	104.12* ±0.29	7.4↑	23.90↑
	14	85.04 ±0.08	94.54* ±1.13	110.47* ±0.08	11.17↑	29.90↑
	21	85.75 ±0.06	95.34* ±0.12	115.30* ±0.05	11.18↑	34.46↑
	28	87.52 ±0.13	98.48* ±0.12	121.48* ±0.14	12.78↑	38.80↑
Serum GPT	7	54.74 ±0.55	67.67* ±6.23	75.25* ±0.03	23.62↑	37.47↑
	14	56.67 ±0.48	73.32* ±0.10	78.67* ±0.34	29.38↑	38.82↑
	21	59.85 ±0.34	83.77** ±1.06	88.59* ±0.87	39.97↑	48.02↑
	28	62.98 ±0.25	90.36* ±0.14	102.6* ±1.5	43.47↑	62.91↑

*Indicates significant ($p<0.05$) and ** indicates significant ($p<0.01$).

IV. DISCUSSION

Haematological indices like haemoglobin content, blood cell counts (RBC and WBC) revealed significant changes due to the treatment. This reveals the toxic nature of the extract. A significant decrease in erythrocyte (RBC) count and haemoglobin percent was observed and this can be attributed to defective haemopoisis (Maurya and Kushwaha, 2010, Choudhary and Deshmukh, 2007). The decrease in haemoglobin content and RBC count can be correlated with paling of the animals, weakness and morbidity. (Cella,*et al*., 2000; Kumar, *et al*., 1999; Choudhari and Deshmukh, 2007).Significant increase in WBC count of treated rats was observed in the present study can be attributed to the stimulation of immune system (Oluwole, 2001). An increase in the WBC count has been reported after chemical stress by various workers (Pandey, *et.al*., 1976; Goel and Garg, 1980; Sastry and Sharma, 1980). A significant increase in the activity of SGPT and SGOT of treated rats

was observed in the present study. Excess of SGOT is common in myocardial infraction (Varley, 1976). The altered levels of these enzymes are found to be affected by the physiological status of the important organs as evidenced by the alteration in transaminase activity levels that leads to an increase in transamination process required during depletion of body proteins. Arora and Saxena (1999), Oser (1965), Ayub Shah and Gupta (2001) have reported that an increase in SGOT and SGPT in albino rats, after administration of synthetic pyrethroid for 21 days, causes change in membrane permeability and hepatocyte dysfunction. An increase in blood cholesterol level of rats on exposure to the extract in the present study may be due to the accumulation of cholesterol as it is associated with retarded oxidative breakdown of sugar under stressed condition (Kabeer, *et al*., 1978).

ACKNOWLEDGEMENT

This research work is supported by the University Grant Commission (UGC), New Delhi. Vide letter F. No. 37-131/2009(SR) dated 12 Dec 2009.

REFERENCES

[1] Arora, S. and Saxena, P.N. (1999). Decis (synthetic Pyrethroid) induced serum biochemical alterations in *Rattus norvegicus.* Proc. Acad. Environ. Biol. 8(suppl), 33(0129,c).

[2] Ayub Shah, M.A. and Gupta, P.K. (2001). Sub acute toxicity studies on peremethrin a synthetic pyrethroid insecticide with particular reference to biochemical changes in rats. Indian J. Toxicol. 8, 61-67.

[3] Choudhary, C.V. and Deshmukh, P.B. (2007). Acute and subchronic toxicity study of somecarpus anacardium on haemoglobin percent and RBC count of male albino rats. Journal of Herbal Medicine and Toxicology, 1(1): 43-45.

[4] Gajalakshmi, S., Divya, R., Divya Deepika, V., Mythili, S. and Sathiavelu, A., (2011). Pharmacological activities of *Annona squamosa*; A review, Int. J. Pharma. Sci. Rev. Res., 10(2), 24-29.

[5] Goel, K.A. and Garg, V. (1980). 2, 4-diamino, 3-amino azobenzene 'DAAB' induced haematobiochemical anomalies in channa punctatus. Bulls. Environ. Contam. Toxicol., 25, 469-476.

[6] Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V and Watal G, (2005), Hypoglycaemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* in experimental animals, J Ethnopharmacol, 99(1), 75-81.

[7] Jain, G.C. and Dixit, V.P. (1982). Effect of Annona squamosa EtOH extract and testicular extract and testicular function of dogs (Canis indicus). II Annual session of science, Abst- 15 Bhavnagar, pp.22.

[8] Kabeer, A.I., Begum, M.D., Sivias, S. and Pumanarao, K.V. (1978). Effect of malathion on free amino acids, total protein, glycogen and some enzymes of plecypod, *Lamellidens marginalis* (Lamark). Proc. Ind. Aca. Sci. 87(12): 377-387.

[9] Maurya, S. and Kushwaha, V.B., (2010). Effect of ethanolic extract of *Parthenium hysterophorus* on haematological parameters in rats, The Bioscan, 5(3), 437-440.

[10] Mortan, J., Sugar Apple. In: J. Morton (Ed), Fruits of warm climate: Miami. Greensboro, Media, pp. 69-72.

[11] Oluwole, F.S. (2001). Effect of garlic on some haematological and biochemical parameters. Afr. J. Biomed. (2001): Vol 4; 139-141.

[12] Oser, B.L. (1965). Hawk's Physiological Chemistry, 14th edn. Mc Gee Hill Book Co., N.Y.

[13] Pandey, B.N., Chandal, A.K. and Singh, M.P. (1976). Effect of malathion on oxygen consumption and blood of channa punctatus (Bloch). The indian J. Zool. 17 (2), 95-100.

[14] Sastry, K.V. and Sharma, K. (1980). Mercury induced haematological and biochemical anomalies in ophiocephalus (channa) punctatus. Toxicol. Lett. 5, 245-249.

[15] Varely, H. (1976). Practical clinical biochemistry, 4th edn. Arnold Helnmann Publisher, Indian Paul's Press. New Delhi.

http://dx.doi.org/10.22161/ijeab/2.1.36 *ISSN: 2456-1878*

Physiochemical Characterization of the Brewers' Spent Grain from a Brewery Located in the Southwestern Region of Parana - Brazil

Sideney Becker Onofre[1,2], Ivan Carlos Bertoldo[1,2], Dirceu Abatti[2], Douglas Refosco[2]

[1] Universidade Comunitária da Região de Chapecó - UNOCHAPECÓ - Centro de Ciências Exatas e Ambientais - CEA - Programa de Pós-graduação em Tecnologia e Gestão da Inovação - PPGTI - Av. Senador Attílio Fontana, 591-E EFAPI - 89809-000 - Chapecó - Santa Catarina - BRASIL.

[2] União de Ensino do Sudoeste do Paraná - UNISEP - Av. União da Vitória, 14 – Bairro Miniguaçu - 85605-040 – Francisco Beltrão - Paraná - BRASIL.

Abstract**—** *Brewers' spent grain is a by-product generated in the production process of breweries formed by the solid part obtained from the wort filtration before boiling. It is mainly comprised of pulp and husk residues of the malt, but it also contains grains of the adjuncts, such as rice, maize and wheat. Quantitatively, brewers' spent grain is the main byproduct of the brewing process and currently it is used as animal feed. The objective of this study was to determine the physiochemical composition of the brewers' spent grain and its potential use in human food. To this end, brewers' spent grain samples were collected from a craft beer brewery located in the southwestern region of the state of Paraná, determining such parameters as moisture, ash, total proteins, lipids, crude fiber, carbohydrates and energy. The results revealed that the moisture and ash levels were 78.23 ± 1.45 and 3.76± 1.23g.100g^{-1}, respectively. The figures for carbohydrates, total proteins, total fats and crude fibers were 1.89±1.21; 4.89 ± 0.29; 2.67 ± 0.68 and 4.19 ± 0.56, represented in g.100g^{-1} respectively. The energy values obtained were 109.23± 4.23 kcal.100g^{-1}. As such, the conclusion can be drawn that brewers' spent grain can be used in both animal and human food.*

Keywords**— Food, Waste, Agriculture, Bromatological Analyses.**

I. INTRODUCTION

According to the Brazilian department of Agriculture (Brasil, 1977), every grain that is subjected to a malting process, i.e., the grain is subjected to partial germination and subsequent dehydration and/or toasting at appropriate technological conditions, should be called malt followed by the name of the grain. Malted barley, or malt, is one of the main raw materials used in the manufacture of beer (Reinold, 1997).

In the first step of the beer manufacturing process, called mashing, two fractions are obtained: a liquid fraction (wort) and a solid fraction (brewers' spent grain), which is characterized as waste. For every hundred liters of beer produced, 20 kg of dry waste is generated, representing 85% of the total solid residue from the production process (Reinold, 1997).

Brewers' spent grain is the brewing residue resulting from the initial beer manufacturing process and it is generated from the filtering of the wort (mixture of ground malt and water) before boiling. This spent grain is basically made up of the husks of the malted barley.

Brewers' spent grain is predominantly fibrous (70 percent of dry weight) and proteinaceous (15 to 25% of dry weight), and it also contains lipids, minerals, vitamins, amino acids and phenolic compounds. Starch is the main source of glucose in the human diet, representing 40 to 80% of the total energy value in daily nutrition and being of considerable importance. Proteins are essential molecules for maintaining the structure and functioning of all living organisms and they have different properties and functions (Aliyu and Bala, 2011; Lima 2010; Robertson *et al*., 2010).

Since brewery waste has a rich composition of organic compounds with a significant nutritional value, it must be treated before it is released to the environment in order to prevent changes to the ecological equilibrium. As such, there is a great incentive to reduce the generation of waste or to promote its reuse in other processes. From the perspective of producing higher value added products and allocating the generated waste to more noble ends, industrial bioprocesses have presented themselves as a potential way of allocating these residues (Pandey *et al.*, 2001), in addition to their potential applications in animal and human food (Mendonça and Oliveira 2012).

According to Aliyu and Bala (2011) and Souza *et al.*, (2011), various applications can be cited, such as: animal and human food and nutrition; energy production through direct burning or through biogas production via anaerobic

http://dx.doi.org/10.22161/ijeab/2.1.36 *ISSN: 2456-1878*

fermentation; production of charcoal; adsorbing material in chemical treatments; cultivation of micro-organisms and obtaining of bio-products through fermentation; support for cellular immobilization; among others.

According to Borges and Neto (2009), Nogueira (2010) and Mega and Andrade (2011), it is estimated that the global annual production of brewing residue (RC) is approximately 30 million tonnes, while Brazilian production accounts for around 1.7 million tonnes/year. From the perspective of sustainability, social and environmental responsibility, these numbers have a severe impact and there is a lack of efficient waste management since its allocation is the responsibility of the generator, who may incur legal penalties if its removal is inappropriate. According to the authors, the inadequate disposal of these residues can cause damage to the environment and its direct elimination in the soil or in sanitary landfills has been shown to be inefficient because there are not enough of these to handle the large amount produced each year.

Considering the nutritional potential of the waste arising from the beer manufacturing process, the objective of this study was to determine the physiochemical composition of the brewers' spent grain in the Southwestern region of the state of Paraná in order to evaluate its use for consumption by humans and household pets.

II. MATERIALS AND METHODS

We used the humid brewers' spent grain from a brewery located in the southwestern region of the state of Paraná. Two kg of sample was collected at the end of the filtration, prior to the removal of the spent grain to the spent grain box. The sample was stored in hermetically closed and cooled packaging and was subsequently transported to the food analysis laboratory of the *Fundação para o Desenvolvimento Científico e Tecnológico* - Fundetec - located in the city of Cascavel - PR - Brazil.

The brewers' spent grain was subjected to physiochemical analyses, in triplicate, regarding the following parameters: moisture (oven drying method at 105º C for 24 hours), ashes (calcination of samples at 550º C), total proteins, lipids, crude fibers, carbohydrates and energy, according to the analytical standards of the *Instituto Adolfo Lutz* (Brazil, 2005).

III. RESULTS AND DISCUSSION

The results of the physiochemical characterization of the brewers' spent grain are shown in Table 1. The values of 78.23± 1.45 and 3.76 ± 1.23 were obtained for the moisture and ash content, respectively, when analyzing the data.

The values found for the moisture and ash content of the brewer's spent grain under analysis (Table 1) are consistent with the literature data. Santos et al. (2003) evaluated the moisture and ash content of 8 batches of brewers' spent grain, consisting of 80% of malted barley and 20% of malted corn, obtaining values between 76.8 and 78.9% for moisture, and between 3.4 and 4% for ashes on a dry basis. Zhaoxia *et al*., (2012) found a water content of 79% and an ash content of 4.4%, for the dry brewers' spent grain from commercial breweries. Robertson *et al*., (2010) determined the moisture content of the brewers' spent grain of the barley from 10 commercial breweries, and found values between 75 and 80%. Dei Cedri (2006) found an ash content of 3.3% for the brewers' spent grain after mashing of the pure malted barley. In other literature reviews, values between 2.3 and 7.9% were found for ashes, and between 75 and 85% for moisture in the composition of the brewers' spent grain (Olajire, 2012; Aliyu and Bala, 2011).

Table.1: Physiochemical composition of the brewers' spent grain (b.u).

Analyzed Parameters	Values Obtained*
Moisture ($g.100g^{-1}$)	78.23±1.45
Ashes ($g.100g^{-1}$)	3.76±1.23
Carbohydrates ($g.100g^{-1}$)	1.89±1,21
Total Proteins ($g.100g^{-1}$)	4.89±0.29
Total Fats ($g.100g^{-1}$)	2.67±0.68
Crude fiber ($g.100g^{-1}$)	4.19±0.56
Energy ($kcal.100g^{-1}$)	109.23±4.23

**Values for the sample expressed as a percentage ($g.100g^{-1}$) of the product on a wet basis (b.u).*

According to Schmidt (1989), brewers' spent grain has a moisture of around 79%. According to Ascheri *et al.*, (2016), brewers' spent grain is characterized by a high moisture of 86% (b.u.) that limits its shelf life to up to 30 days for its fresh consumption. The high amount of water in the wet residue may result in other limiting factors, such as difficulties in long distance transport and storage.

Regarding carbohydrates, total proteins, total fats and crude fibers, the values obtained were 1.89±1.21; 4.89 ± 0.29; 2.67 ± 0.68 and 4.19 ± 0.56, represented in $g.100g^{-1}$ respectively. The energy values obtained were 109.23± 4.23 $Kcal.100g^{-1}$.

When the data obtained in this study is compared with data from Murdock *et al.*, (1981); Polan *et al*., (1985), Rogers *et al.*, (1986); NRC (1986) and Costa *et al.*, (1994) one van see that the content of total proteins, total fats and crude fiber is similar to the literature.

The carbohydrate content obtained for the brewers' spent grain ($1.89g.100g^{-1}$) is in agreement with the literature data, which indicates that brewers' spent grain is

predominantly a fibrous material (Aliyu and Bala, 2011; Lima 2010; Robertson *et al.*, 2010; Mussato *et al.*, 2006) that is poor in fermentable sugars. In addition, the washing until exhaustion of this residue for the recovery of the brewing wort extract, reduces the sugar content to its minimum.

The differences between the values obtained in this study and the literature are perfectly understandable when one takes into account that the proximate composition of the brewers' spent grain is a function of several factors, such as: barley variety, harvest time, grains used in the malting process, and the technological process used in the brewery, among others.

The total protein values found in this study were lower than those reported by Lima *et al.*, (2006) for crude green corn, rice and peas, and higher than those reported for tomatoes, paprika, avocado, pineapples, cashews, jackfruit and custard apples. Brewers' spent grain was also superior regarding the crude fiber content, coming second only to avocado and green peas.

Despite the great application of brewers' spent grains in animal feed, it can also be used for human consumption. Because according to Dongowski *et al.*, (2012), the high fiber value and the protein and sugar residues turn these spent grains into potential ingredients for use in bakery products, such as breads and cookies, where an increase in fibers, in particular, could bring benefits to consumers from a nutritional and functional point of view. These authors analyzed and characterized a bread with 10% brewers' spent grain (which was subjected to a drying and milling process) and concluded that after the addition of the residue, the bread took on a dark color with the appearance of whole bread. It also became more acid because spent grain has an acid pH.

Mattos (2010) also worked with brewers' spent grain and characterized a bread with 30% brewers' spent grain (which was not subjected to a drying and milling process) and he concluded that after the addition of the residue, the bread took on an appearance and texture similar to whole bread.

According to Cabral Filho (1999), the high availability, continuous generation and physiochemical characteristics of brewers' spent grains from the manufacture of beer are factors that corroborate its potential use as human food. One should also consider that the reuse of this brewing residue contributes to environmental sustainability by giving a proper destination to it, adding social and nutritional value to human food because of the increasing demand for nutritious and healthy food.

The obtained results reveal that brewers' spent grains can be used as human food since it has a similar, and in some cases even superior, composition when compared with other food items commonly consumed by human beings.

IV. CONCLUSION

Understanding the chemical properties of food is of fundamental importance to assess the availability of nutrients and the best characteristics for processing.

Brewers' spent grain has a high water content, and is therefore conducive to microbial development and rapid deterioration. On the other hand, it showed to have similar ash, protein, carbohydrate, fat and crude fiber contents as other foods, and it could therefore be used in animal and human foods.

REFERENCES

[1] Aliyu, S., Bala, M. 2011. Brewer's spent grain: A review of its potentials and applications. *African Journal of Biotechnology*, 103(3): 324-331.

[2] Ascheri, D.P.R., Burger, M.C., Malheiros, L.V., Oliveira, V.N. 2007: *Curvas de secagem e caracterização de hidrolisados de bagaço de cevada*. http://www.abq.org.br/cbq/2007/trabalhos/10/10-380-261.htm/ (Acesso 20 setembro 2016).

[3] Borges, M.S., Souza Neto, S.P. 2009. Meio ambiente x Indústria de cerveja: um estudo de caso sobre práticas ambientais responsáveis. In: Congresso Nacional de Excelência em Gestão, 5., 2009, Niterói. *Anais*... Niterói, Brasil.

[4] Brasil. 2005. Instituto Adolfo Lutz. *Normas analíticas do Instituto Adolfo Lutz:* Métodos químicos e físicos para análise de alimentos. 4ª ed. Instituto Adolfo Lutz, São Paulo, Brasil, 1018p.

[5] Brasil. 1977. Ministério da Agricultura, Pecuária e Abastecimento. Portaria n° 166 de 12 de abril de 1977. *Padronização, classificação e comercialização do Malte cervejeiro ou Cevada malteada para fins cervejeiros.* Brasília. Brasil.

[6] Cabral Filho, S.L.S. 1999. *Avaliação do resíduo de cervejaria em dietas de ruminantes através de técnicas nucleares e correlatas*. Dissertação de (Mestrado) – Universidade de São Paulo, São Paulo. São Paulo. Brasil.

[7] Celus, I., Brijs, K., Delcour, J.A. 2006. The effects of malting and mashing on barley protein extractability. *Journal of Cereal Science*, 44: 203-211.

[8] Costa, J.M.B., Mattos, W.R.S., Biondi, P., Carvalho, D.D. 1994. Composição Químico bromatológica do resíduo de cervejaria. *Boletim da Indústria Animal*. 51(1): 21-26.

[9] Dongowski, G. et al. 2012. Dietary fiber-rich barley products beneficially affect the intestinal tract of rats. *Journal of Nutrition*, 132: 3704-3714.

[10] Lima, D.M., Colugnati, F.A.B., Padovani, R.M., Rodriguez-Amaya, D.B., Salay, E., Galeazzi, M.A.M. 2006. *Tabela brasileira de composição de*

alimentos / NEPA-UNICAMP.- T113 Versão II. -- 2. ed. -- Campinas, SP: NEPA-UNICAMP.

[11] Lima, U.A. 2010. *Matérias-primas dos Alimentos.* São Paulo: Ed Blucher.

[12] Mattos, C. 2010. *Desenvolvimento de um pão fonte de fibras a partir do bagaço de malte.* 41p.Monografia em Engenharia de Alimentos, Universidade Federal do Rio Grande do Sul. Porto Alegre, RS. Brasil.

[13] Mega, J.F., Andrade, A.A. 2011. Produção de Cerveja no Brasil. *Revista CITINO – Ciência, Tecnologia, Inovação e Oportunidade*, 12(1): 123-129.

[14] Mendonça, L.M., Oliveira, V.S. 2012. *Utilização do resíduo úmido de cervejaria na alimentação de cabras aglo nubiana no final de lactação.* Dissertação de Mestrado. Universidade Federal de Sergipe, Brasil.

[15] Murdock, F.R., Hodgson, A.S., Riley Jr, R.E. 1981. Nutritive value of brewers grains for lactating dairy cows. *Journal of Dairy Science.Champaing.* 64(9): 1826-1832.

[16] Mussatto, S.I., Dragone, G., Roberto, I.C. 2006. Brewer's spent grain: generation,characteristics and potential applications. *Journal of Cereal Science*, 43(1): 1-14.

[17] Nogueira, A.D. 2010. Lúpulo: A Essência da Cerveja. *Revista Engarrafador Moderno.* São Paulo: Ed. Aden. p.22-29.

[18] NRC - National Research Council. 1986. *Nutrient Requeriment of Beef Cattle.*7th ed. Washington: National Academic Press.

[19] Olajire, A.A. 2012. The brewing industry and environmental challenges. *J. Cleaner Prod.*, 23(12): 1-21.

[20] Pandey, A., Soccol, C.R., Nigam, P., Soccol, V.T. 2000. Biotechnological potential of agro-industrial residues I: sugarcane bagasse. *Bioresour. Technol.*, 74(1): 69-80.

[21] Polan, C.E., Herrington, W.A., Wark, W.A. 1985. Milk production response to diets supplemented with dried grains, wet brewers grains, or soybean meal. *Journal of Dairy Science*, 68(8): 2016-2026.

[22] Reinold, M.R. 1997. *Manual Prático de Cervejaria.* 1.ed. São Paulo: Aden.

[23] Robertson, J.A., I'anson, K.J.A., Treimo, J., Faulds, C.B., Brocklehurst, T.F., Eijsink, V.G.H., Waldron, K.W. 2010. Profiling brewers' spent grain for composition and microbial ecology at the site of production. *Food Sci. Technol.*, 43: 890-896.

[24] Robertson, J.A., Monredon, F.D., Dysseler, P., Guillon, F., Amado, R., Thibault, J.F. 2010. Hydration Properties of Dietary Fibre and Resistant Starch: a European Collaborative Study. *LWT - Food Science and Technology*, 33: 72-79.

[25] Rogers, J.A., Conrad, H., Dehority, B.A., Grubb, J.A. 1986. Microbial numbers, rumen fermentation, and nitrogen utilization of steers fed wet or dried brewers grains. *Journal if Dairy Science.* 69: 745–53.

[26] Santos, M., Jiménez; J.J., Bartolomé; B., Gómez-Cordovéz, C., Del Nozal, M.J. 2003. Variability of brewers' spent grain within a brewery. *Food Chemistry*, 80: 17–21.

[27] Schmidt, H. 1989. *Katechismus der Brauerei-Praaxis*, Nürnberg, 15ª ed.: Verlag Hans Carl, Nürnberg, Doitland.

[28] Souza, L.C., Zambom, M.A., Alcalde, C.R., Radis, A.C., Fernandes, T., Taffarel, L.E. 2011. *Inclusão do resíduo úmido de cervejaria na dieta de vacas em lactação.* Publicado: 48a Reunião Anual da Sociedade Brasileira de Zootecnia, Santa Maria. Brasil.

[29] Zhaoxia, L., Jinlong, Y., Dan, S., Cheng, D. 2012. Thechniques optimization of combined enzymatic hydrolisis on brewers' spent grain from Novozymes. *Journal of Life Sciences*, 6: 1232-1236.

http://dx.doi.org/10.22161/ijeab/2.1.37 *ISSN: 2456-1878*

In Vitro screening of larvicidal and insecticidal activity of methanolic extracts of *Artocarpus heterophyllus, Artocarpus altilis* and *Piper betle*

Sindhu S Nair*, Vaibhavi Kavrekar

Department of Biotechnology, Mount Carmel College Autonomous, Bengaluru-560052, India

Abstract— *The aim of this work was to evaluate the larvicidal and insecticidal activity of the selected plants namelyArtocarpus altilis, Piper betle and Artocarpus heterophyllus. The leaves of Artocarpus altilis and Artocarpus heterophyllus and roots of Piper betle were subjected to methanolic solvent extraction for the isolation of various bioactive constituents. The evaluation of larvicidal activity was carried out using late third instar larvae of Drosophila melanogaster. The insecticidal activity of extracts was studied against adult Bruchus pisorum, Tribolium castaneum, Sitophilus oryzae and was evaluated by direct contact application method .Nucleic acids and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating the different activities of cells. Piper betle and Artocarpus heterophyllusextractshad a reducing effect on the nucleic acid and protein content in the larvae in a dose dependent manner whereas Artocarpus altilis extract did not exhibit any significant larvicidal activity. Piper betle and Artocarpus heterophyllusextractsshowed good insecticidal activity whereas A.altilis extract showed poor insecticidal activity. The results of the present study clearly indicate that Piper betle and Artocarpus heterophyllus extracts can be developed as ecofriendly larvicides and were also quite effective as insecticides for providing a better and excellent alternate for the control of insects.*

Keywords— Insecticidal, larvicidal, Bruchus pisorum, Tribolium castaneum, Sitophilus oryzae, Drosophila melanogaster.

I. INTRODUCTION

Aromatic and medicinal plants form a very large group of medicinally and economically important plants which serve as the basic raw materials for medicines, cosmetics, perfumes and food additives. A recent aspect of interest in plant drug research is the new concept of a non-specifically increased resistance of an animal to diseases attributable to other substances, besides the active principles responsible for specific biological activity (Fabricant and Farnsworth, 2001). This will probably justify the use of many of the plant drugs as household remedies by indigenous people of many countries from ancient times and hence warrants their evaluation in more detail. The fact that only very few percent of the six lakhs species of plants on the planet has been investigated, indicates the opportunity provided and challenges thrown to phytochemists. More recently there is an emerging trend in research and development to support the bio activities of medicinal plants (Adlercreutz H and Mazur W., 1997).

Reports in the scientific literature indicate that plant derived compounds serve as potential sources of novel antimicrobial, anticancer, anti inflammatory, antioxidant and anti HIV agents.Insects present a problem in stored grain throughout the world because they reduce both the quantity and quality of grain. The stored pest red flour beetle, *Tribolium castaneum*is an example of a major pest in storage of grain based products. This species is for long associated with human stored food and has been found in a wide range of commodities includinggrain,beans, flour, peas, dried fruits, spices and nuts. A greater awareness of the hazards associated with the use of synthetic organic insecticides has led to an urgent need to explore suitable alternative products for pest management and control. Insecticidal activity of several plants against several insects has been reported by many workers (Camps F and Coll J., 1993).

The natural products from several floral species have been demonstrated to act as toxicants, repellents, and antifeedants against a number of coleopteran that attack stored products. Seeds and floral extracts of several plants have been reported to have toxic and potent growth reducing activity to insects (Mehta *et al*., 1995). The detrimental effects of plant extracts on insects can be manifested in several manners including mortality, toxicity, growth inhibitor, anti feedant, reduction of fecundity and fertility and suppression of reproductive behavior. Exposure of toxic agents to insects can cause changes even at the molecular level. Nucleic acids (DNA and RNA) and protein contents are considered as important biomarkers of the metabolic potential of cells as they play the main role in regulating the different cellular

activities. Changes in the amount of nucleic acid can be used to detect the effect of toxic agents on cell proliferation and cell death (Cares *et al*., 2009).

The harmful effects of insects and other pests such as mosquitoes, cockroaches, rodents, parasitic worms, flies has been well known and challenged by man. Pesticides are any substances or mixture of substances used for preventing, destroying, repelling or mitigating any pest. They include chemicals like lead, sulphur, mercury and arsenic. DDT (dichlorodiphenyltrichloroethane) was effectively used to control malaria and typhus diseases (Gullan, P. J. and Crantson, P. S.,1994). It was the first synthetic organic pesticide discovered and was used for agricultural purposes.There is no doubt that the use of insecticides has immensely contributed to the increase in agricultural productivity and improvement in human health, particularly the eradication of diseases. However it has been established that use of synthetic organic pesticides particularly the chlorinated hydrocarbons such as DDT and derivatives has led to serious effects on human health, environmental pollution, and death of non target organisms including animals, plants, and fish. This situation led to the ban of DDT in 2004 (Jacobson M., 1982).

Natural products from plants have attracted researchers in recent years as potent sources of new pesticides. The use of higher plants by the natives of various parts of the world as insecticidalagents has been well known (Edeoga*et al*., 2005. One of the early plants to be reported as insecticidalagent was tobacco (*Nicotiana tabacum*). The use of tobacco leaves to kill aphids led to the isolation of the alkaloid called nicotine. The chemical investigation of plant, *Rhododendron hortense* showed the presence of an active conmponent called *rotenone*, with considerable insecticidal activity. Plants of the genus *Chrysanthemum* are the sources of very successful insecticidal extract, *pyrethrum*, and the active constituents called pyrethrins. There is significant evidence that a number of plants possess pesticidal activity and this has been confirmed by investigations by various research groups in different parts of the world. The toxicity of the ethanol extracts of the leaves of twenty plant species from different families to *Callosobruchus maculatus* and *Callosobruchus chinensis* were studied (Jilani G and Su HCF., 1983). It was observed that mortality reached a maximum level in 72 hours of exposure to the leaves oils which indicated a high level of lethality. Similarly the protectant effectiveness of some plants native to Nigeria against the maize weevil, *Sitophilus zeamais* Motsch, and the cowpea weevil, *Callosobruchus maculatus*, respectivelyhave been established (Vanhecke *et al*., 1981).

On the basis of the results of various pesticidal screenings, it has been established that a number of plants have broad pesticidal activity and have been commonly used in traditional agricultural applications in many parts of the developing countries. Various investigations have shown that in most cases the insecticidal activity is usually distributed among the various parts of the same plant though the lethality and quantities of the active components may vary (Miyazawa *et al*., 1993).

In the past decades apart from the *pyrethrum* which has attained international and commercial importance due to its high efficacy and broad spectrum insecticidal activity very few natural insecticides have been developed. The tropical plant *Azadirachta indica*, popularly known as the neem tree is effectively used to control over twenty five different species of insect pests(Zettler JL and Cuperus GW., 1990). The activity has been associated with the presence of active compound called *azadirachtin* which is found to be highest in the kernel than in the leaves and other tissues of the plant. The compounds from *Piper longum, Piper retrofractum and Piper guineense* are known to be active against *Callosobruchus maculatus*, the garden insect, *Zonocerus variegatus* L, and the mosquito larvae causing 96-100% mortality rate in 48 hours mostly as solution sprays. From the chloroform and petroleum extracts of *P. guineense* fruits two *Piper amides, guineensine* and *piperine* were isolated. Piperinehas been shown to be a synergist rather than an insecticide in the crude extracts and a number of plants produce *polyphenols* called tannins which confer bitter taste on such plants and consequently herbivores stay away from eating such plants (Yang RZ and Tangs CS., 1988).

II. MATERIALS AND METHODS

Collection of plant material and preparation of plant extracts

Middle aged leaves of *Artocarpus heterophyllus*, fruit of *Artocarpus altilis* and roots of *Piper betle* were collected from South Bangalore, Karnataka and used for the extraction of bioactive compounds. They were positively identified from the Botany Department of Mount Carmel College, Bengaluru. The plant materials were washed thoroughly with distilled water, shade dried, powdered and stored in air tight containers for extraction of phytochemicals. The plant materials were subjected to solvent extraction using 300 mL of 80 % methanol. The extract was then filtered and concentrated using rotary vacuum evaporator at 45-50 °C and stored at 4°C for further investigations.

Investigation of larvicidal activities of plant extracts

Rearing medium

Adult *Drosophila melanogaster* were collected and reared on the artificial diet at 25^0C in the culture bottle. Artificial diet contained: brewers' yeast (60 g), glucose (80 g), agar

http://dx.doi.org/10.22161/ijeab/2.1.37 *ISSN: 2456-1878*

(12 g), and propionic acid (8 mL) in 1000 mL double distilled water.

Method of treatment

The stored crude methanolic plant extracts were used for the study.The different extract concentrations 100, 200,300, 400 and 500 ppm were used for the test. Twenty late third instar larvae of *Drosophila melanogaster* were selected for each set of treatment. Seven numbers of glass beakers of 250 mL capacity were taken and labelled for different concentrations of plant extracts and in addition one was maintained for check and one for control. In case of control, distilled water and for check, methanol was added in place of extract. Larvae were dipped into the solution for two minutes and then transferred back in the rearing medium (composition mentioned above). Each experiment was conducted in triplicates along with the control group. Mortality of larvae followed by the exposure was recorded after 24hours up to 48hours. The mortality due to treatment of 3rd instar larvae with different concentrations of the plant extracts was recorded after 24 and 48 hours (Audu *et al*., 2007).

The LC_{50} value was determined as following:

$$LC_{50} = LC_{100} - \frac{\sum \text{Mean death X Concentration difference}}{\text{No of organisms per group}}$$

The experiment was repeated three times on subsequent days.

Biochemical Analysis

Homogenate preparation

After 48 hours of exposure larvae were homogenized (10%, w/v) in 50 mM Tris–HCl buffer (pH 7.5) on ice using glass homogeniser. The homogenates were centrifuged at 4°C for 10 minutes at 15,000 g in a refrigerated centrifuge. The corresponding supernatants were either used fresh or kept frozen at -20°C until further use for determining the concentration of different biomolecules.

Total protein content estimation

The protein samples (tissue extracts) were mixed with the protein reagent (alkaline copper sulphate solution), incubated for 10 minutes at room temperature followed by addition of Folin-ciocalteau reagent. The reaction mixture was further incubated at room temperature for 30 minutes. The absorbance of blue colour was monitored at 660 nm using spectrophotometer (Lowry *et al*., 1951). Simultaneously, a blank was also processed containing all the reagents except the protein. Bovine serum albumin (BSA) was used as a standard.

Estimation of nucleic acids

Total RNA was estimated by the orcinol method using yeast RNA as a standard. DNA estimation was done by Diphenylamine method. Pentose of RNA reacts with orcinol reagent forming a bluish green colour at 660 nm. DNA gives colour with diphenylamine reagent at 600 nm. Diphenylamine produces a blue colour by reaction with deoxyribose moiety in DNA (Baig *et al*., 2010).

Investigation of insecticidal activities of plant extracts

The insecticidal activity of the methanolic plant extracts was determined by direct contact application using filter paper. Each fraction (200 mg) was dissolved in 3 mL methanol and was applied by micropipette to 90 mM diameter filter paper. After drying for 24 hours each filter paper was placed in a petridish and 10 adults of *Bruchus pisorum*, *Tribolium castaneum*, *Sitophilus oryzae* were placed in each petridish and covered with a lid. A check group treated with solvent was prepared to determine the effect of solvents. A control batch was kept for the determination of environmental effects. Another group was treated with a solution of reference insecticide Permethrin (235.9 µg/cm^2). All these were kept without food for 24 hours. The number of survivals and % mortality was calculated according to the formula (Audu *et al*., 2007).

$$\text{Mortality (\%)} = \frac{100 - \text{Number of survivors in sample}}{\text{Number of survivors in control}} \text{ X } 100$$

All treatments were repeated three times and reported as the average.

III. RESULTS AND DISCUSSION

Though larvicides play a vital role in controlling fruit flies (*Drosophila melanogaster*), these also show a negative impact in areas of beneficial and non target organisms. In view of an increasing interest in developing plant origin larvicides as an alternative to chemical larvicides, this study was undertaken to assess the larvicidal potential of the plant extracts against fruit fly larvae.In the present study, the effects of various extracts on the level of nucleic acids and protein were studied in *D. melanogaster* larvae in a dose dependent manner. The larvae of *Drosophila melanogaster* (twenty in each set) were treated with different concentrations of methanolic plant extracts for 24 and 48 hours. The control and check represent larval treatment with water and methanol, respectively. The effect of extracts on larval mortality is shown in figures 1 and 2.

Nucleic acids and protein contents are regarded as important biomarkers of the metabolic potential of cells, as these play the main role in regulating the different activities of cells. Since insects have very little carbohydrate, protein is used to meet the increased energy demand. Proteins are mainly involved in the architecture

http://dx.doi.org/10.22161/ijeab/2.1.37 *ISSN: 2456-1878*

of the cell which is the chief source of nitrogenous metabolism. The decreases in total protein level with the increasing dose of the plant extracts suggest the high protein hydrolytic activity due to elevation of protease activity. Inhibition of DNA synthesis, thus, might affect both protein as well as protein synthesis machinery. *Piper betle* and *Artocarpus heterophyllus*extractshad a reducing effect on the nucleic acid and protein content in the larvae in a dose dependent manner whereas *Artocarpus altilis* extract did not exhibit any significant larvicidal activity (Figures3-11). The findings showed that *Piper betle* and *Artocarpus heterophyllus*extractscan be developed as ecofriendly larvicides.

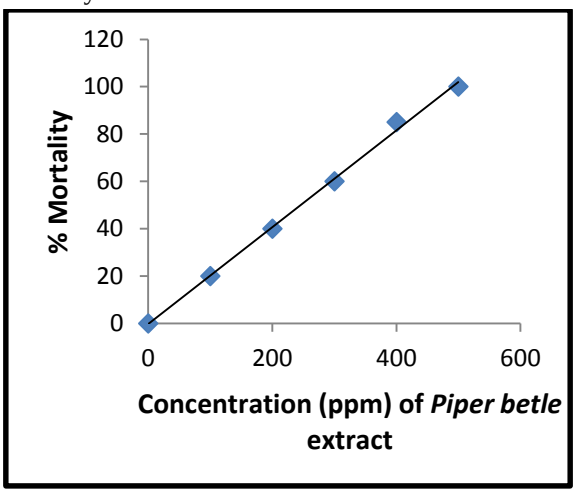


Fig.1: Mortality curve of D. melanogaster larvae for the determination of LC_{50} of Piper betle extract (values are expressed as mean ± SD, n = 3)

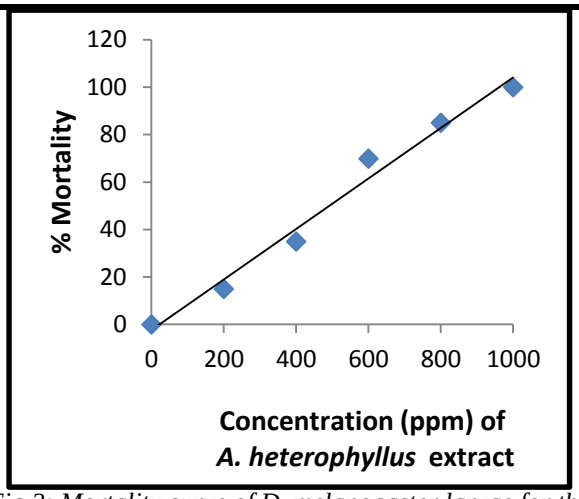


Fig.2: Mortality curve of D. melanogaster larvae for the determination of LC_{50} of A. heterophyllus extract (values are expressed as mean ± SD, n = 3)

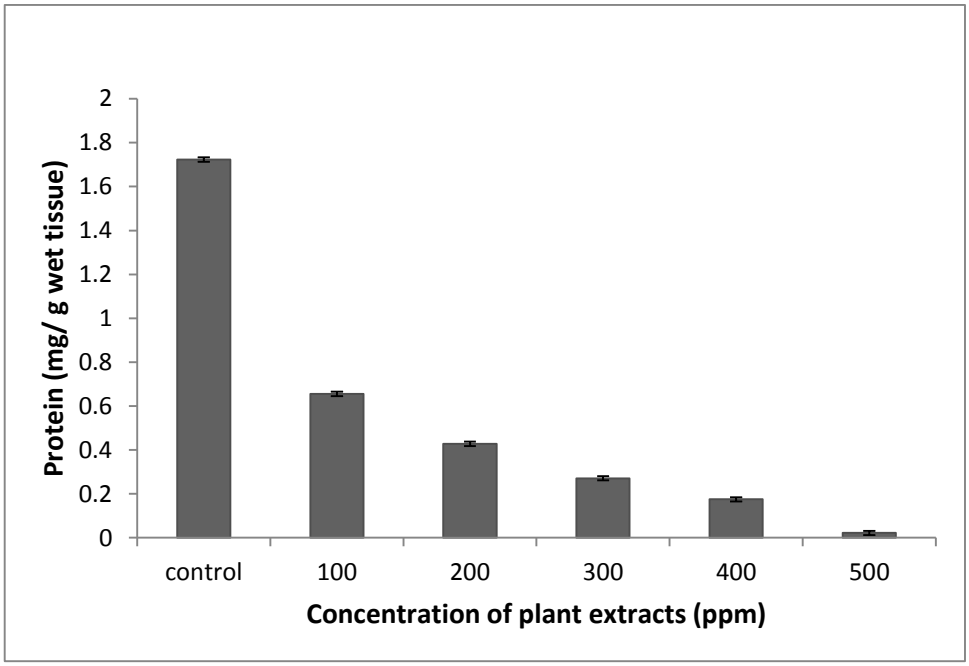


Fig.3: Effect of Piper betle extract on the level of protein after 48 hours exposure of Drosophila melanogaster larvae(values are expressed as mean ± SD, n = 3)

http://dx.doi.org/10.22161/ijeab/2.1.37 ISSN: 2456-1878

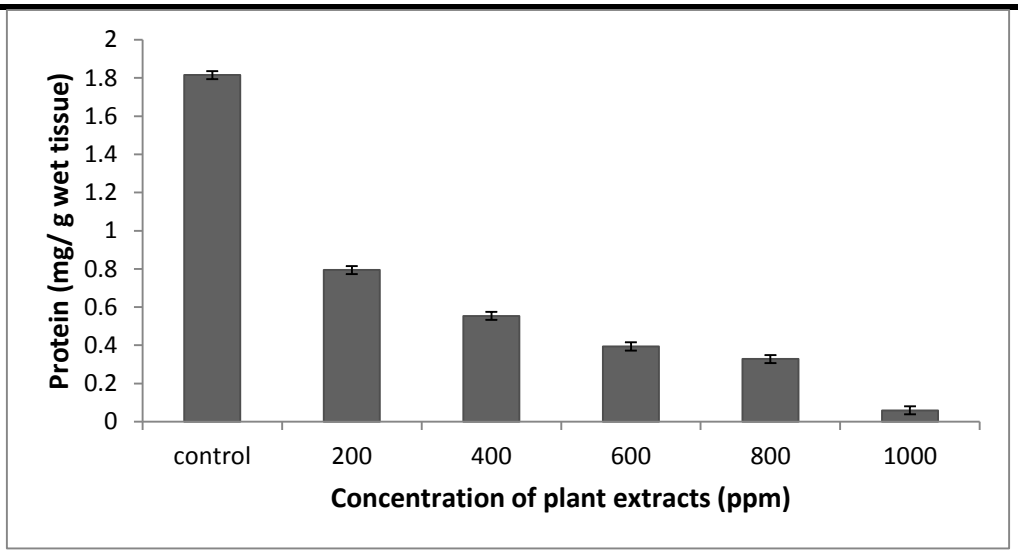


Fig.4: Effect of Artocarpus heterophyllus extract on the level of protein after 48 hours exposure of Drosophila melanogaster larvae (values are expressed as mean ± SD, n = 3)

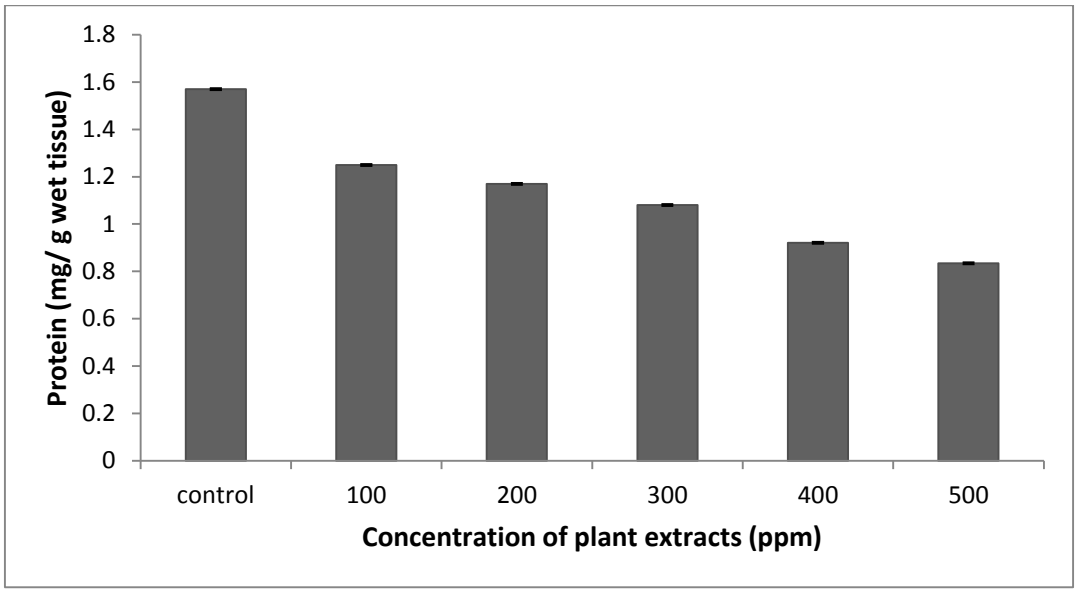


Fig.5: Effect of Artocarpus altilis extract on the level of protein after 48 hours exposure of Drosophila melanogaster larvae(values are expressed as mean ± SD, n = 3)

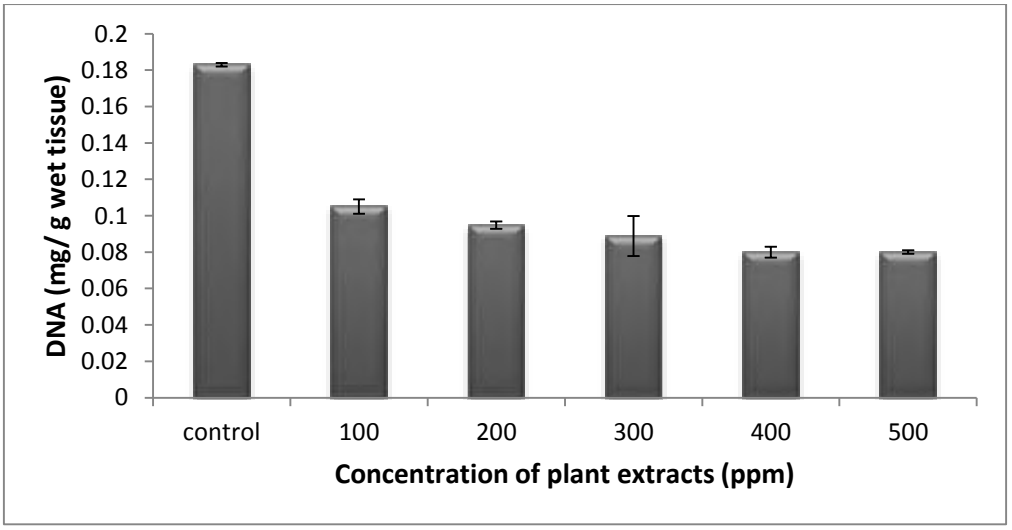


Fig.6: Effect of Piper betle extract on the level of DNA after 48 hours exposure of Drosophila melanogaster larvae(values are expressed as mean ± SD, n = 3)

http://dx.doi.org/10.22161/ijeab/2.1.37 ISSN: 2456-1878

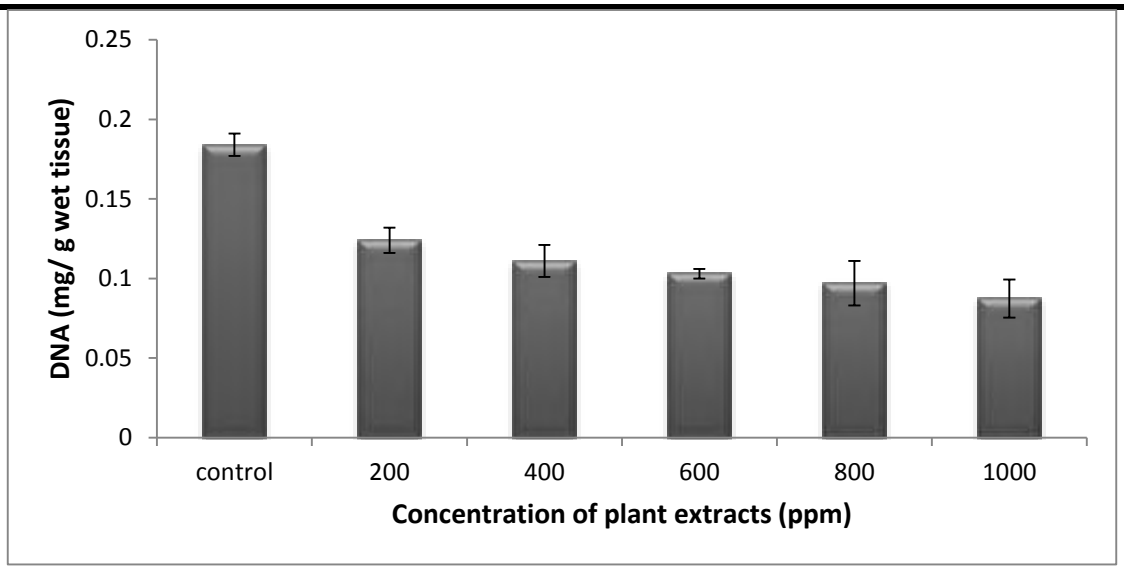


Fig.7: Effect of Artocarpus heterophyllus extract on the level of DNA after 48 hours exposure of Drosophila melanogaster larvae (values are expressed as mean ± SD, n = 3)

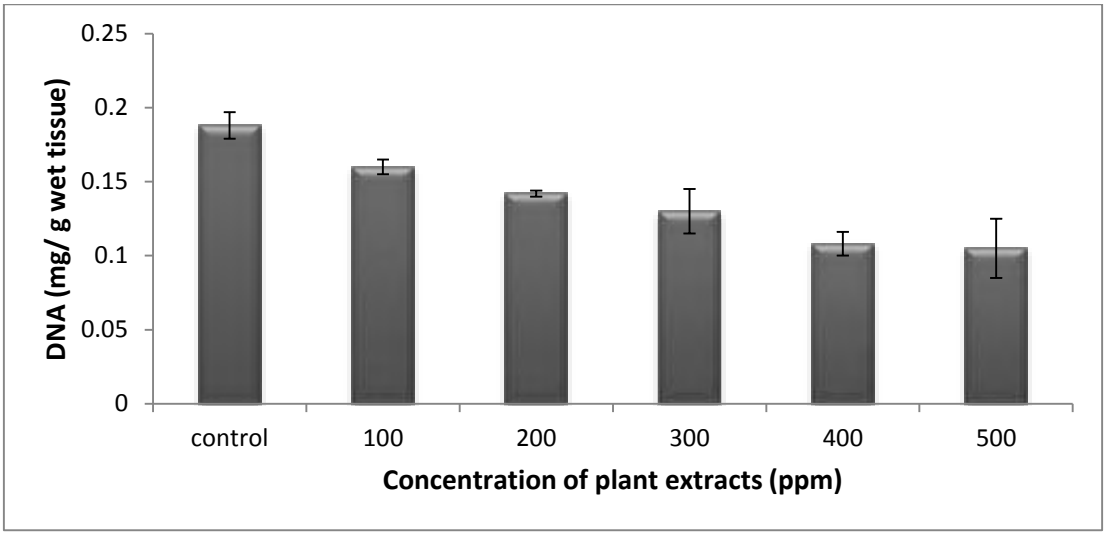


Fig.8: Effect of Artocarpus altilis extract on the level of DNA after 48 hours exposure of Drosophila melanogaster larvae (values are expressed as mean ± SD, n = 3)

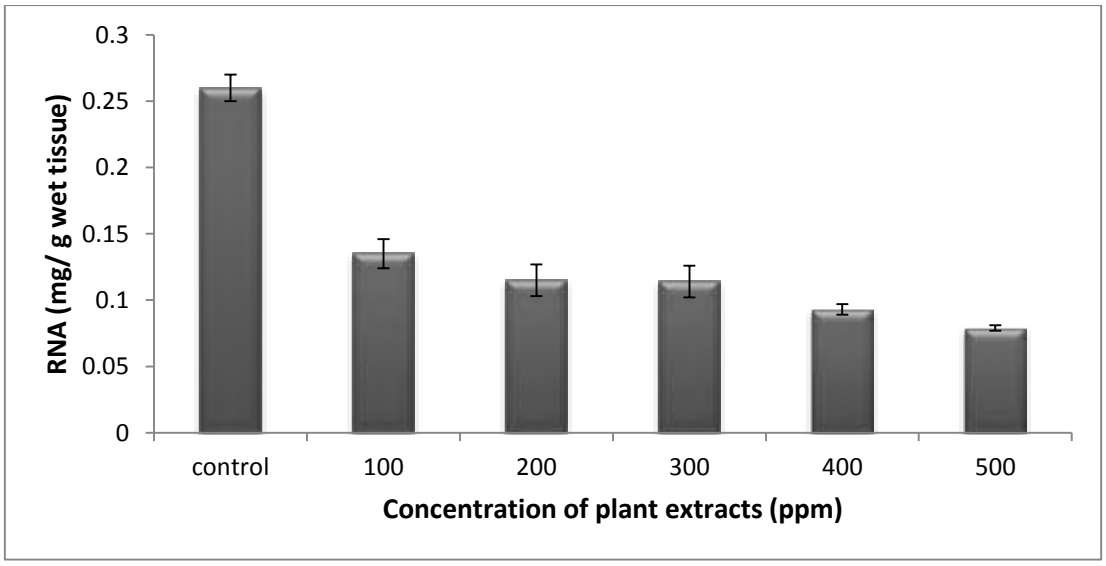


Fig.9: Effect of Piper betle extract on the level of RNA after 48 hours exposure of Drosophila melanogaster larvae(values are expressed as mean ± SD, n = 3)

http://dx.doi.org/10.22161/ijeab/2.1.37 *ISSN: 2456-1878*

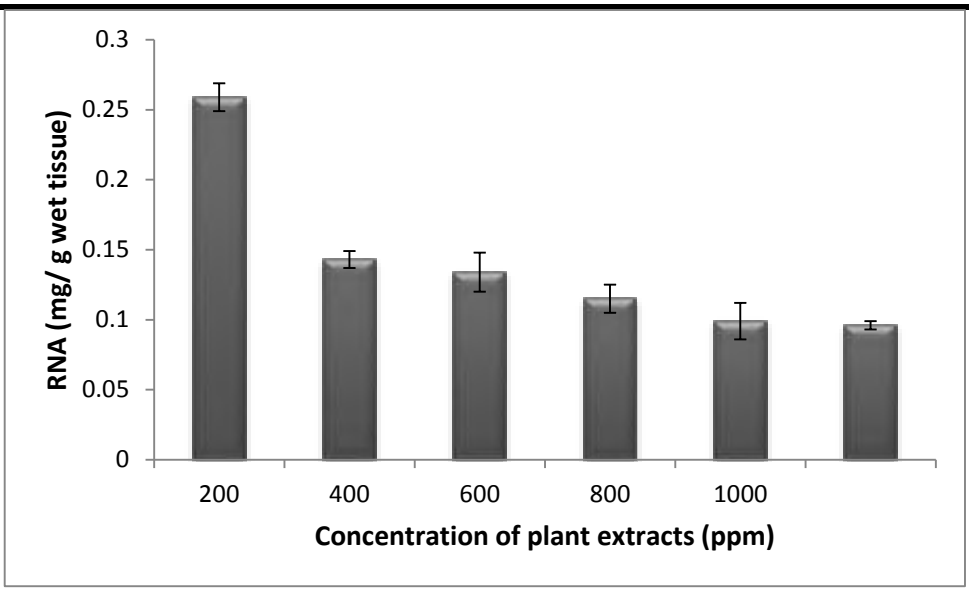


Fig.10: Effect of Artocarpus heterophyllus extract on the level of RNA after 48 hours exposure of Drosophila melanogaster larvae(values are expressed as mean ± SD, n = 3)

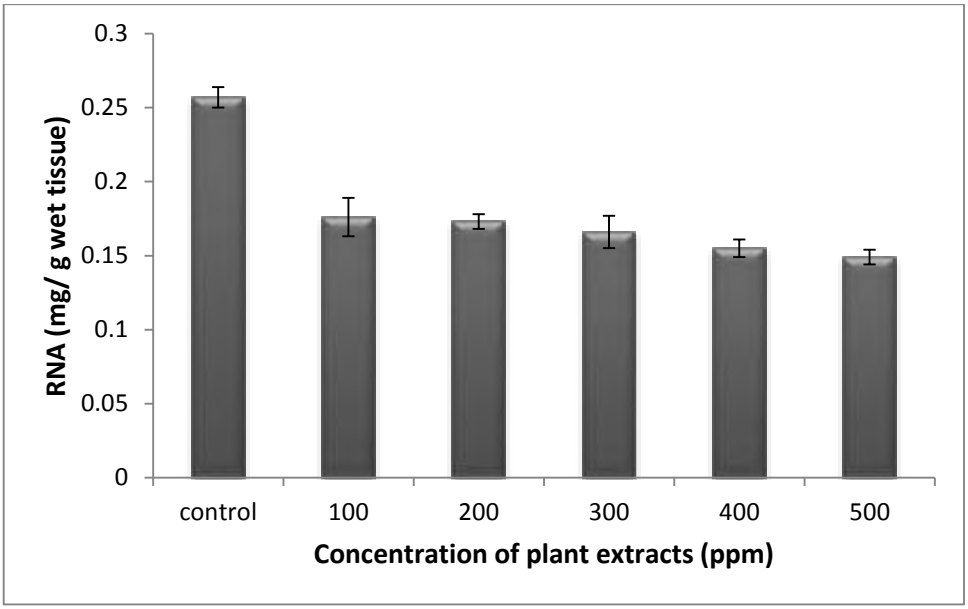


Fig.11: Effect of Artocarpus altilis extract on the level of RNA after 48 hours exposure of Drosophila melanogaster larvae(values are expressed as mean ± SD, n = 3)

Screening of plant extracts for deleterious effect on insects is one of the important approaches in the search of novel biological insecticides. The use of synthetic compounds to control insect pests is associated with several adverse effects that include loss of efficacy, insect resistance,human and eco-toxicity, contamination of water and soil and toxicity to non target species. Therefore, there is an urgent need to develop safe, convenient, environmental and low cost alternatives. Plant extracts are considered to beless toxic, non-pollutant and easily biodegradable. The insecticidal activity of extracts was studied against *Bruchus pisorum, Tribolium castaneum*, *Sitophilus oryzae. Piper betle* and *Artocarpus heterophyllus*extractsshowed good insecticidal activity whereas *A.altilis* extract showed poor insecticidal activity (Figure 12).

http://dx.doi.org/10.22161/ijeab/2.1.37 *ISSN: 2456-1878*

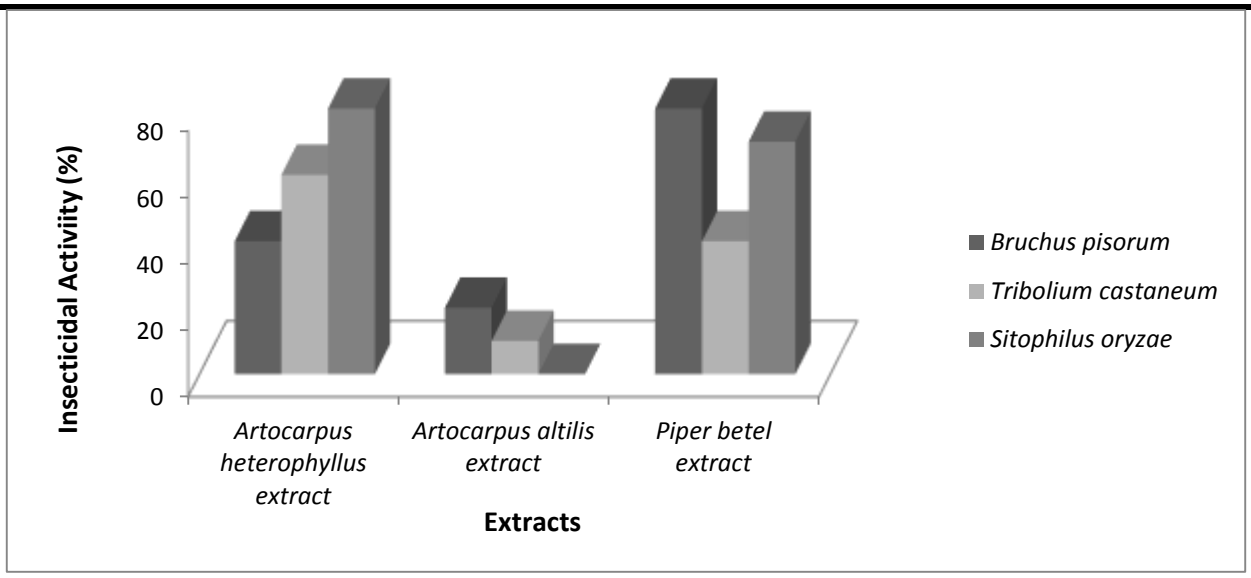


Fig.12: Insecticidal activity of plant extracts against Bruchus pisorum, Tribolium castaneum and Sitophilus oryzae(values are expressed as mean ± SD, n = 3)

To overcome the increasing problems associated with the use of toxic synthetic products there has been an urgent need of developing safer, alternative crop protectants such as botanical insecticides,anti feedants and repellents. The insecticidal activity of a large number of polyphenolic compounds, essential oils and other plant extracts has been assessed against several major agricultural pests. The results of the present study indicate the antifeedant property of the *Piper betle* and *Artocarpus heterophyllus*extractswhich may be due to the different compounds present in the extract possessing different bioactivities. The results obtained from the present study clearly indicate that *Piper betle* and *Artocarpus heterophyllus* extracts could serve as potential candidates for developing botanical insecticides for efficient control of insects.

REFERENCES

[1] Adlercreutz H, Mazur W. (1997). Phyto-oestrogens and Western diseases. Ann Med.29:95–120

[2] Audu SA, Mohammed I, Kaita HA. (2007). Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). Life Science Journal 4(4): 75-79

[3] Camps F, Coll J. (1993). Insect alleochemicals from *Ajuga iva* plants. Phytochemistry 32:1361-1370

[4] Cares MG, Vargas Y, Gaete L, Sainz J, Alarcon J. (2009). Ultrasonically assisted extraction of bioactive principles from *Quillaja Saponaria Molina*. Physics. Procedia. 3: 169-178

[5] Jacobson M. (1982). Plants, insects and man- their interrelationships. Econ. Bot. 36: 346-354

[6] Jilani G, Su HCF.(1983). Laboratory studies on several plant materials as insect repellent for protection of cereal grains. J. Econ. Entomol. 76: 154-157

[7] Lowry O H, NJ Rosebrough, AL Farr, RJ Randall. (1951). Protein measurement with the Folin-Phenol reagents. J. Biol. Chem. 193: 265-275

[8] Mehta, P. K., Vaida, D. N., & Kashyap, N. P. (1995). Antifeedant properties of some plant extracts against brinjal hadda beetle Henosepilachna vigintioctopunctata. Journal of Entomological Research.19(2): 147-150

[9] Miyazawa, M, Ota H, Ishikawa Y, Kameoka H. (1993). An insecticidal compound from *Illicium verum*. Chem. Express. 8: 437-440

[10] Yang RZ, Tangs CS. (1988). Plants used for pest control in China: a literature review. Econ. Bot. 42: 376-406

[11] Vanhecke P, Persoone G, Claus C, Sorgeloos P. (1981). Proposal for a short-term toxicity test with *Artemia nauplii*. Ecotoxicol. and Environ. Safety 5: 382-387

[12] Zettler JL, Cuperus GW. (1990). Pesticide resistance in *Tribolium castaneum* (Coleopteran: Tenebrionidae) and *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in wheat. J. Econ. Entomol. 83: 1677- 1681

[13] Edeoga H O, Okwu D E, Mbaebie B O. (2005). Phytochemical constituents of some Nigerian medicinal plants. AfrJ Biotechnol. 4:685–688

[14] Fabricant D S, Farnsworth N R.(2001). The value of plants used in traditional medicine for drug discovery. Environ Health Perspect.109: 69–75

[15] Gullan, P. J. & Crantson, P. S. (1994). Insects: A. Outline of Entomology. Chapman and Hall London, U. K., 491.

http://dx.doi.org/10.22161/ijeab/2.1.38 *ISSN: 2456-1878*

Vulnerability of the Livestock Sector in Changing Climate Conditions: A Case from India

Nirma Bora

Abstract— *In India, livestock sector plays an important role in socio-economic development of rural households. Over 70 percent of the country's rural households own livestock and a majority of livestock owning households are small, marginal, and landless farmers. The reality of climate change and the fact that life in the poorest and vulnerable economies will be worst affected is set to have far-reaching consequence on the animal and its owners. At the same time, livestock have always shouldered a portion of the blame for rising greenhouse gas (GHG) emissions. However, recent extensive scientific evidence and report by FAO and universities in the US has brought to light the fact that the large GHG emission figure of livestock emission was big data hype. The developed countries play clever by shifting blame for anthropogenic GHG emission away from the fossil fuel based power generation, transportation, industries and lifestyle of the global North to activities in developing countries such as paddy cultivation and animal husbandry.*

Keywords— Livestock, emission, climate change, vulnerability, developed countries, meat, GDP.

Highlights

- World demand for livestock products growing strongly
- vulnerability of livestock increasing in a changing climate
- Increased share of livestock in budgetary allocations, subsidised fodder, availability of water, strengthened veterinary services.

Acknowledgement

This policy brief was supported by PAIRVI, a New Delhi based Human Rights organization. The author likes to thank the Director of PAIRVI, Mr. Ajay Jha, who provided insight and expertise that assisted the paper and comments which greatly improved the manuscript.

I. INTRODUCTION

Evidence from the Intergovernmental Panel on Climate Change (IPCC, 2007) is now over whelming convincing that climate change is real, and it will become worse affecting the poorest and vulnerable people the most (IFAD, 2009). The IPCC predicts that by 2100 the increase in global average surface temperature may be between 1.8 and 4.0 °C. With global average temperature increase of only 1.5 – 2.5°C degrees, approximately 20-30 percent of plant and animal species are expected to be at the risk of extinction (Fischlin et al, 2007). While some species will be able to migrate or change their behavior to accommodate climate change, other species may go extinct (EPA).

Of the planet's 1.3 billion poor people, at least 90% are located in Asia and sub-Saharan Africa (Thornton et al., 2002). The livestock sector in these economies will be specifically affected by climate changes through: changes in the pattern and quantity of rainfall, an increase in temperature, changes in winds, changes in seasonality, more frequent catastrophic events, a decrease in feed and fodder production, reduced water availability, changing patterns and distribution of disease, changes in the marketing and prices of commodities.

Traditionally, however, livestock keepers have been capable of adapting to livelihood threats and indeed—for some people— livestock keeping is itself an adaptation. It is important, however, to recognize that the outcomes of climate change are uncertain and the precise adaptations will vary from location to location and person to person. Strengthening resilience of the livestock sector relies on building the adaptive capacity of livestock keepers and taking an ambitious approach to address the livestock management.

II. LIVESTOCK RESOURCE

India has one of the largest livestock population of around 520.6 million of which cattle (cows , bulls, oxen) constitutes 12.7%, buffalo 56.7%, goats, 14.5% and sheep 5.9 % (FAOSTAT, 2008). India ranks first with respect to the population of buffaloes, second in cattle and goats, third in sheep, fifth in ducks and chickens and tenth in camel population in the world (GOI, 2011-12). The national distribution of livestock and its growth pattern is shown in Table 1.

Table.1: Trends in Livestock Growth

S.No.	Species	Livestock Census (In millions)		Growth Rate (%)
		2003	2007	2003 over 2007
1.	Cattle	185.2	199.1	7.50
2.	Buffalo	97.9	105.3	7.58
3.	Sheep	61.5	71.6	16.41
4.	Goat	124.4	140.5	13.01
5.	Other Animals (Horses, camels, pigs, mules, yak, mithuns)	16.02	13.1	-19.13
	Total Livestock	**485.2**	**529.5**	**9.13**

Source: Compiled by data collected from Livestock Census, DAHD

Significance of livestock for India

Animal Husbandry has been making a significant contribution to the national economy and socio-economic development in the country. In mixed farming systems livestock reduce the risks resulting from seasonal crop failures as they add to the diversification of production and income sources. In rural India, where over 15-20% families are landless and about 80% of the land holders belong to the category of small and marginal farmers, livestock is the main source of livelihood (Hegde, BAIF). The potential of the livestock sector is evident from its economic contribution to the total GDP, which stood at 4.11% at current prices during 2012-13 (MOSPI, 2015). In the arid states like Rajasthan, 8 percent of G.D.P. of the State is contributed by livestock sector alone (Govt of Rajasthan). In the semi arid state of Gujarat, livestock contributes to around 5.08 % of the total SGDP (DOAH, 2013).

In 2010-11, the total output from livestock in India was higher (at Rs 3,40,500 crore) than the value of food grains (Rs 3,15,600 crore) and fruits and vegetables (Rs 2,08,800 crore), and this is going to go up substantially (Mahapatra, 2012). Table 2 shows the livestock sector growth surpassing the other agricultural sub sectors.

Table.2: Growth Trends in Agriculture Sub-Sectors

Sub-Sectors	Ninth Plan 1997-2002	Tenth Plan 2002-2007	Eleventh Plan 2007-2012
Non-Horticulture Crops	1.7	2.1	2.8
Horticulture Crops	3.8	2.6	4.7
Livestock	3.6	3.6	4.8
Fishing	2.7	3.3	3.6

Source: Central Statistical Organisation

To understand the significance of livestock in developing economies we must look beyond GDP and examine the kinds of livestock benefits that are excluded from national accounts. The role of livestock also extends to being an important source of draught power in rural Indian households. Bullock power continues to be used in agricultural operations and transport of agricultural products to nearby markets. Animal energy is renewable, saves fossil fuels, and prevents emission of greenhouse gases. The fossil fuel equivalent of animal energy used in the Indian agriculture has been found to be 19 million tonnes of diesel in 2003 (Birthal & Dikshit, 2010). Considering the same amount of fuel was used to run tractors in the absence of working animal stock, it would have released 6 million tonnes of carbon dioxide (Birthal & Dikshit, 2010).

The dung-manure is another important input contributed by livestock in agriculture. It is estimated that approximately 50% of the total dung produced is utilized as manure while the rest is used as domestic fuel or lays waste on roadside. Above all, livestock contributes to the diet of 1.25 million Indians and many more globally. Milk, meat, and eggs, the "animal-source foods," though expensive, are one of the best sources of high quality protein and micronutrients that are essential for normal development and good health.

In other agro pastoral economies of the world too, value of the contribution from the livestock sector is significantly higher than hitherto believed. While in India, livestock

production currently contributes about 25.6 percent of the agricultural GDP, in Eastern Europe and Central Asian (EECA) countries and Latin America and the Caribbean (LAC) countries, the contribution is as high as 44.5 per cent and at 42.7 percent (Biasca, 2012). If non-monetized contributions (draught power and manure) were to be included, reflecting the importance of integrated crop-livestock farming systems, the contribution of livestock to agricultural GDP would increase further.

Vulnerability of Livestock to Climate Change

Climate change will impact humans and animals both. While humans are more capable of adapting to the impact of climate change, animals are not. When their habitats change irrevocably — the grazing land and water bodies dry up or cool mountains heat up — animals may simply go extinct. Reports have indicated that developing countries are more vulnerable to the effects of climate change due to their high reliance on natural resources, very limited capacity to adapt institutionally and financially, and high poverty levels (Thornton et al., 2006). Animal health in such a habitat may be affected by climate change in four ways: heat related diseases and stress, extreme weather events, adaptation of animal production systems to new environments, and emergence or re-emergence of infectious diseases, especially vector borne diseases that are critically dependent on environmental and climatic conditions

The widespread impact of climate change on livestock in the country is being demonstrated year after year in the form of heavy toll on animal life. Be it the 1999 tropical cyclone that hit the state of Orissa claiming 4.45 lakh livestock or the 2013 floods in Uttarakhand where another 9470 livestock got washed away and 649 cattle shed were damaged, climate change has resulted in livestock losses triggering urgency to respond (MoHA, 2013). Post disaster, crippling shortage of fodder coupled with other hardships forces poor farmers to sell their livestock for peanuts. The people of Kashmir faced a similar plight in 2014 when severe floods in the region claimed life of 10,050 milch animals, besides 33,000 sheep and goats (Firstpost, 2014). Following the calamity, residents of many villages reluctantly sold their livestock at cheap rates to meat sellers since they had no fodder and most cowsheds were either damaged or destroyed.

The unpredictable weather conditions have also resulted in poor availability of pasture and grazing land; and feed and fodder scarcity. In 2003, there was a deficit of 157 million tons of green fodder, 44 million tons of dry fodder, and 25 million tons of concentrates in India (Dijkman et al., 2010*).* The area under permanent pastures and grazing land represents a mere 3.3% of total area and has been declining steadily from 12 million ha in 1981- 82 to 10.2 million ha in 2001-02 (FAI, 1982, 2002).

Besides, the warmer and wetter climate and the densely populated nature of the country in terms of both human beings and livestock has increased the occurrence of vector-borne diseases[1] and spread of zoonotic viral infection (Chogle, Feb 2012*).* According to a study, Ethiopia, Nigeria, and Tanzania in Africa, as well as India in Asia, have the highest zoonotic disease burdens[2](Grace et al., 2012).

Research indicates that there is more in store for the animal as heat stress is predicted to reduce the total milk production for India by 1.6 million tons in 2020 accounting about Rs 23.65 billion, at current price rate. The decline in milk production will be higher in crossbreeds (0.63%) followed by buffalo (0.5%) and indigenous cattle (0.4%) (Upadhayay, 2004-07*).*

Contribution of livestock to climate change

The major greenhouse gases emitted by livestock are methane and nitrous oxide. Livestock mainly emit methane due to anaerobic fermentation in their digestive system while nitrous oxide is released from its manure. These emissions became widely talked about when in 2006 the United Nations concluded that the livestock industry was a big contributor to climate change. The Food and Agriculture Organization (FAO), agency of the United Nations that leads international efforts to defeat hunger, in its report titled 'Livestock's Long Shadow' quantified the emissions from livestock as 18% of the total anthropogenic emissions of the world.[3] Ignoring the contamination and emission by industries and transport, it held livestock business among the 'most damaging sectors' to the earth's increasingly scarce resources, contributing among other things to water and land pollution. However, if the trends in global GHG emissions are considered by sector, it is the electricity/heat that contributes to 37 percent and manufacturing, construction, and industries that contributes to 19 percent of the global GHG emission (TSP dataportal).

Much later after seven years, the 2013 Assessment Report of the FAO, revised figures for livestock emission. It now estimates that the global livestock sector accounts for as much as 7.1 gigatonnes of CO_2-equivalent every year,

[1] Vector–borne diseases are infection transmitted by the bite of infected blood-sucking arthropod species such as mosquitoes, ticks, bugs, and black flies.

[2] Zoonotic diseases are (also called zoonoses) are infectious diseases that can be spread from animals to humans.

[3] Global emission from transport stand at 13% based on 4th Assessment Report of IPCC (2007).

http://dx.doi.org/10.22161/ijeab/2.1.38 *ISSN: 2456-1878*

representing 14.5 percent of all human-related greenhouse gas emissions (Gerber, 2013). Nevertheless, the revised model too calculated livestock sector emission by assessing all sources of emissions along the livestock supply chain. The figures by FAO included not just emission from the animal but the total the amount of greenhouse gases emitted from every aspect of raising meat and dairy. FAO did not do the same when estimating the greenhouse gases from cars (Lutey, 2012). The latter report ignored greenhouse gases actually created during the car's production and instead zeroed in on tailpipe emissions. Besides, it is not livestock per se which are responsible for increased greenhouse gasses; it is the corn/ soybean/ chemical fertilizer/ feedlot/ transportation system under which industrial animals are raised.

Even within the United Nations, there is large discrepancy on global emissions from livestock. In 2013, Food and Agricultural Organization (FAO) of the UN estimated the total global emissions from livestock sector as 14.5 percent (Gerber, 2013). This number was quite low in the 2012 United Nations Environment Programme (UNEP) Report that measured the total emissions from agriculture as 11 percent of which livestock emissions were mere 4.7 percent (UNEP, 2012). Another UK based environmentalist reports that direct emission of methane and nitrous oxide from livestock makes up around 9 percent of total man-made greenhouse-gas emissions. It is emissions from elsewhere in the livestock supply chain, such as transport and feed production, that boosts this figure to 18%. (Kalauher, 2014). Due to large variations in the emissions figures given by different UN agencies and scientists, neither validity nor reliability of the data could be established. Consequently, in the absence of reliable data it is highly undesirable to hold the developing economies accountable for their survival emissions and push them for emission reduction targets equal to the developed countries.

Policy Measures- Combating or contributing to Climate change

The last few decades has seen the Indian livestock sector emerging as one of the fastest growing sub-sectors of agriculture. However, the two entities that have largely been by-passed by the benefits of this growth are the livestock themselves and the small and marginal farmers who rear them. Livestock sector policies and programmes since 1990s has largely been dominated two major development narratives. The first narrative is the productivity myth whereas the second is the efficiency narrative. While both these objectives fast-tracked growth, they did not translate into livestock sector policies, which ensured inclusiveness and efficiency of the sector. Be it the breed development schemes and allied services or market deregulation and privatization, livestock sector policies have largely tended to benefit the already better off livestock holders.

Analyses of major national policies addressing livestock in India reveals that they are apparently biased towards the productivity-enhancement. Priority has been given to those livestock sub-sectors which have showcased huge successes - namely the dairy sector through Operation Flood and the meat industry through the Pink Revolution. Even the very recent National Livestock Policy, 2013, has primarily been formulated to improve productivity of the livestock sector and facilitates dissemination and adoption of technologies for improving efficiency and exploitation of production potential.

Furthermore, the National and various State Action Plans on Climate Change (NAPCC and SAPCC), intended to undertake activities and programmes aimed at climate change adaptation and mitigation, have adopted a very casual approach in dealing with the livestock sector. While a few, like Uttarakhand and Madhya Pradesh, have studied and well documented the climate change impact on livestock and suggested adaptation strategies, rest like Jharkhand and Rajasthan either have excluded the sector from their approach strategy or have dealt more with mitigation measures for reducing livestock methane emission rather than adopting an inclusive approach where support is extended to livestock and its owners. Beside, no assistance has been provided to owners of small ruminants as focus is on bigger milch cattle and higher milk production.

Even the National Mission on Sustainable Agriculture (NMSA), one of the eight missions under NAPCC launched in 2010, proposes extending genetic engineering to livestock. It refuses to learn from the ongoing plight of owners of genetically modified breeds who are more in need of fodder and forage, water, and veterinary aid than owners of local breeds are. It has been observed that some of the traditional Indian breeds of cows like Sahiwal, Tharparkar, Red Sindhi, Rathi, Gir, Kankrej, have traits that enable them to survive under low input, withstand more heat, travel long distances for water, and face resistance to disease.

In the name of better income to livestock owners, the government's ambitious export policies are also adding to climate concerns. With meat production at 6.3 million tons in 2010, India's annual per capita meat consumption stands at only 4 kg while for China, UAE and Australia its 58 kg,74 kg, and 111 kg respectively, thereby making evident where all the meat goes(FAO, 2013). Of the total beef

production in India, the country consumes only 53.8 % while the remaining 46.1% is exported to countries like China (routed through Vietnam), Saudi Arabia, Egypt, Thailand etc., thereby making India the top beef exporting country in the world.[4] In 2010, 36.1 Mt of CO_2-equivalent emissions were related to meat produced in one country but consumed in a different country (The Conversation, 2014)). Therefore, raising livestock, for slaughtering later, comes at a heavy price for India. It includes stresses such as deforestation, desertification, "excretion of polluting nutrients, overuse of freshwater, inefficient use of energy, diverting food for use as feed and emission of GHGs" (Janzen, 2011).

Another emerging problem is the divergence of agricultural land for production of grains for livestock rather than for human beings. Moreover, his produce is not meant to feed domestic cattle but meet the feed demand abroad. Vandana Shiva, Indian environmental activist, says in her book, *Stolen Harvest*, "Europe's intensive livestock economy requires seven times the area of Europe in other countries for the production of cattle feed. In a complementary economy, the cattle eat the straw and agricultural waste that humans cannot. But, in a competitive model such as the livestock industry, grain is diverted from human consumption to the intensive feed for livestock. It takes eight kilograms of grain to produce one kilogram of meat." By using our agri- land for producing feed–grains meant for livestock industry in some foreign land, India is creating a sort of imbalance that will divert grains away from our own people. India, thereby, has 25% deficit in dry fodder, 65% in green fodder and 60% in feed concentrates.

States in India have urged the government to implement immediate measures to tackle the scarcity of fodder in the country. They have pitched for a the creation of a Fodder Corporation of India much in line with the Food Corporation of India(FCI). In face of the 2,00,000 tonnes of deoiled rice bran (DORB) and oil cakes worth Rs 8,500 crore exported every year, state governments have urged the centre to sought a ban on export of oilseed cake and discontinue harvesting of wheat and other fodder crop using combine harvester (Kumar, 2013). This feed could be retained in our livestock feed system to keep feed prices in check.

As meat supply and consumption increase around the world, more sustainable food systems must be encouraged. A study in the UK found that emissions from beef amount to 16 kg CO_2-eq/kg beef compared to 0.8 kg CO_2-eq/kg of wheat (Garnett, 2009). In another study in Sweden, authors conclude that "it is more "climate efficient" to produce protein from vegetable sources than from animal sources", and add that "beef is the least efficient way to produce protein, less efficient than vegetables that are not recognized for their high protein content, such as green beans or carrots" (Carlsson-Kanyama and González 2009).

III. RECOMMENDATIONS

- **Decentralize Policy Planning**: The tendency of the government to centralize planning has remained unchanged and still exercises strong control. Decentralized policy planning actually being practiced is a myth largely; making bureaucracy unable to innovate. Policy implementers face limitations due to hegemonic directives, while at the same time government staff adhering to tacit protocols create resistance to innovative top-down policies and limit engagement with farmers.
- **Increase the Share of Livestock in Budgetary Allocation**: The livestock sector is under-invested and neglected by the financial and extension institutions. Even the 2013-14 budgetary allocation for Animal Husbandry, Dairy Development and Fisheries has been very dismal. While the share for agriculture and allied sector increased by 18 % from 2012 that of Animal Husbandry, Dairy Development, and Fisheries remained low at 12.3% [5](Singh, 2013). Even under the National Mission on Sustainable Agriculture (NMSA), Livestock and Fisheries combined have been allocated 9,000 crores of the total 1,08,000 crore budgetary support to the intervention (NMSA, 2010). Only 6% of the animal heads (excluding poultry) have insurance cover. Livestock extension remains grossly neglected. Only about 5% of the farm households in India have access to information on livestock (GOI, *2012-17*). Improving information and knowledge and then providing training on adaptation-based livestock management at grassroots level is expected to bring about changes that are more significant.
- **Check Excess Promotion Foreign Breeds and Support Local Breeds**: The all-India breeding policy was drawn up under the Third FYP (1961-66) and accepted by the central and state governments (GoI, 1961). The policy emphasised crossbreeding nondescript, indigenous species with exotic stocks to

[4] India produced 3.643 million metric tons of beef in 2012, of which 1.963 million metric tons was consumed domestically and 1.680 million metric tons was exported.

[5] The 2013-14 budgetary allocation for agriculture stands at INR 187.81 billion while the peanut share allotted within it to Animal Husbandry, Dairy Development, and Fisheries is a total of INR 18.17 billion (Singh, 2013).

http://dx.doi.org/10.22161/ijeab/2.1.38 *ISSN: 2456-1878*

increase milk production (Singh, 2011). However, more than three decades of crossbreeding, has revealed that most exotic breeds have not been able to maintain high levels of productivity for a long duration.

- **Make fodder banks or Subsidize Fodder in Drought Periods**: The existing fodder resources of the country can meet 216.62 million out of the 416 million cow units while there is no arrangement to sustain the remaining 48.08 (Kothari & Mishra, DADH**)**. Climate change will further effect livestock production by altering the quantity and quality of feed available for animals. Better quality diets for the ruminants, will increase their feed-conversion efficiencies and thus reduce the amount of methane generated. Fodder storage will also improve food security through construction of larger grain storage facilities.
- **Strategize the Availability of Water for livestock:** Few states that face crippling water crises for both human and animals are Rajasthan, Maharashtra AND Eastern Uttar Pradesh. In this regard, Madhya Pradesh SAPCC strategizes efforts to enhance availability of water for livestock by integrating the concern with watershed management practices. Other states need to follow similar suit strategizing water needs.
- **Create a Disaster Recovery Plan**: Currently, the *gaushalas* are poorly managed with no working arrangements between *gaushalas* and local / state government and Animal Husbandry Department. This makes it essential for the government to provide all support to organizations volunteering to take care of cattle and willing to organise cattle-camps during the natural calamities, such as drought or massive rainfall.
- **Strengthen Veterinary Services:** Climate change may increase the prevalence of parasites and diseases that affect livestock. Improved opportunities for delivering animal health and production services to farmers particularly traditional smallholder farmers is needed in the changing climate scenario. Establishing ambulatory and advisory services at doorstep (as prioritized in Uttarakhand SAPCC) should be made available. On the other hand, indigenous knowledge based on ethno veterinary practices can address some of the health care problems on a local and low-cost basis.
- **Coordinate and Collaborate between Livestock Institutions:** It is a well-known fact that every state in India has a number of organizations for the development of the livestock sector. The state Department of Animal Husbandry (DAH), veterinary colleges and universities, livestock development agencies and milk unions are the most notable among these. Collaboration between these different organisation in the livestock sectors like is critical for betterment of the livestock sector.
- **Strengthen Non-Performing Cooperatives**: The success of dairy cooperatives has been largely confined to a few states in India such as Gujarat, Punjab, Andhra Pradesh and Rajasthan, where brands like Amul, Verka, Vijaya and Saras have become household names. However, a large number of dairy cooperatives, unions and federations are defunct and are not able to create value for their members. Cooperatives in Uttar Pradesh (Parag Dairy), Kerala (Milma), and Madhya Pradesh (Uttam Dairy) are largely loss making (Vivek, 2000*)*. A lot needs to be done to strengthen such non-performing cooperatives. Also, dairy cooperatives need to be promoted and strengthened in hilly and backward districts of the country.
- **Promote Low-Carbon Diet Initiatives:** As far as reducing enteric emission in the country from the large ruminants is concerned, the government must realise that global demand for livestock products is on a rise and this demand in rich countries in many cases is met by imports of livestock products or feed grains from the developing world like India. This practice has made India into exporting 21% of its total meat production, thereby increasing the levels of greenhouse gas emissions in the country (NMPPB, 2008). Methane emissions can be reduced by reducing the number of extra livestock being raised to meet the demands of developed countries and by promoting a low meat diet. The best approach initially can be advertising campaign making people aware that increased livestock production can be severely damaging to their habitats.

IV. CONCLUSION

Livestock are an important and sometimes overlooked element of the livelihood strategies of the poor. With world demand for livestock products continuing to grow strongly across the world and vulnerability of the sector increasing in a changing climate, the developing countries need to strategically plan policies to meet the challenges. This would include measures to strengthen the veterinary service, support local breeds, create a disaster recovery plans, control GHG emission 'transferred' by developed economies, meet the feed deficit, and promote low-carbon diet initiatives. Above all, livestock plays a vital role in the agricultural and rural economies of the developing world

like Africa, Asia and Latin America, where the poor and the landless derive a higher proportion of household income from livestock sources than do other households. Needless to say, developed countries should reconsider holding the developing economies accountable for emissions from agriculture, as their 'lifestyle emission' is no match to the 'survival emissions' of agro-pastoral economies. Blaming them for the comparatively small percentage of global emission they create to provide food security, seems a gimmick/dodge to target a small-time emitter and shrewdly overlook the big one.

REFERENCES

[1] Animal Welfare Board of India (July 2013). Uttarakhand Floods and Relief Measures. Vol 9 Issue 6. MoEF, Govt of India.

[2] Bloomberg News. (16 Jan 2013). *Camel Slaughter Plan Rejected.* Retrieved June 3, 2013 from http://www.bloomberg.com/news/2013-01-16/australian-panel-rejects-plan-to-kill-camels-for-carbon-credits.html

[3] Biasca, Ronalee.(Rev.Sep 2012). The role of livestock for ACP countries: Challenges and opportunities ahead,p7.

[4] Birthal, Pratap S. & Dikshit AK. (July-Dec 2010). Environmental Value of Draught Animals. *Agricultural Economics Research Review, Vol. 23*, pp 227-232.

[5] Chogle, A.R. (Feb 2012). Zoonotic Diseases in India : Focus on Research Options for Anthrax Control. *Journal of the Association of Physician in India, Vol 60.*

[6] Dijkman, Jeroen et al. (2010). Livestock in a changing landscape: Experiences and Regional Perspective, Vol 2, FAO, p 59.

[7] DOAH, 2013. Retrieved on 7 May 2015 from http://doah.gujarat.gov.in/Images/pdf/animalhusbandary/ankdakiy-aheval12-13.pdf

[8] Mitloehner, Frank Dr. (31 March, 2011). Dr. "Debunking"Livestock Long Shadow." Interview. *Drovers Cattle Network News Source*.Retrieved April 29, 2015 from <http://www.cattlenetwork.com/cattle-news/latest/Debunking-livestocks-long-shadow-118996039.html>.

[9] Ehui, S., S. Benin, T. Williams and S. Meijer (2002), Food Security in Sub-Saharan Africa to 2020, Socio-economics and Policy Research Working Paper 49, International Livestock Research Institute, Nairobi

[10] EPA. (Sep 1999). Enteric Fermentation. *U.S. Methane Emissions 1990 – 2020: Inventories, Projections, and Opportunities for Reductions*, p 6-2.

[11] EPA, Adaptation Overview. Retrieved June 3, 2013 from http://www.epa.gov/climatechange/impacts-adaptation/adapt-overview.html

[12] Fischlin et al. (2007). Ecosystems, their properties, goods, and services, Chap 4. *Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the IPCC* (p 213). Cambridge: Cambridge University Press.

[13] FAOSTAT, 2008.

[14] Firstpost (29 Sep2014). 'After floods Jammu and Kashmir to submit memorandum of losses to Centre'. As retrieved on 15 May 2015 from http://www.firstpost.com/politics/floods-jammu-kashmir-submit-memorandum-losses-centre-1735607.html

[15] Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A. & Tempio, G. (2013) Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome.

[16] Government of Rajasthan. As retrieved on 12 May 2015 from http://animalhusbandry.rajasthan.gov.in/activities.aspx

[17] Hegde, Dr. N.G. Livestock Development for Sustainable Livelihood of Small Farmers, *BAIF*, Pune.

[18] ICAR. Based on calculation of various livestock related schemes under the title Schemes as retrieved on 27 Sep, 2013 from http://www.kiran.nic.in/index.html

[19] IFAD. (Nov 2009). Livestock and climate change. Retrieved on June, 3, 2013 from http://www.ifad.org/lrkm/factsheet/cc.pdf

[20] IPCC. (2007). Fourth Assessment Report.

[21] Janzen, H.H. (2011). What place for livestock on a re-greening earth? *Animal Feed Science and Technology*, pp 166-167, 783-796.

[22] Kalaugher, Liz (14 Nov 2014). Meat is moving Green House Gas Emissions. Retrieved on 1 May 2015 from http://environmentalresearchweb.org/cws/article/news/59290

[23] Kumar, Rahul (9Aug 2013). Export of animal feed deprives our livestock of nourishment, critical to ensure right to food. *The Economic Times.*

[24] Lutey, Tom. (13 Nov 2012). Prof debunks flatulence as major cause of global warming. Retrieved on 29

April 2015 from http://billingsgazette.com/news/state-and-regional/montana/prof-debunks-flatulence-as-major-cause-of-global-warming/article_3340e03d-a03a-5469-ae2d-fa06f23cecb6.html

[25] Mahapatra, Richard. (Feb 2012). Livestock marks shift in agriculture. *Business Standards*. Retrieved June 3, 2013 from http://www.business-standard.com/article/economy-policy/livestock-marksshift-in-agriculture-112020100085_1.html

[26] MOSPI, 2015. Retrieved 7 May 2015 from http://mospi.nic.in/Mospi_New/upload/SYB2015/CH-10-LIVE-STOCK%20AND%20FISHERIES/Livestock_and_fisheries_writeup.pdf

[27] Thornton, P.K., Kruska, R.L., Henninger, N., Kristjanson, P.M., Reid, R.S., Atieno, F., Odero, A. & Ndegwa, T (2002). Mapping poverty and livestock in the developing world. Nairobi, ILRI. Thornton, P.K.,

[28] Thornton, P.K., Jones, P.G., Owiyo, T.M., Kruska, R.L., Herrero, M., Kristjanson, P., Notenbaert, A., Bekele, N., & Omolo, A. (2006). Report of the DFID. *ILRI*, p 200.

[29] TPS data. Retrieved May 13, 2015 from http://www.tsp-data-portal.org/Breakdown-of-GHG-Emissions-by-Sector-and-Gas#tspQvChart

[30] Upadhayay,R.C. (2004-07). Final report of the Network Project on Climate Change. *National Dairy Research Institute*. NDRI

[31] UNEP. (2012). The Emissions Gap Report 2012. United Nations Environment Programme (UNEP), Nairobi, p21.

http://dx.doi.org/10.22161/ijeab/2.1.39 *ISSN: 2456-1878*

Effect of Mining Activities on Vegetation Composition and nutrient status of Forest Soil in Benue Cement Company, Benue State, Nigeria

Unanaonwi O E*, Amonum J I

*Department of Biology, Federal University Otuoke, Bayelsa State, Nigeria
Department of Forest and Forest Production, Federal University of Agriculture Makurdi, Nigeria

***Abstract*—** *Mining is essential in the economic development plan of any country endowed with mineral resources. This is due to both internal and external economic benefits that are made available to countries that are involved in the extraction of mineral resources. Internally, there is creation of employment and revenue generation among others while externally; a substantial foreign exchange is available to such countries. However, looking at the socio-economic importance of the industry, most countries lose sight of the ensuing effect that might accrue to an area as a result of mining activities. This study sought to provide an empirical data to ascertain whether or not mining activities has affected tree diversity of the area in general and on vegetation and soil nutrients in particular. In the study diversity indices (Shannon, margalef and Pielou's evenness) all indicated higher values for adjacent site 5 km away from the factory. Soil health indicators investigated revealed significant differences except Potassium, with adjacent site having higher mean values. This study has indicated that tree diversity was higher in the adjacent site and also that soil 5 km away from the factory was healthier than soil within factory site. Construction of shield over factory site is suggested.*

Keywords*— *Mining activities, Forest soil, nutrient status, Ecological indices, Soil health.

I. INTRODUCTION

Mining is any activity that involves excavating the earth surface for the purpose of exploiting its mineral wealth. This could be for local economic and industrial development or for export purposes (David, 2002). If properly coordinated, its positive socio-economic impact cannot be overemphasized as it provides natural resources for consumption, offers employment, as well as a source of revenue and foreign exchange. It also leads to the development of some socio-economic infrastructures like roads, schools, hospitals, among others (Hilson, 2002).

The industry has been, and in many cases remains important to the socio-economic development of many developed and industrialized countries such as Australia, Canada, Sweden, and the United States. Various cities and regions have built their wealth and industrial development at least in part, on mining. Historical examples include Monterrey in Mexico and Colombia among others (Akande and Idris, 2005; Singh, 2007).

In developing countries also, mining will continue to provide technological development and employment. Large-scale mineral exploitation has contributed over 90% of all foreign exchange earnings, 60% of Gross National Product, 50% of total government revenue and 30% of total employment in some Southern African Countries (Olaleye *et al*., 2010). Similarly, small scale-mineral exploitation provides a source of livelihood for those in rural and semi-urban Africa. The exploitation of mineral resources has assumed prime importance in several developing countries including Nigeria which is endowed with abundant mineral resources; this has contributed immensely to the socio-economic status of the country (Adekoya, 2003).

However, if mining activities are not properly organized, it can result to various environmental problems. The industry's operations ranging from prospecting to excavation are seen to be causing several environmental problems ranging from erosion, pollution, formation of sinkholes, soil nutrients loss, bio-diversity loss, heavy metal and organic contamination of groundwater and surface water (Kiranmay, 2005). He also asserted that mining causes massive damage to landscape and biological community as plant communities get disturbed and subsequently become impoverished thus presenting a very rigorous condition for its growth. Dumping of mine products will result into destruction of surrounding vegetation, and severe soil and water pollution.

Soil is a natural medium or only slightly disturbed materials that took centuries to develop under permanent forest cover.

http://dx.doi.org/10.22161/ijeab/2.1.39 *ISSN: 2456-1878*

A succession of genetic soil layer is present, ranging from the very important surface organic layer down to the mineral parent material (Ibanga *et al*., 2008). It is a mixture of minerals, organic matter, gases, liquids, and countless organisms that together support plant life. It is a medium for plant growth; a means of water storage, supply and purification, it is a habitat for organisms all of which modify the soil.

Soil Nutrients play a vital role in enhancing the growth of forest because plants require essential soil nutrients such as nitrogen, calcium, potassium, phosphorus, among others which are assimilated from the soil to complete their vegetative and reproductive life circles (Julio and Carlos, 1999). Other essential elements such as carbon, hydrogen and oxygen are readily available to plants because they are freely obtained from carbon dioxide and water and converted to carbohydrates during photosynthesis (Olaleye *et al*., 2010).

Vegetation, which refers to the plant cover of the earth, displays patterns that reflect a wide variety of environmental characteristics as well as temporal aspects operating on it (Kumi-Boateng and Issaka, 2012). This is due to the fact that it supports critical functions in the biosphere by regulating the flow of numerous bio-geochemical cycles like that of water, carbon, and nitrogen; it is also of great importance in local and global energy balance. Removal of vegetation cover strongly affects soil characteristics, including soil fertility, chemistry and texture (Adewoye, 2005; David and Mark 2005).

Although vegetation is of high environmental and biological importance, it is often under intense human pressure in mining areas especially where surface mining and illegal small scale mining activities are prevalent, resulting into changes in land-use/land-cover of mine areas. Directly or indirectly, mining has been seen to be a major factor responsible for vegetation loss in mining areas the world over (Adewoye, 2001; David and Mark 2005). Directly, it is caused by vegetation clearance for various mining activities and indirectly, with dust pollution as volume of dust is discharged into the air during the process of quarrying. This eventually gets deposited on the leaves of plants and flowers as well as the soil supporting the plants. The overall effect of this is that the photosynthetic and fruiting ability of the plants is impaired. When calcium, surphur-dioxide among other chemical constituents enter the plants through the stomata pores it leads to the destruction of chlorophyll and disruption of photosynthesis in plants subsequently leading to stunted growth or death (Ujoh and Alhassan, 2014).

Irrespective of the socio-economic importance of mineral resources, Aigbedion and Iyayi (2007) stated that the three stages of mineral development (i.e exploration, mining and processing), have caused different types of environmental damages, which include ecological disturbance, destruction of natural flora and fauna, soil nutrient loss, land degradation and water, among others. According to Olaleye (2010), the scale of operations involved in each stage of mineral development however determines the intensity and extent of soil degradation and vegetation loss. For example, recent environmental impact studies of limestone mining in Sagamu, Ogun State, Nigeria, has also revealed a decline in kola nut output from the plantations within a few kilometers radius of the mine (Adekoya, 2003; Tolulope, 2004; Aigbedion and Iyayi, 2007).

According to Aigbedion and Iyayi (2007) A similar situation exists in all the limestone and marble quarries in differing proportions at Ewekoro, Nkalagu, Ashaka, Kalambaina, Okpilla, and Jakura among others. On the discovery of limestone traces in Mbayion, Gboko Local Government Area, Benue State of Nigeria in 1960, a cement plant was established within the region which commenced operation in 1980. Subsequently, in 2004 with Dangote Industries Plc. as the new management of the company, an aggressive upgrading and rehabilitation of the plant was carried out. This has subsequently transformed the company into a new state-of-the-art cement factory with two 1.4 million tonnes lines (Vetiva Research, 2010). Due to increase in quarrying activities caused by the upgrade of the processing plant within the study area, the natural vegetation belt of the area which is characterized with the presence of tall grasses and tall trees is being threatened as it has to be cleared to give room for mining activities The consequences of vegetal deterioration within the study area are however enormous with various environmental and economic implications as agriculture is the main source of income for the people living within the study area. Against this backdrop, the assessment of the effect of mining within the area especially as it affects forest soil and vegetation becomes necessary.

II. MATERIALS AND METHODS

2.1 Study area

The study was conducted in Mbayion in Gboko Local Government Area of Benue State, which is located in the Northern part of the State. It is situated between latitudes 07° 08' and 07° 31' N of the equator and longitudes 08° 37' and 09°10' E of the Greenwich Meridian. It is made up of five (5) Districts namely: Mbatyiav, Mbayion, Mbatyerev,

Yandev and Ipav. Mbayion which is the study area, is situated between latitudes 7016' and 7028'N of the equator and longitudes 8048' and 9000'E of the Greenwich Meridian. It shares common boundaries with Takar Local Government in the North, Yandev in North-East, Ipav in South-East, Ushongo Local Government in the South, Mbatiav in South-West and Mbatierev District in the North-Western part of the Local Government as shown in Fig.1 and 2.

2.2 Soils and Vegetation

The predominant factors that have influenced the distribution of soils within the study area are relief and vegetation cover. Within the area, the predominant soil is tropical ferruginous soils; coarse loamy soils; laterite soils as well as sandy soils (Benue State Economic Empowerment and Development Strategy (BENSEED), 2004). According to BENSEED (2004), the presence of clay soils near streams and valleys, are however mixed with a reasonable amount of sandy soil and as such, most parts of the area is adequately drained and free from water. With respect to vegetation, the area belongs to the Guinea Savanna belt which is made up of a lot of grasses interspersed with trees. Trees grow side by side with tall grasses giving the area a luxuriant vegetation cover (Ibanga *et al*., 2008). This vegetation belt serves as a transitional belt between the tropical rain forest in the South and the open grassland in the North of Nigeria. Within this vegetation belt, several grain and root crops are produced in commercial quantities and the study area is known for the cultivation of crops like maize, guinea corn, millet, rice, yam, cassava among others. Tree crops like oranges, mangoes among others are also produced commercially in Gboko local government (Ministry of Information and Orientation, 2012).

2.3 Method of Data Collection

2.3.1 Soil and Vegetation composition Sampling procedure and Data Collection

To assesses vegetation composition, two areas were purposely selected:- vegetation within the cement factory and vegetation within the adjacent site 5km away. Plot of size 100m x 100m (1 hectare) were demarcated. This was further demarcated to sub-plots size of 20m x 20m from which 5 sample plots were randomly selected for the study. Tree species found within sample plots were recorded under the following parameters:- diameter at base (db), diameter at breast height (dbh), and total height.

For soil analysis, 6 plots of size measuring 10m x 10m were laid within the cement factory and the adjacent forest to collect soil samples. Four auger points per site were drilled at random to a depth of 0-45cm, using soil auger in four directions on a transect line, for site 1, site 2, site 3, site 4, for both the soil within and outside the factory. The soil samples from the four auger points per location were poured together and mixed thoroughly in a polythene bag. An appreciable quantity was poured into a polythene bag, labeled and taken to the laboratory for analysis.

2.4 Data Analysis

2.4.1 Data Analysis for Vegetation Cover

Floristic compositions in the two sites were estimated using diversity indices such as species richness, diversity and evenness. Species richness was computed using Margalef (1951) as cited by Spellerberg (1991) and Magurran (2004). It is measured by the formula:

$$D = \frac{(S-1)}{\ln N}$$

Where, **D** = species richness index (Margalef index), **S** = number of species and **N** = the total number of individuals.

Species diversity was estimated using Shannon- wiener diversity index as cited by Spellerberg (1991); Turyahabwe and Tweheyo (2010); Ruszazyk *et al*;(1992) cited by Radha *et* al; (2016).

Shannon- wiener diversity index equation is stated as:

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

Where H′ = species diversity index, **pi** = the proportion of individuals or the abundance of the i^{th} species expressed as a proportion of the total abundance. The use of natural logs is usual because this gives information in binary digits.

Species evenness was estimated using Pielou's evenness (equitability) index (Pielou, 1975) cited by Turyahabwe and Tweheyo (2010) as followed:

$$J' = \frac{H'(observed)}{H_{\max}}$$

J′ = Pielou's evenness index. Where **H′** (observed) / $\mathbf{H_{max}}$, where $\mathbf{H_{max}}$ is the maximum possible diversity, which would be achieved if all species were equally abundant (=**Log S**)

The indices were computed for all plant species in various growth forms (trees, sapling, shrubs and herbs) in each plot of vegetation location.

http://dx.doi.org/10.22161/ijeab/2.1.39 *ISSN: 2456-1878*

2.4.2 Data analysis for soil sample

Soil P^H was determined using p^H Meter, Available phosphorus was determined using the method of Liu. (Liu, 2000).Total nitrogen was determined using Micro kjeldahl Method, Potassium determined using Flame photometry and organic carbon determined using wet oxidation Method (Black and Walkley, 1934). Paired test was used to analyzed soil variables such as Soil P^H , soil organic carbon and organic matter, soil Nitrogen, soil Phosphorus and Potassium. The level of significance in each case was set at $P< 0.05$.

III. RESULTS AND DISCUSSION

In this study, a total of 27 tree species representing 13 families were encountered. Out of this number 12 tree species were recorded in the factory site and 15 tree species were recorded in the adjacent site as presented in Table 1. A total of 53 individuals were recorded in the study with 23 encountered in the factory site and 30 encountered in adjacent site. Our result presented in table 2 also shows that adjacent site recorded the higher species richness (D= 4.248) while factory site recorded a lower value (D= 3.683). Species richness is the number of species in an area, and in this study, the adjacent plot 5 kilometers away from the factory was more diverse. This could be as a result of cement dust inhibiting certain plant species from thriving.

Shannon diversity index was higher in off-site ($H^1 = 2.464$) while factory site recorded ($H^1 = 2.415$). Shannon Diversity index has been reported to fall between 1.5 and 3.5, and that it rarely surpasses 4.5, where a value near 4.6 indicates that numbers of individuals are distributed evenly (Radha *et al*., 2016). Species evenness value indicates that factory site recorded the higher value (0.9325) while the adjacent site recorded low (0.7834). However, since species evenness ranges from one to zero, where 1 indicates complete evenness and 0 no evenness, the two sites from our result, shows no evenness in species composition.

Overall tree population was observed to be more in the adjacent plot in comparison with plots within the cement factory. Dust deposit on leaves of plants is cable of blocking the stomata pore thereby hindering transpiration resulting to decrease in biomass production. It may lead to reduction in growth for certain species that could not survive. Leaves and bark injuries caused by dust deposit have been reported by Farmer *et al*.,(1991), which has led to alteration of tree community composition and structure. Natural regeneration becomes difficult for many forest trees due to hard crust formed on the forest floor by dust deposit. Sometimes the crust is so heavy and hard that young plants are smothered. Table 1 show that trees in diameter classes 61 and above were lacking in the adjacent plot, while they were visible within these class ranges in the factory site. Trees within these dbh classes are mature and useable by the local population. These trees are extracted by individuals without restriction unlike the ones within the factory which are protected.

Table 3 shows the mean values of soil nutrients indicators between soil within the cement factory and from adjacent plot 5km away from the cement factory. Soil within the cement factory recorded mean values of 6.21 for soil P^H , 0.70 for Organic Carbon, 1.22 for Organic Matter content, 0.10 for Nitrogen, 3.38 for Phosphorus, 3.23 for Calcium, Magnesium 1.58, Potassium 0.26 and Sodium 0.67. Mean values for same soil parameters from the adjacent plot, 5km away from factory recorded 6.45 for P^H , 0.79 for Organic Carbon, 1.39 for Organic matter, Nitrogen 0.16, Phosphorus 3.92, Calcium 3.98, Magnesium 1.83, Potassium 0.29 and Sodium 0.80.

T-test shows that all soil parameters investigated in the two locations were significantly different from each other except soil Phosphorus.

3.1 Soil health and determinants

The concerns on the sustainability of forestry and agricultural systems have increased recently because the world population is ever increasing and so is the demand for food. To feed seven billion people while sustaining the environment is a big challenge for the present generation. Sustainable forestry and agriculture aims at meeting the needs of the present without compromising the productive potential for the next generations. Sustainable yields can only be reached with the maintenance or recovery of the soil health. Thus, a healthy soil has been defined as "The continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health " (Doran and Safley, 1997).

To assess the sustainability of a production system, changes in chemical, physical, and biological properties, and the effects on the soil's capacity to support plant growth and exert environmental functions, must be monitored (Doran and Safley, 1997).

Soil health definition cannot be generalized for all kinds of soil and soil-use as criticized by Sojka and Upchurch (1999). Thus, indicators of soil health must be selected according to soil use and management, soil characteristics and environmental circumstances. Parameters investigated in this study were chemical properties of soil in the two locations it is important to discuss the general and most used chemical indicators of soil health. Chemical attributes of soil health are correlated with the

capacity to provide nutrients for plants and/or retaining chemical elements or compounds harmful to the environment and plant growth. Soil pH, cation exchange capacity (CEC), and organic matter and nutrient levels are the main chemical attributes used in soil health assessment, especially when considering the soil capacity for supporting high yield crops (Kelly *et al*., 2009), although our study did not examine soil CEC.

Chemical attributes have been correlated with plant yields and thus the variations of a particular indicator are easily interpreted, and allow a quick improvement of the soil chemical properties by liming and/or fertilization. These soil chemical indicators can also be useful in considering the soil's capacity for sustaining forest production and sustainability, maintaining nutrient cycling, plant biomass and organic matter (Schoenholtz *et al*., 2000). Idowu *et al*. (2008) concluded that the most important chemical parameters to be assessed were pH, available N, P, K, Mn.

Soil organic carbon is also a key attribute in assessing soil health, generally correlating positively with crop yield (Bennett *et al*., 2010). The soil organic carbon affects important functional processes in soil like the storage of nutrients, mainly N, water holding capacity, and stability of aggregates (Silva and Sá-Mendonça, 2007). In addition, the soil organic carbon also affects microbial activity. Hence, a key component of soil fertility, especially in tropical conditions, which interacts with chemical, physical, and biological soil properties and must be considered in assessments of soil health.

Nitrogen is the most required plant nutrient, which is found in several chemical forms in soil (Cantarella, 2007), resulting in a very dynamic behavior. Soil nitrogen has been assessed mainly as mineral N, especially nitrate, organic N or potentially mineralizable N, as stored in the soil organic matter. Despite the importance in plant nutrition and environment, the use of nitrogen as parameter for assessing soil health is subjected to factors that affect its dynamics in soil, like climatic conditions, turning inadequate the diagnosis of the real availability for plants, based on soil chemical analysis (Cantarella, 2007).

Phosphorus (P) is also a key nutrient for agricultural yields and is essential in assessments of soil health. Along with nitrogen, P is the main nutrient that limits the agricultural yields, especially in highly weathered, oxidic soils, where the major part of the total soil P is fixed in clay minerals and oxides. The available P in the soil solution is present as orthophosphates, but the microbial P and organic-P are also stocks that can rapidly become available. Procedures for assessment of P availability have been well established (Pankhurst *et al.*, 2003; Zhang *et al*., 2006a).

Soil chemical parameters have been traditionally used for assessment of potentially available nutrients for crops, and are based on worldwide well established analytical methodologies. Among them, organic matter, pH, and available nutrients and also some potential hazardous chemicals have been used to establish levels of soil health.

IV. CONCLUSION

In this study biodiversity indices and some chemical soil health indicators were employed to investigate the effects of mining activities on soil nutrient status and vegetation composition within the cement factory and 5 km away from the factory. The study revealed that the adjacent site which was 5 km away from the factory has a higher level of biodiversity than the factory site indicating low plant population and low number of species. All the diversity indices used in the study indicated higher values for the adjacent site. The study further proved that all the Soil health indicators investigated were significantly different from the two sites with the adjacent plot recording higher mean values, except Potassium. Our results suggest that soil chemical properties and vegetation composition and structure are being impaired by mining activities. This may have negative effects on vegetation and soils within close range to the factory, thereby effecting rural livelihood.

REFERENCES

[1] Acheampong, E., Impact Assessment of Mining Activities by Ashanti Goldfields, Bibiani Limited on the Environment and Socio-Economic Development of Bibiani, *Undergraduate Dissertation, Faculty of Social Sciences, Kwame Nkrumah University of Science and Technology*, 2004.

[2] Adekoya, J. A, Environmental Effect of Solid Minerals Mining. *Journal of Physical Science*, Kenya, Pp. 625–640, 2003.

[3] Adewoye, O. O, Assessment of Beneficiation Routes of Tantalite Ores from key locations in Nigeria, *Journal of Mineral Material Characterization and Engineering*, 4: 67 –76, 2005.

[4] Aigbedion, I. N, and Iyayi, S. E, Environmental Effect of Mineral Exploitation in Nigeria. *International Journal of Physical Sciences*, Vol. 2 (2), Pp. 033-038, 2007.

[5] Akande, J.M, and Idris, M.A, Environmental Effects of Gemstone Exploration in Ofiki,Oyo State, Nigeria. *Journal of Science Engineering of Technology (JOSET)*, Vol. 12(1), Pp. 5858 – 5869, 2005.

[6] Bennett, L.T., Mele, P.M., Annett, S., Kasel, S, Examining links between soil management, soil health, and public benefits in agricultural landscapes: an Australian perspective. *Agriculture, Ecosystems and Environment* 139, 1-12,2010.

[7] Benue State Economic Empowerment and Development Strategy (BENSEED), Second draft Strategy Report. http://www.ng.undp.org/documents/SEEDS/Benue_State.pdf., 2004. Accessed 12th February 2010.

[8] Bray-Kurtz,Available Phosphorus- Bray No.1 Method. http://vlab.amrita.edu/?sub=2&brch=294&sim=1550&cnt=1==bray

[9] Cantarella, H, Nitrogenio = Nitrogen. p. 375-470. In: Novais, R.F., Alvarez, V., Barros, N.F., Fontes, R.L.F., Cantarutti, R.B., Neves, J.C.L., eds. Fertilidade do solo = Soil fertility.Sociedade Brasileira de Ciencia do Solo, Vicosa, MG, Brazil. (in Portuguese),2007.

[10] David, L. and Mark, B, *Practical Conservation Biology*. Australia: CSIRO publishing, Pp. 25-30, 2005.

[11] David, K. K, A Report of the Civil Society Review of the Implementation of Agenda 21 in Kenya, Kenya NGO Earth Summit 2002 Forum, *Journal for Natural Resources Minerals*, 2002.

[12] Doran, J.W., Safley, M, Defining and assessing soil health and sustainable productivity. p. 1-28. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R., eds. Biological indicators of soil health. CAB International, Wallingford, UK, 1997.

[13] Farmer AM, Bates J W abd Beal J N, effects of dust on vegetation and its consequences for nature conservation in Great Britain. CSD Note 57, Nature Conservancy Council, Peterborough, 1991.

[14] Hilson, G, An Overview of Land-use Conflicts in Mining Communities. *Land-use Policy*, 19(1): 16 – 17, 2002.

[15] Ibanga, I. J., Umoh, N. B., and Iren, O. B, Effects of Cement Dust on soil Chemical Properties in the Calabar Environment, South Eastern Nigeria. *Communication in Soil Science and Plant Analysis, 39*(3-4),2008.

[16] Idowu, O.J., van Es, H.M., Abawi, G.S., Wolfe, D.W., Ball, J.I., Gugino, B.K., Moebius, B.N., Schindelbeck, R.R., Bilgili, A.V, Farmer-oriented assessment of soil quality using field, laboratory, and VNIR spectroscopy methods. *Plant Soil* 307, 243-253, 2008.

[17] Julio, H. and Carlos, B, Estimating rates of nutrient depletion in soils of agricultural lands of Africa. *Tech. Bulletin-T.48.* Alabama: U.S.A,1999.

[18] Kelly, B., Allan, C., Wilson, B.P, Soil indicators and their use by farmers in the Billabong Catchment, southern New South Wales. *Australian Journal of Soil Research* 47, 234-242, 2009.

[19] Kiranmay, S., Impact of Coal Mining on Vegetation*: a Case Study in Jaintial Hills District of Meghalaya, India.* Unpublished ,*International Institute for Geo-information Science and Earth Observation (ITC). Enschede, the Netherlands and Indian Institute for Remote Sensing, National Remote Sensing Agency (NRSA) Department of Space, Dehradun*, 2005.

[20] Kumi-Boateng, B., Boye, C.B., and Issaka, Y. (2012). *Land Use/Land Cover Mapping Using Remote Sensing for Urban Development-A Case Study of Tarkwa and Its Environ.* Proceedings of ASPRS 2012 Conference on Opportunities for Emerging Geo-Spatial Technologies, San Diego, California, U.S.A, 26-30 April, Pp. 1-112, 2012.

[21] Liu, F.,Handbook of Environmental Monitoring for Agriculture. China Standard Press, Beinjing, pp.699, 2001.

[22] Magurran, A.E, Measuring Biodiversity, University of Ulster, Times Higher Education Supplement, 2004.

[23] [22]Margalef,D.R,InformationTheoryEcology,1951. http://www.vliz.be/imisdocs/publications/139371.pdf Retrived 28th Decamber, 2016

[24] Olaleye, V. F. and Oluyemi, E. A, Effects of Cement Flue Dust from a Nigerian Cement Plant on Air, Water and Plankton Quality. *Journal of Environmental Monitoring Assessment*, 162: 153-162, 2010.

[25] Pankhurst, C.E., Magarey, R.C., Stirling, G.R., Blair, B.L., Bell, M.J., Garside, A.L., Management practices to improve soil health and reduce the effects of detrimental soil biota associated with yield decline of sugarcane in Queensland, Australia. *Soil and Tillage Research* 72, 125-137, 2003.

[26] Pielou, E.C, Naturalist's Guide to the Arctic. University of Chicago Press Books. Pp344, 1995.

[27] Radha R, Susheela P, Subhashini K, Comparative syudies on butterfly diversity and species richness in selected agricultural fields in Tamil Nadu. Inter journal of Environment, Agriculture and Biotechnology vol-1 ssue-4. 2016.

[28] Schoenholtz, S.H., Vam, Miegroet, H., Burger, J.A, A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities. *Forest Ecology and Management* 138, 335-356, 2000.

[29] Silva, I.R., Sa Mendonca, E. (2007). Materia organica do solo = Soil organic matter. p. 275-374. In: Novais, R.F., Alvarez, V.H., Barros, N.F., Fontes, R.L.F., Cantarutti, R.B., Neves, J.C., eds. Fertilidade do solo = Soil fertility. Sociedade Brasileira de Ciencia do Solo, Viosa, MG, Brazil (in Portuguese), 2007.

[30] Singh, A., Re-vegetation of Coal-mine Spoils Using Prosopis Juliflora in Singrauli Coalfield is a Harmful Practice from an Ecological Viewpoint., *Journal of Current Science*, 93: 1204, 2007.
[31] Sojka, R.E., Upchurch, D.R, Reservations regarding the soil quality concept. *Soil Science Society of America Journal* 63, 1039-1054, 1999.
[32] Spellerberg, N, Monitoring Ecological change, Cambridge University press, pp334, 1991.
[33] Tolulope, A. O, Oil Exploration and Environmental Degradation: the Nigerian Experience, *Environmental Informatics Archives*, 2:387-393, 2004.
[34] Turyahabwe, N, and Tweheyo M.,Does foresnntenure affect land characteristics? *International Forestry Review* vol. 12(4), 2000.
[35] Ujoh, F. 1. and Alhassan, M.M, Assessment of Pollutants in Streams around a Cement Plant in Central Nigeria. *International Journal of Science and Technology. Volume 4:3,* 2014.
[36] Vetiva Research, Scheme of Merger Document, DCP Analyst Presentation, Vetiva Capital Management Limited. Initiation of Coverage, Dangote Cement PLC: The Emergence of a Titan, 2010.
[37] Zhang, P., Li, L., Pan, G., Ren, J., Soil quality changes in land degradation as indicated by soil chemical, biochemical and microbiological properties in a karst area of southwest Guizhou, China. *Environmental Geology* 51, 609-619, 2006.
[38] Wakley, A, and Black I. A, Measuring Organic carbon, 1934. Retrieved 29th December, 2016 . http://bcodata.whoi.edu/LaurentianGreatLakes_Chemistry/bs116.pdf--black 1934.

Table.1:. Species composition in dbh classes within factory site(A) and adjacent site (B)

DBH Classes	site A	site B
>20	0	0
21-40	11	22
41-60	6	11
61-80	2	0
81>	1	0
Number of Species	12	15

Table.2:result of Species Diversity within site A and site B

Variables	Site A	Site B
Number of species	12	15
Number of individuals	23	30
Shannon H	2.415	2.464
Evenness	0.9325	0.7834
Margalef	3.683	4.248

Table.3: Result of means and T-test values of soil nutrients indicators within factory and adjacent site

Variables	Mean values site A	Mean values site B	SE	Prob. at 5% level	Paired T-test
P^H	6.21	6.45	-	0.001	15.24*
OC	0.70	0.79	0.01772	0.007	5.192*
OM	1.22	1.39	0.01855	0.001	-9.274*
N	0.10	0.16	0.003614	0.016	-4.040*
P	3.38	3.92	0.18055	0.040	-2.991*
Ca	3.23	3.98	0.10851	0.002	-6.986*
Mg	1.58	1.83	0.02939	0.001	8.437*
K	0.26	0.29	0.00490	0.005	-5.715^{ns}
Na	0.67	0.80	0.0235	0.003	6.487*

http://dx.doi.org/10.22161/ijeab/2.1.39 *ISSN: 2456-1878*

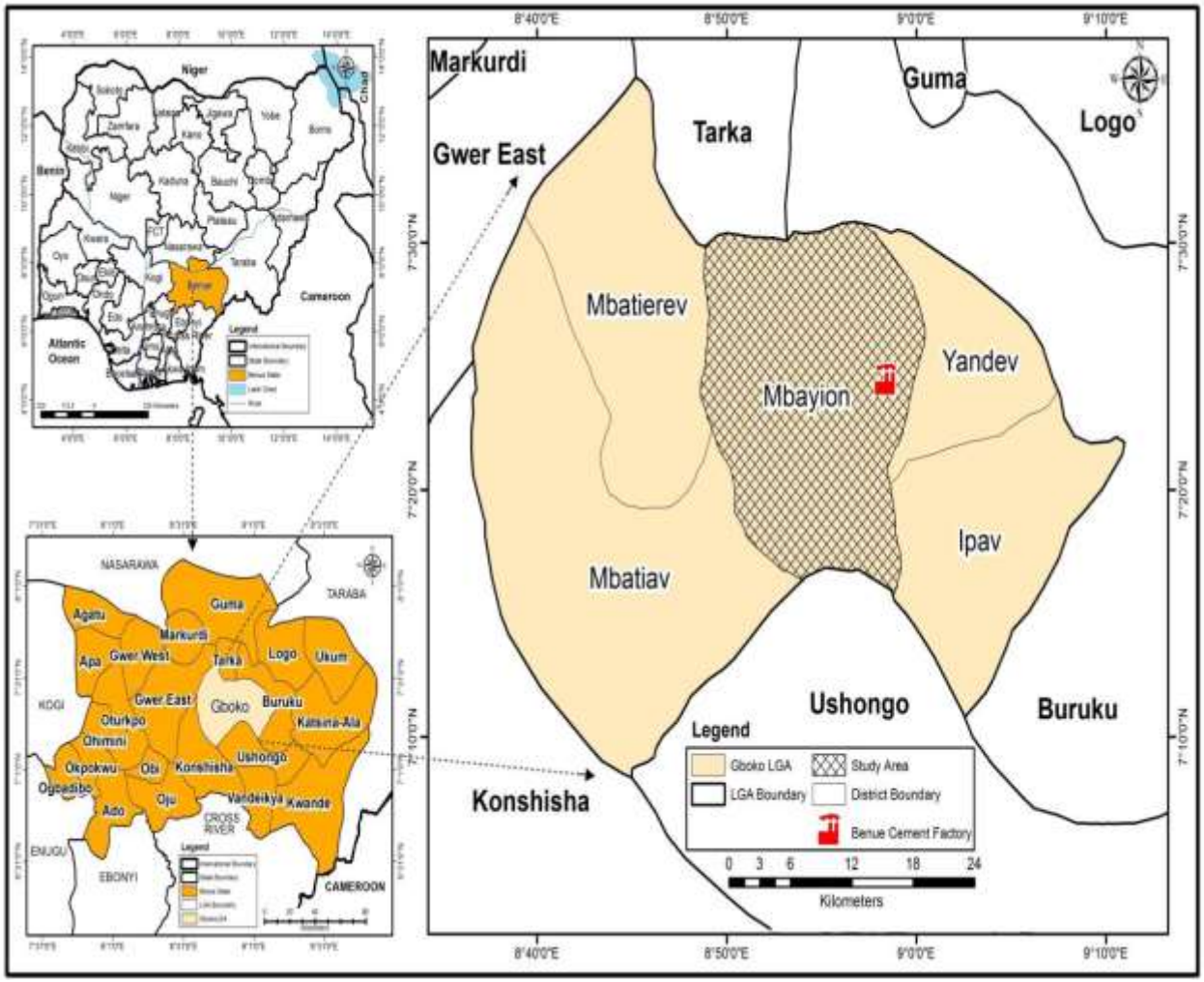


Fig.1: Gboko LGA Showing Study Area

Source: Modified from the Administrative Map of Gboko LGA, 2014.

http://dx.doi.org/10.22161/ijeab/2.1.39 *ISSN: 2456-1878*

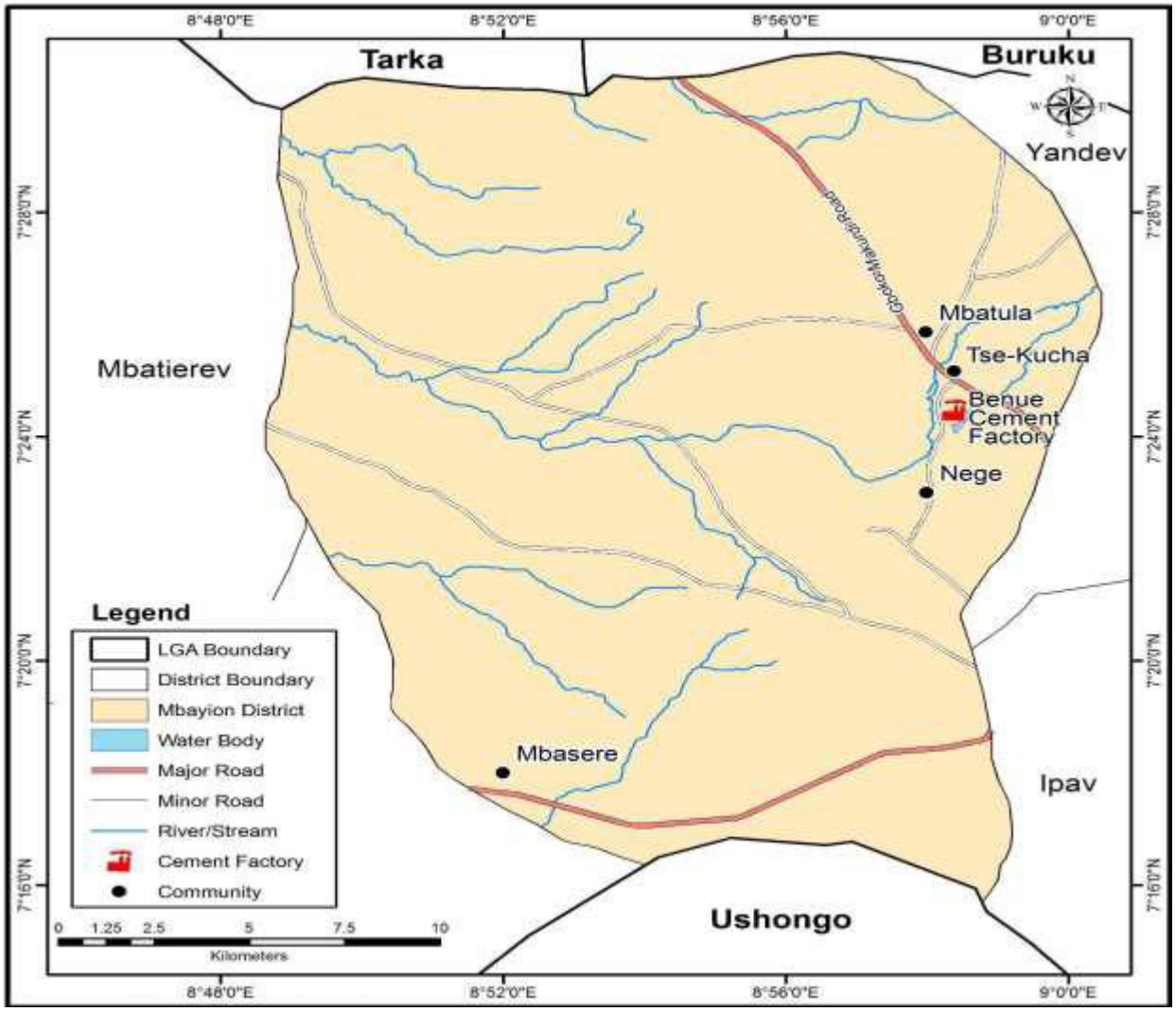


Fig.2: Mbayion District (Study Area)

Source: Modified from the Administrative Map of Gboko LGA/Google Maps, 2014.

http://dx.doi.org/10.22161/ijeab/2.1.40 *ISSN: 2456-1878*

Lime Pretreatment Associated Compositional and Ultrastructural Changes in Selected Root and Vegetable Processing Residues

Mithra. M. G, Padmaja. G

Division of Crop Utilization, ICAR- Central Tuber Crops Research Institute, Kerala, India

Abstract— *The study aimed at exploring the suitability of processing residues from selected root and vegetables for bioethanol production, which are otherwise environmental pollutants. The effect of lime pretreatment at high (HT), low (LT) or room (RT) temperatures on compositional and ultrastructural changes in peels of root crops (sweet potato, elephant foot yam and tannia) and vegetable processing residues (peels from ash gourd and mixed vegetable waste) was studied. Pretreatment resulted in the removal of very little polysaccharides, including starch from these biomasses. Hemicellulose was removed to a higher extent in 24 h RT pretreatment (11.6-12.3%) compared to 7.3-8.5% removal in HT pretreatment. Maximum lignin removal (ca. 33-38%) occurred in RT pretreated (24 h) samples. Approximately 22-25.7% lignin was removed during HT pretreatment (121 °C) for 30 min. which increased to 28-31% when prolonged to 60 min. Pretreatment Efficiency (PE) was low (4.2-14.7%) in HT pretreatment, while 5.7-13.5% and 5.2-14.2% PE was observed in LT and RT pretreatments respectively. Scanning electron micrographs of lime pretreated biomass indicated that starch being a major ingredient of the biomass under study, preferential saccharification of starch by amylases might be necessary to expose the cellulose and hemicellulose for their subsequent saccharification to release fermentable sugars.*

***Keywords—Composition, Lime pretreatment, Processing residues, Root crops, Ultrasructure, Vegetable crops*.**

I. INTRODUCTION

There is an ever-increasing global concern over the rapid depletion of fossil fuel resources, enhanced demand for transportation fuel in developed and developing countries and the environmental challenges caused by the emission of greenhouse gases (GHGs) resulting from the burning of coal and fuel, which is implicated as the main factor for global warming [1, 2]. Bioethanol from renewable resources is recognized as the best transportation fuel which could help reduce dependency on fossil fuels [3]. Despite the cost-effectiveness of corn and sugar based ethanol, the ethical conflicts on the diversion of food to fuel have necessitated the search for potentially cheap and inedible feedstock for bioethanol production [4-6]. Owing to the low cost and abundant availability, lignocellulosic biomass (LCB) has been widely recognized as the most viable and sustainable feedstock for biofuel production. It is reported that bioethanol from cellulosic and other biomass resources has the potential to reduce GHG emission by 86% [7]. Nevertheless, the sustainability of second generation (2G) ethanol produced from LCBs, despite its potential to replace oil-based fuels depends on the economically feasible production, by overcoming the technological barriers such as recalcitrance to degradation, enzyme costs for effective conversion to sugars, high pretreatment costs and its associated problems *viz.*, formation of inhibitors, cost of chemicals for neutralization etc. [8-10].

Although lignocellulosic materials generally comprise agricultural residues, woody biomasses and dedicated crops such as switchgrass, Bermuda grass etc., there is also a major global contribution from the processing residues due to the increased industrial activities. While as high as 90% of the LCBs are constituted by cellulose, hemicellulose and lignin, processing residues contain starch also as a main component [2, 11, 12], indicating the need for different approaches in their handling for ethanol production. The three main steps in the conversion of LCBs to ethanol are pretreatment, saccharification to monomeric sugars and fermentation. The aim of pretreatment is to detach lignin and hemicellulose from the cellulose, reduce the crystallinity and increase the porosity of cellulose, thereby enhancing its accessibility to cellulases [13, 14]. An efficient pretreatment method should reduce the formation of fermentation inhibitors, preserve the potential sugar yielding carbohydrates in the residue, improve the release of sugars prior to and during enzymatic saccharification and minimize energy requirement [14, 15-18]. Dilute acids and alkali have been used for pretreatment by several researchers on a wide variety of LCBs and extensive reviews have appeared on such techniques and their comparative advantages/disadvantages [3, 6, 14, 19-21]. Major

disadvantages of acid treatment include the need for corrosion-resistant reactors, less efficiency of lignin removal and formation of inhibitors such as furfural, 5-hydroxymethyl furfural and acetic acid [4, 22, 23]. Hence, lime (calcium hydroxide) pretreatment has been attempted for several lignocellulosic feedstocks [11, 24-27]. Lime pretreatment has regained interest as a promising pretreatment technique because it is a cheap chemical that could be safely handled, needs only low temperatures and pressures and could be recovered easily. Besides, lime also facilitates the removal of lignin and acetyl groups and reduces the chances of formation of fermentation inhibitors [11, 28, 29]. The divalent calcium ions in calcium hydroxide are reported to effectively crosslink with lignin, thereby preventing its non-productive binding with cellulase [30, 31].

Sweet potato (*Ipomoea batatas* Lam) is the second most important root crop with a world production of 103.11 million tonnes [32] and China is the leading producer accounting for almost 80% of the global production. During the processing of sweet potato for starch or flour preparation, approximately 5-6% goes as waste peel and is reported to contain 79% carbohydrate [33]. Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) is a most popular root crop grown and consumed in South Asian countries such as India, Malaysia, Indonesia and the Philippines [34] and during processing, considerable loss (*ca.* 15%) of peel occurs due to the non-uniform surface morphology of the roots. Tannia (*Xanthosoma sagittifolium* (L.) is a tropical root crop grown widely in West Africa, tropical America and Asia [35]. Processing of cormels leads to the generation of peels (10-13%) as refuse and consist of the thin skin along with the outer cortex of the roots [35, 36] and except for compositional studies, its value addition has not been reported. Ash gourd (*Benincasa hispida* Cogn.) is cultivated as a vegetable in India, Japan, China and Australia [37]. Approximately 25% goes as peel waste during commercial processing for sweet manufacture in India, causing major disposal problems [38]. It is estimated that 73-96% of the typical family's waste comprises of biodegradable materials in lower income groups and 26% in the higher groups in India [39]. Out of the biodegradable wastes generated, a major part is accounted by kitchen/domestic waste, while hotels also contribute significantly to this fraction of solid waste. With a view to exploring the potential of these processing wastes (which are also rich in starch besides cellulose and hemicellulose) for bioethanol production, the effect of lime pretreatment at high, low and room temperatures on compositional and ultrastructural alterations in three root crop processing wastes (peels from sweet potato, elephant foot yam and tannia) and two vegetable wastes such as ash gourd peel and mixed vegetable wastes (comprising the inedible parts such as peels, seeds and pulp part covering them and damaged parts of common vegetables collected from the households and restaurants) was investigated. As different from the typical LCBs, these wastes also contain appreciable amounts of starch, which comes along with the peel during the peeling operation, enabling them to be categorized as lignocellulo-starch biomass (LCSB). Nevertheless, their ultrastructural and compositional differences as well as the alterations brought about by pretreatments have hitherto not been reported. Hence this study aims at a detailed understanding of the changes brought about during lime pretreatment on the polysaccharide and lignin components so that the best treatment could be identified for further saccharification studies.

II. MATERIALS AND METHODS

2.1 Samples

Peels collected from sweet potato, elephant foot yam, tannia and ash gourd by manual peeling were washed in running tap water to remove the adhering dirt and sand, immediately drained and dried in the sun for 24-36 h, followed by drying in an air oven to reduce the moisture content to <10%. It was then powdered in a hammer mill (particle size: *ca.* 2-3 mm) and packed in air tight containers till use. In order to utilize the whole waste residues for bioethanol production, the unscreened biomass was used for the various experiments. Besides, mixed vegetable wastes were collected from households and restaurants and these were also dried, powdered and stored for further studies.

2.2. Enzyme Source

Spezyme® Xtra (α-amylase) and Stargen™ 002 (Granular starch hydrolyzing enzyme) were supplied by M/s Genencor International Inc. USA (presently Danisco US Inc., USA). Spezyme contained a thermostable α-amylase (E.C. 3.2.1.1) with an activity of 14,000 α-amylase units (AAU)/g (1.0 AAU = amount of enzyme required to hydrolyze 10.0 mg starch/min under the assay conditions) [40]. Stargen™ 002 contains *Aspergillus kawachi* α-amylase (E.C. 3.2.1.1) expressed in *Trichoderma reesei* and a gluco-amylase (E.C. 3.2.1.3) from *Trichoderma reesei* that work synergistically to hydrolyze granular starch substrate to glucose. It has an activity of 570 Glucoamylase units (GAU)/g and one GAU is the amount of enzyme that will liberate one gram of glucose per hour from soluble starch substrate under the conditions of the assay [41].

2.3. Pretreatments

Three types of lime pretreatments were attempted in this study such as (i) treatment with lime (calcium hydroxide; 0.1 g/g biomass) at high temperature (121 °C) and

pressure of 0.102 MPa for 30 min. and 60 min. (HT pretreatment) (ii) treatment at low temperature (50 °C) for 6 h and 24 h (LT pretreatment) and (iii) treatment at room temperature (30 ±1 °C) for 24 h and 48 h (RT pretreatment). In the first experiment, the unscreened biomass residues (10 g) were suspended in 100 ml lime solution (10% w/v) in a 250 ml Erlenmeyer flask and exposed to heat in a Pressure Cooker (M/s TTK Prestige India Ltd.) for 30 min. and 60 min. (as separate lots and time after the pressure build up) at 121 °C and pressure 0.102 MPa. The flasks after pH adjustment to 6.0 with Conc. Hydrochloric acid (HCl), were cooled, volume made up to the nearest and filtered. Part of the residue (2.0 g each) at each time period was lyophilized (Thermo-Savant Freeze Drying Chamber FDC-206) for ultrastructural studies using the scanning electron microscope. The remaining residue was dried in an air oven at 50 °C for 20 h followed by high temperature drying at 100 °C for 1 h and stored after cooling to room temperature for further studies.

In the second experiment, one set of biomass slurry was incubated at 50 °C in a thermostatic water bath (Julabo SW22) for 6 h, while the second set was incubated for 24 h. In the third experiment, one set of biomass slurry was incubated at room temperature (30 ±1 °C) for 24 h, while the second set was incubated for 48 h. After the incubation, the pH was adjusted to 6.0 using concentrated HCl and volume raised to the nearest. The filtrates and residues were stored as in the first experiment, for further studies.

2.4. Compositional Studies

The pretreated residues were subjected to compositional analysis comprising starch, total and reducing sugars, cellulose, hemicellulose, ash and lignin by standard procedures. Detailed compositional analyses of the native biomasses selected were reported earlier [42]. In the present study, only the composition of the pretreated biomass has been undertaken as per the methods described under:

2.4.1. Starch

Starch being a major component of the biomass residues under study, the total starch content in the pretreated biomasses was determined using the hydrolytic enzymes such as Spezyme and Stargen as per the procedure standardized earlier [43]. Biomass slurry (0.5g/20 ml) was digested with Spezyme (0.5 ml equivalent to approximately 7000 α-amylase units) for 30 min. at pH 5.5 and 90 °C after which the temperature and pH were brought to 40 °C and 4.5 respectively and digested for 24 h with Stargen (0.5 ml or 285 Glucoamylase units). The reducing sugars released were assayed by the titrimetric method of Moorthy and Padmaja [44]. Enzyme and substrate blanks were also kept to nullify the reducing sugars originally present in the enzyme and biomass samples respectively. Starch content was calculated from the reducing sugar values using the Morris factor, 0.9.

2.4.2. NDF and ADF

The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the method of Goering and Vansoest [45] with slight modifications to take care of the interference from starch. Native/pretreated residue (0.5 g) was mixed with 0.5 g sodium sulphite and 50 ml cold neutral detergent solution and after boiling the pH was adjusted to 5.5 and Spezyme (0.5 ml) added and boiling continued for 1 h. After incubation for 1 h, the pH and temperature were brought down to 4.5 and 40 °C respectively and incubated with 0.5 ml Stargen for 24 h. The contents after filtration through Whatman no.1 filter paper (Grade 1; 11 µm pore size) and washing with acetone were dried in an air oven at 100 °C for 8 h. The dry weight of residue (W1) was used to calculate NDF as:

$$\text{NDF (\%)} = \frac{\text{W1 x 100}}{\text{Sample weight}} \quad (1)$$

ADF was determined from the NDF fraction by treating 0.5 g of it with 50 ml acid detergent solution (20 g cetyl trimethyl ammonium bromide in 1 l of 1 N sulfuric acid) and heating for 1 h after the onset of boiling. The contents after filtration were washed and dried at 100 °C overnight. ADF in the NDF fraction was calculated from the residue weight (W2) using the formula and worked back to express as percentage of the original biomass:

$$\text{ADF (\%) in NDF} = \frac{\text{W2 x 100}}{\text{Sample weight of NDF}} \quad (2)$$

2.4.3. Structural Carbohydrates and Lignin

Hemicellulose content in the pretreated residue was determined as the difference of Neutral detergent fibre (NDF) and acid detergent fibre (ADF). Cellulose content in the ADF fraction from the pretreated residue was determined using acetic-nitric reagent by the method of Updegroff [46] with slight modification to avoid interference from starch by using the ADF fraction from the pretreated residue, which was found to give highly reliable results. Ten milliliters of acetic/nitric reagent (10:1 mix of 80 % acetic acid and concentrated nitric acid) were added to 0.5 g ADF in a long test tube which was then boiled for 30 min. at 100 °C in a boiling water bath. The slurry after dilution with de-ionized water was filtered through Whatman no. 1 filter paper and the filtrate was discarded. Residue after washing with distilled water was hydrolysed with 67% sulfuric acid (10 ml) at room temperature for 1 h. The sugars released were

estimated using anthrone reagent and cellulose content in the pretreated biomass calculated using pure cellulose standard was worked back to the original biomass based on the weight of the dry solids remaining after pretreatment. The ash content was determined in the ADF fraction from treated residue by the standard procedure [47], by keeping in a muffle furnace at 550 °C for 6 h. In order to eliminate the error due to the held up proteins in the lignin fraction, the crude protein content in the ADF fraction (from each pretreated residue) was determined by the Kjeldahl method [47] and subtracted from the ADF values to get the true ADF content. The lignin content of the pretreated biomass was calculated as:

Lignin (%) = True ADF (%) - [(cellulose + ash) %] (3)

2.4.4. Characterization of Pretreated liquor

The reducing sugar content in the filtrate was quantified by the same titrimetric method, while the reducing sugars held back in the residues were computed from the substrate blank values from starch estimation.

2.5 Pretreatment Efficiency

The total reducing sugar content (pretreated liquor + residue) after nullifying the original reducing sugar (RS) content in the native biomass was used to compute the Pretreatment Efficiency on the basis of the potential sugar yielding carbohydrates (cellulose, hemicellulose, starch and total sugars) as:

$$PE\ (\%) = \frac{[(RSpt + RSr) - Rsob] \times 100}{[C+HC+S+TS \text{ in original biomass (\% dwb)}} \quad (4)$$

Where RSpt = RS released from the biomass due to pretreatment (expressed as % of the original biomass); RSr = RS held back in the residue (expressed as % of the original biomass); RSob = RS (%) originally present in the biomass; C: cellulose; HC: hemicellulose; S: starch and TS: total sugars; (C+HC+ S+TS represent the total potential sugar yielding carbohydrate fraction).

2.6 Ultrastructural Studies

The ultrastructure of native as well as pretreated biomass was studied on HITACHI Scanning Electron Microscope S-2400). Dry powder (native) and lyophilized powder (pretreated) were applied on the double side carbon pasted on an aluminium stub. A thin gold-platinum coating was applied for 3 min. using E-1010 Ion Sputter Unit under 10 Pa vacuum and discharge current of 10 mA. The SEM photographs were visualized at 500x magnification.

2.7 Statistical Analysis

The various biochemical constituents were expressed as percentage of the original biomass based on the water insoluble residue weight obtained from each pretreatment. Three replicates were kept for each experiment and duplicate analyses were performed on each replicate. Statistical analysis was performed by Analysis of Variance (ANOVA) for statistical testing of the mean values and was followed by least significant difference (LSD) for pair-wise comparison of mean values by using the statistical package, SAS 9.3 [48].

III. RESULTS AND DISCUSSION

The effect of pretreatment of unscreened powders of selected root and vegetable processing wastes as well as mixed vegetable wastes with lime at high temperature (121 °C; 0.102 MPa) for 30 min and 60 min, low temperature (50 °C; 6 h and 24 h) and room temperature (30 ± 1 °C; 24 h and 48 h) on the compositional and ultra structural changes were studied.

3.1 Compositional Changes due to Pretreatment

3.1.1. Polysaccharides and Lignin

The changes in cellulose, hemicellulose and starch during pretreatment of peels of sweet potato (SP), elephant foot yam (EFY), tannia and ash gourd as well as mixed vegetable waste (MVW) is given in **TABLE** 1. Very small quantities of polysaccharides were removed during pretreatment. Except in SP peel and MVW, there was insignificant change in cellulose from the native, in all the three types of treatments and time periods. Cellulose removal ranged from 6.28-9.09% in the biomass residues in 24 h RT pretreatment while in the HT pretreatment (60 min.), there was only negligible removal (1.0-2.45 %). Hemicellulose was also removed to a higher extent in 24 h RT pretreatment (11.6-12.3%) compared to 7.3-8.5% removal in HT pretreatment (Fig. 1a and b). Maximum lignin reduction occurred in the RT (24 h) pretreated samples, followed by LT (24 h) treatment for most biomasses, which was insignificant with the HT pretreatment at 121 °C for 60 min. (**TABLE** 1). Chang et al. [49] reported lime loading of 0.1 g Ca $(OH)_2$ /g dry biomass for bagasse and wheat straw as optimum where no glucan or xylan removal occurred. Based on enzyme digestibility of pretreated LCBs, lime pretreatment conditions were optimized by different researchers as 120 °C for 1 h for bagasse [49], 100-120 °C for 2 h for switchgrass [50] and 120 °C for 4 h for corn stover [24]. Kim and Holtzapple [51] observed that after 16 weeks of lime (0.5g/g dry biomass) pretreatment of corn stover at 55 °C, only 6.3% glucan was solubilized, while 21% xylan was solubilized.

http://dx.doi.org/10.22161/ijeab/2.1.40 *ISSN: 2456-1878*

Table 1: Polysaccharide and lignin changes in lime pretreated root and vegetable processing residues (expressed as g/100 g original material on dry basis).

Parameters	Original Biomass [42]	Lime pretreatment					
		High temperature (121 °C)		Low temperature (50 °C)		Room temperature (30 ±1 °C)	
		30 min.	60 min.	6 h	24 h	24 h	48 h
(a) Sweet potato peel							
Cellulose (C)	13.31^{a}	12.31^{b}	13.17^{ab}	12.53^{ab}	12.87^{ab}	12.10^{b}	12.59^{ab}
Hemicellulose (HC)	13.32^{a}	11.96^{b}	12.32^{b}	12.17^{b}	12.06^{b}	11.72^{b}	12.13^{b}
Starch(S)	32.05^{a}	31.86^{a}	31.11^{b}	31.96^{b}	30.71^{c}	30.61^{c}	31.84^{a}
Lignin (L)	8.15^{a}	6.37^{b}	5.62^{c}	6.46^{c}	5.40^{c}	5.29^{c}	5.43^{c}
(b) Elephant foot yam peel							
C	15.63^{a}	14.61^{a}	15.47^{a}	14.68^{a}	15.00^{a}	14.53^{a}	14.65^{a}
HC	14.00^{a}	12.70^{bc}	12.98^{b}	12.90^{bc}	12.59^{cd}	12.34^{d}	12.81^{bc}
S	28.96^{a}	28.71^{a}	28.21^{b}	28.71^{a}	27.73^{c}	27.51^{c}	28.67^{a}
L	7.01^{a}	5.28^{bc}	4.92^{cd}	5.73^{b}	4.60^{de}	4.37^{e}	4.66^{de}
(c) Tannia peel							
C	17.32^{a}	16.19^{a}	17.11^{a}	16.21^{a}	16.69^{a}	16.12^{a}	16.25^{a}
HC	14.48^{a}	12.97^{bc}	13.25^{bc}	13.61^{b}	12.98^{bc}	12.71^{c}	13.73^{b}
S	30.46^{a}	30.12^{b}	29.48^{c}	30.22^{ab}	29.19^{d}	29.11^{d}	30.10^{b}
L	8. 26^{a}	6.25^{c}	5.71^{d}	6.72^{b}	5.53^{d}	5.46^{d}	5.69^{d}
(d) Ash gourd peel							
C	18.67^{a}	17.55^{a}	18.21^{a}	17.60^{a}	18.12^{a}	17.49^{a}	17.63^{a}
HC	18.30^{a}	16.36 ^{b}	16.87 ^{b}	16.93 ^{b}	16.67^{b}	16.17^{b}	16.78^{b}
S	19.91^{a}	19.71^{a}	19.25^{b}	19.89 ^{a}	19.30^{b}	19.20^{b}	19.77^{a}
L	10.70^{a}	7.95^{bc}	7.55^{cd}	8.46^{b}	7.09^{d}	7.06^{d}	7.11^{d}
(e) Mixed vegetable waste							
C	11.71^{a}	11.03^{c}	11.59^{ab}	11.07^{bc}	11.30^{abc}	10.91^{c}	11.66^{a}
HC	11.97^{a}	10.70^{b}	11.00^{b}	10.99^{b}	10.78^{b}	10.50^{b}	10.92^{b}
S	28.10^{a}	27.88^{a}	27.22^{b}	27.97^{a}	27.01^{b}	26.96^{b}	27.90^{a}
L	7.55^{a}	5.80^{b}	5.41^{bc}	5.85^{b}	5.01^{c}	4.99^{c}	5.22^{bc}

*Each value is mean from three replicates; statistical comparison for each parameter for each biomass was made with the respective native untreated samples; means with different alphabets in each row are significantly different at $p < 0.05$.

Saha and Cotta [12] reported that lime (0.1g/g biomass) pretreatment of rice hulls at 121 ºC for 1 h yielded more sugars during enzymatic saccharification than lower loading rate of lime and exposure periods. Most of the starch remained unhydrolyzed in the lime pretreated biomass (**TABLE** 1). The percentage hydrolysis ranged from 3.6% to 5.0% in the RT (24 h) pretreated biomasses, while it was 2.6% to 3.3% in the HT pretreated biomasses (Fig. 1 a and b). Dilute sulfuric acid (DSA) pretreatment was earlier found to hydrolyze 85-94% of starch in these biomasses exposing the cellulose fibers for saccharification [42]. Nevertheless, lime pretreatment at 121 °C retained most of the starch along with cellulose, while 11% of the hemicellulose got solubilized. Saha and Bothast [52] reported that no glucose was released from starch during hot water pretreatment of corn fiber at 121°C for 1 h. Starch changes during pretreatment of biomasses have not hitherto been reported, as most of the LCBs do not contain starch. Lime pretreatment resulted in the retention of high percentage of solid biomass (**TABLE** 2). Except in the case of sweet potato peel and MVW, there were no significant differences in solids recovery in the various treatments. There are several reports on the high biomass recovery after lime pretreatment of sugarcane bagasse [25, 49, 52]. It was found that delignification was not influenced by high temperature, as it was non-significant for RT and LT for 24 h and HT for 60 min. for most residues. Lignin removal ranged from 34-37.6% at RT (24 h) and on prolonging the time to 48 h, there were only insignificant changes in lignin. Approximately 22-25.7% lignin was removed from the various residues during HT pretreatment (121 °C) for 30 min. and 29-31% lignin

http://dx.doi.org/10.22161/ijeab/2.1.40 *ISSN: 2456-1878*

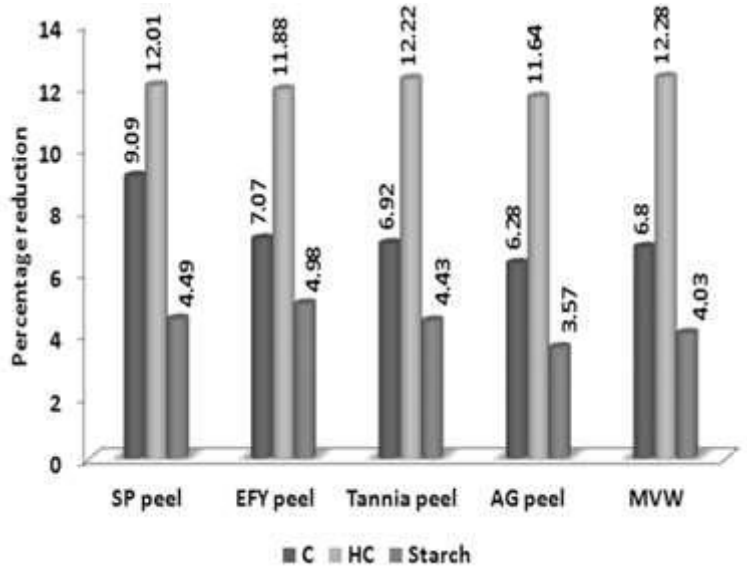


Fig. 1a: Percentage removal of C, HC and starch from biomass due to RT pretreatment with lime (24 h).

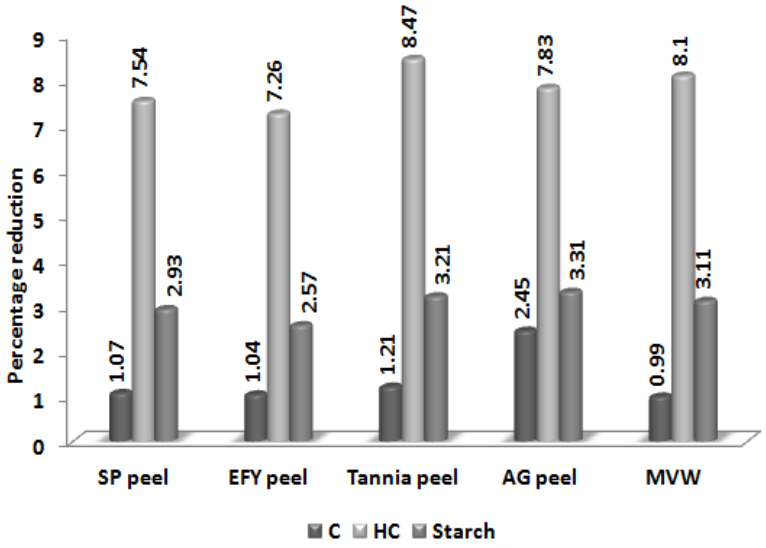


Fig.1b: Percentage removal of C, HC and starch from biomass due to HT pretreatment with lime (60 min.).

Table.2: Percentage solids remaining in lime pretreated root and vegetable processing residues.*

Biomass residue	**Percentage solids remaining after lime treatment**					
	HT (121 °C)		**LT (50 °C)**		**RT (30 ±1 °C)**	
	30 min	**60 min**	**6 h**	**24 h**	**24 h**	**48 h**
Sweet potato peel	92.50^{ab}	91.35^{ab}	94.25^{ab}	90.10^{ab}	88.13^{b}	95.79^{a}
Elephant foot yam peel	95.85^{a}	93.60^{a}	96.08^{a}	92.48^{a}	91.55^{a}	97.50^{a}
Tannia peel	90.90^{a}	90.00^{a}	92.25^{a}	89.00^{a}	87.40^{a}	93.50^{a}
Ash gourd peel	94.80^{a}	91.43^{a}	95.73^{a}	92.23^{a}	91.03^{a}	97.04^{a}
Mixed vegetable waste	95.00^{bc}	94.73^{dc}	95.75^{b}	94.01^{d}	91.00^{e}	98.64^{a}

*Each value is mean from three replicates; means with different alphabets in each row are significantly different at $p < 0.05$.

removal occurred from different biomasses by extending the time to 60 min. (Fig. 2a-e). Lignin removal was much less (18.3-22.5%) when biomass residues were pretreated with lime at 50 °C for 6 h. Nevertheless, on prolonging the reaction time to 24 h, 33-34% removal was observed. Although high temperature is reported to remove more lignin from biomass, the lower extent of removal in the present study might have resulted from the lower exposure time at HT compared to 24 or 48 h at RT.

Kim and Holtzapple [51] found that lignin and hemicellulose were selectively removed and cellulose crystallinity increased with delignification of lime pretreated corn stover. There are several reports that the divalent calcium ions of lime have high affinity for lignin and could effectively crosslink lignin [30, 31]. Lime is also reported to remove acetyl groups and lignin-carbohydrate ester linkages, thereby enhancing cellulose digestibility [14]. Xu et al. [26] reported that although calcium ions cross linked lignin under alkaline conditions, lignin complex remained in the residue without getting solubilised and hence the lignin content in the pretreated residue was high. They had also found that only 16-35% reduction in lignin occurred in lime pretreated switchgrass which corroborated with our results. Under alkaline conditions, lignin molecules become negatively charged due to the ionization of carboxyl, methoxy and hydroxyl groups, which then have a high affinity for calcium [3].

3.2. Reducing sugars and Pretreatment Efficiency

Reducing sugars in the pretreated liquor from lime pretreated residues indicated that there was only small increase from the original value in all the three pretreatments, which resulted primarily from the hemicellulose hydrolysis, followed by the mild starch hydrolysis leading to exposure of reducing groups (**TABLES** 3 and 1). In the case of the various biomasses, maximum increase was noticed in RT (24 h) pretreatment followed by LT (24 h).

Accordingly, the Pretreatment Efficiency (PE) computed based on the potential sugar yielding carbohydrates was also low for the various treatments (**TABLE** 4). Approximately 4.2-14.7% PE was observed in the HT pretreatment, while 4.6-13.5% and 5.2-14.2% PE were observed in LT and RT pretreatments respectively. Among the biomasses, the lowest PE was observed for EFY peel, which might be due to the structural variations among the biomasses. Prolonging the reaction time for all the treatments resulted in significant decrease in PE for RT and HT pretreatments, possibly as a consequence of conformational changes in starch whereby some of the exposed reducing groups were reverted. This is also supported by the low RS values in the pretreated liquor from RT (48 h) and HT (60 min.) for most biomasses. Kim and Holtzapple [51] reported that delignification and deacetylation could remove the barriers to enzymatic hydrolysis and even though the crystallinity of biomass was increased slightly on delignification, it had less effect on the ultimate sugar yields. Wang et al. [7] reported much lower solid loss in lime pretreatment of coastal Bermuda grass than NaOH pretreatment. They also found that reducing sugar release during enzymatic saccharification of lime (0.1g/g biomass) pretreated (room temperature) Bermuda grass was less at 48 h, compared to 34 h and also lower at 24 h compared to 6 h at 50 °C. Based on the delignification, slightly higher starch hydrolysis and energy expenditure considerations, RT pretreatment with lime (24 h) and HT pretreatment (60 min.) could be considered as the best pretreatments. Even though energy expenditure is more on the HT pretreatment for 60 min., starch gelatinization occurring at 121 °C might be advantageous for effective saccharification in the next stage. Nevertheless, saccharification studies presently underway could only confirm the relative advantage of lime pretreatment techniques over others such as dilute sulfuric acid and steam pretreatment reported earlier for these residues [42].

Removal of the pretreated liquor by filtration before saccharification might be more difficult due to starch gelatinization. However, since the biomasses under study have a high percentage of starch, treatment of pretreated slurry as a whole might be advantageous compared to the treatment of water insoluble solids.

http://dx.doi.org/10.22161/ijeab/2.1.40 *ISSN: 2456-1878*

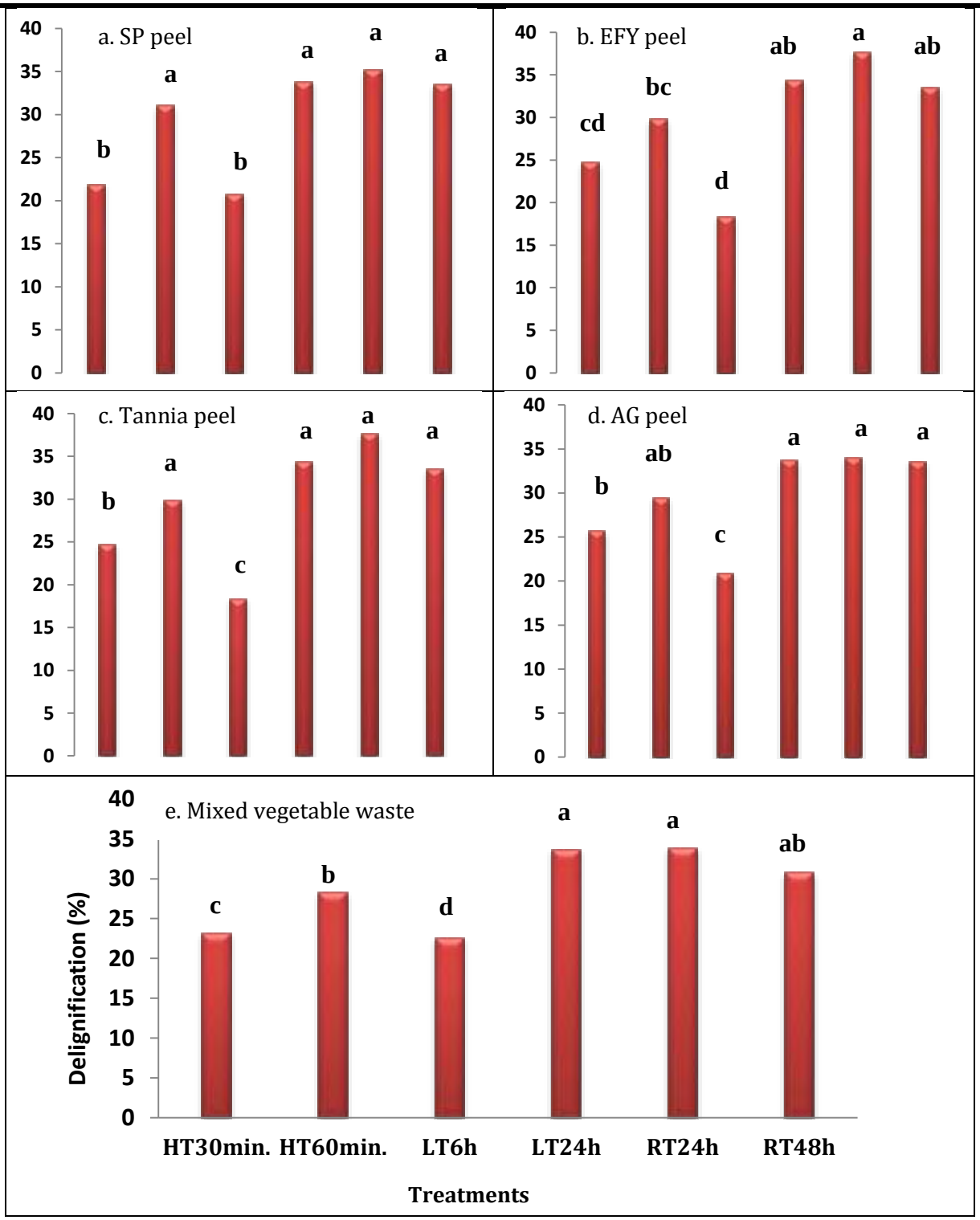


Fig. 2a-e. Delignification in processing residues subjected to lime pretreatment. Statistical comparison was between treatments and bars with different alphabets on the top are significant at $p < 0.05$.

Table 3: Reducing sugar content (g/L) in the pretreated liquor from lime pretreated residues.*

Type of lime pretreatment and time	Sweet potato peel	Elephant foot yam peel	Tannia peel	Ash gourd peel	Mixed vegetable waste
(a) Native biomass without pretreatment [42]					
Initial	6.22[f]	2.58[f]	1.34[f]	5.19[f]	7.50[f]

(b) HT pretreatment (121° C and 0.102 MPa)					
30 min	8.73^{c}	5.18^{c}	1.67^{e}	8.52^{b}	9.52^{c}
60 min	9.20^{b}	4.44^{f}	2.32^{c}	7.72^{e}	9.20^{e}
(c) LT pretreatment (50 ° C)					
6 h	8.19^{e}	4.78^{e}	1.56^{f}	7.65^{f}	9.27^{d}
24 h	9.20^{b}	5.74^{b}	2.60^{b}	7.97^{c}	10.05^{b}
(d) RT pretreatment (30 ±1 °C)					
24 h	9.97^{a}	6.69^{a}	2.70^{a}	9.13^{a}	10.87^{a}
48 h	8.25^{d}	4.98 ^{d}	1.70^{d}	7.82^{d}	8.93^{f}

*Statistical comparison was made for each parameter with the respective values in the original (native) biomass for each sample; means with different alphabets in each column are significant at $p < 0.05$.

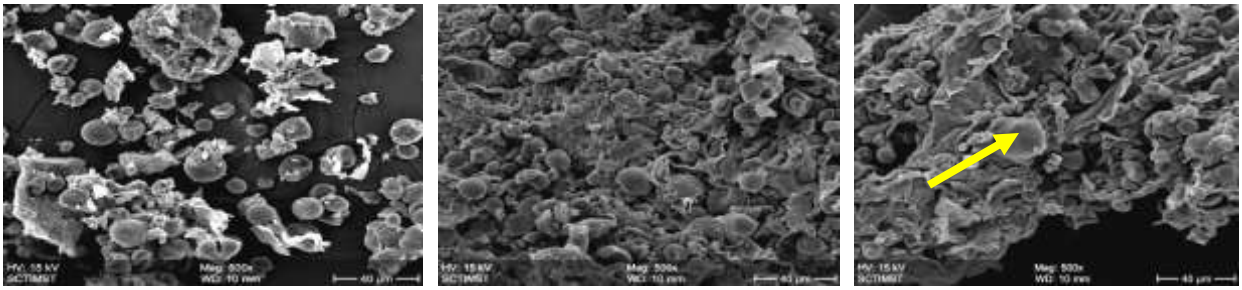
Table 4: Pretreatment Efficiency (%) in sugar release from lime pretreated biomass.*

Type of lime pretreatment and time	**Sweet potato peel**	**Elephant foot yam peel**	**Tannia peel**	**Ash gourd peel**	**Mixed vegetable waste**
(a) HT pretreatment (121 °C and 0.102 MPa)					
30 min.	12.51^{b}	5.21^{b}	6.17^{f}	14.73^{a}	13.53^{b}
60 min.	12.36^{c}	4.22^{d}	7.70^{c}	12.18^{e}	12.17^{d}
(b) LT pretreatment (50 °C)					
6 h	11.18^{e}	4.60^{c}	6.68^{e}	12.53^{d}	11.62^{e}
24 h	11.61^{d}	5.73^{b}	8.65^{a}	11.84^{f}	13.46^{c}
(c) RT pretreatment (30 ± 1 °C)					
24 h	12.67^{a}	7.94^{a}	8.55^{b}	13.72^{b}	14.19^{a}
48 h	11.12^{f}	5.17^{b}	7.22^{d}	12.59^{c}	11.49^{f}

* Computed as given in Methods (Equation 4) based on the potential sugar yielding carbohydrates; means with different alphabets in each column are significant at $p < 0.05$.

3.3 Ultrastructure of Pretreated Biomass

Scanning electron microscopy (x500) was done to understand the ultrastructural changes brought about in the biomass due to lime pretreatment. In the case of peel residues from the three root crops, large number of intact and deformed starch granules was visible (Fig. 3). Starch damage occurring during the grinding and milling operations might have led to alteration in the morphology of starch granules [42]. Broken cell structures were also evident, indicating the absence of rigid fibers in the native biomasses under study, as different from the typical LCBs. Rigid fibrous pattern was earlier reported for cassava leaf and stem powders from our laboratory, while such structures were absent in the peel samples which were dominated by starch [53]. Native ash gourd peel presented a surface morphology with open holes and broken fibers. Such holes normally found on removal of hemicellulose and lignin during pretreatment indicated the possibility of native enzymes which might be acting during the drying time (24 h). Nevertheless, the compositional profile indicated the presence of 18.3% hemicellulose and 10.7 % lignin in ash gourd peel powder with slightly lower starch content (19.9%) than the other residues (28-32%). Mixed vegetable waste also had open pores, with many pores being plugged in by starch granules (Fig. 3m).



http://dx.doi.org/10.22161/ijeab/2.1.40 *ISSN: 2456-1878*

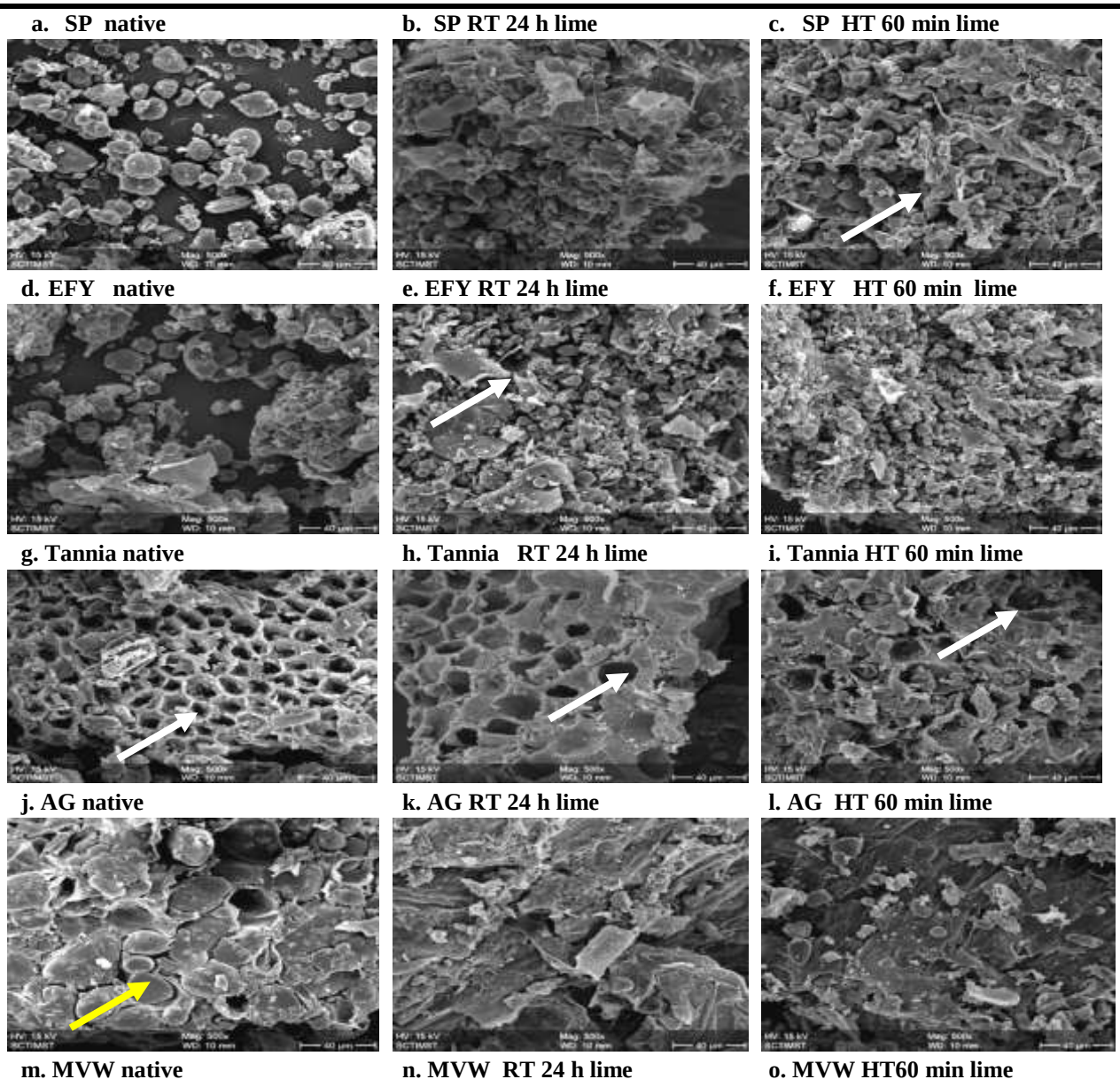


Fig. 3 (a-o): SEM photographs of lime pretreated (RT for 24 h and HT for 60 min.) biomass samples (x500); white arrows indicate the deformed cell pores; yellow arrows indicate the plugging of holes by starch

Lime pretreatment at room temperature resulted in greater distribution of intact and broken starch granules on the surface in the case of the root crop peels (Fig. 3 b, e and h). Apertures resulting from the removal of hemicellulose and lignin as reported in the case of lime (0.5g/g biomass at 55 °C) pretreatment of poplar [29] or for 2.5% potassium hydroxide (KOH) treated sugarcane bagasse [54] or 2.0% KOH pretreated corn cobs [55], were not visible in root crop residues subjected to RT lime pretreatment, probably because of the masking of the pores by the enormous starch granules. Broken fiber particles were evident especially in EFY and tannia peels (Fig. 3 e and h). Ash gourd peel which had several well defined holes in the native biomass, changed to a surface morphology having stretched holes with larger diameter. Besides, some of the pores were sealed by filmy material, which might be partially solubilized hemicellulose/starch. Ash gourd peel was reported to contain *ca*. 8.5% ash, a major part of which was contributed by the chalky wax on the peels. Ghosh and Baghel [56] reported that the wax coating contained pentacyclic triterpene, isomultiferol acetate etc. as major components. Besides a number of methyl pyrazines have been reported from the extracts of the whole fruit (with peel) [56]. The interaction of such compounds with lignin or carbohydrates during lime pretreatment is not understood. The starch plugged cavities seen in the native MVW disappeared on lime pretreatment at room temperature. Largely fragmented or broken fibers were seen with a few starch granules (Fig. 3 m and n). Deacetylation during lime treatment might have

facilitated the deconstruction of cellulose changing to amorphous form, without much change in the absolute content of cellulose. Gelatinized and swollen starch granules were seen in the HT pretreated (60 min.) biomass samples (Fig. 3 c, f, i, l and o). As the gelatinized starch was spread over the surface, broken fiber structures were not very clear, especially in the SEM of root crop peels. In the case of ash gourd peel, open pores were all deformed with coating of gelatinized starch over some of the holes (Fig. 3 l). Swollen starch along with fiber particles were seen in HT pretreated MVW (Fig. 3 o). Scanning electron micrographs of lime pretreated biomass indicated that starch being a major ingredient of the biomass under study, preferential saccharification of starch by amylases might be necessary to expose the cellulose and hemicellulose for their subsequent hydrolysis by cellulases.

IV. CONCLUSION

The present study dealing with a novel approach on the understanding of lime pretreatment effect on starch containing lignocellulosic biomass hitherto not known, showed that RT and HT pretreatments of the biomasses gave high biomass yield coupled with high delignification (34-38% and 29-31% respectively) when compared to the other treatments. Considering the low energy expenditure, slightly higher starch hydrolysis and high lignin removal, these pretreatments are considered the best for the biomasses in the present study. Two clear indications from the compositional and ultrastructural studies were (i) preferential hydrolysis of starch during enzymatic saccharification shall be advantageous, as it exposes the cellulose and hemicellulose for further enzymatic cleavage and (ii) starch swelling in RT pretreatment and gelatinization in HT pretreatment being major changes, whole slurry saccharification would be necessary to get high fermentable sugar yield.

ACKNOWLEDGEMENTS

The authors are thankful to the Kerala State Council for Science, Technology & Environment (KSCSTE) for the financial support and to the Director, ICAR- CTCRI for the facilities provided for the study. The help extended by Dr. J. Sreekumar, Principal Scientist (Agricultural Statistics) for the statistical analyses are acknowledged. The enzymes, Spezyme and Stargen were received by courtesy from M/s Danisco US Inc., USA. The support extended for the SEM analysis by the Sree Chithra Tirunal Institute of Medical Sciences & Technology (SCTIMST), Kerala is gratefully acknowledged.

REFERENCES

[1] Fuglestvedt, J, Berntsen, T, Myhre, G, Rypdal, K and Skeie, R. B. 2008. “Climate forcing from the transport sectors”. Proceedings of the National Academy of Science, U.S.A, 105, 454-458.

[2] Yat, S. C, Berger, A and Shonnard, D. R. 2008. “Kinetic characterization of dilute surfuric acid hydrolysis of timber varieties and switchgrass”. Bioresource Technology, 99, 3855-3863.

[3] Sarkar, N, Ghosh, S. K, Bannerjee, S and Aikat, K. 2012. “Bioethanol production from agricultural wastes: An overview”. Renewable Energy, 37, 19-27.

[4] Sun, Y and Cheng, J. 2002. “Hydrolysis of lignocellulosic materials for ethanol production: a review”. Bioresource Technology, 83, 1–11.

[5] Taherzadeh, M. J and Karimi, K. 2008. “Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review”. International Journal of Molecular Science, 9, 1621-1651.

[6] Limayem A and Ricke S.C. 2012. “Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects”. Progress in Energy and Combustion Science, 38: 449-467.

[7] Wang, W, Keshwani, D. R, Redding, A. P and Cheng, J. J. 2008. “Alkaline pretreatment of coastal bermudagrass for bioethanol production”. ASABE Paper No. 08, St. Joseph, Mich, USA.

[8] Chandel, A.K, Rudravaram, R, Narasu, M.L, Rao, V and Ravindra, P. 2007. “Economics and environmental impacts of bioethanol production technologies: an appraisal”. Biotechnology and Molecular Biology Revew, 2(1), 14-32.

[9] Yang, B and Wyman, C. E. 2008. “Pretreatment: The key to unlocking low cost cellulosic ethanol”. Biofuels, Bioproducts and Biorefining, 2, 26-40.

[10] Alvira, P, Tomas-Pejo E, Ballesteros, M and Negro, M. J. 2010. “Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review”. Bioresource Technology, 101, 4851-4861.

[11] Wyman, C. E. 1999. “Biomass ethanol: Technical progress, opportunities and commercial challenges”. Annual Review of Energy and the Environment, 24, 189-226.

[12] Saha, B. C and Bothast, R. J. 1999. “Pretreatment and enzymatic saccharification of corn fiber”. Applied Biochemistry and Biotechnology”, 76, 65-77.

[13] MCMillan, J. D. “Pretreatment of lignocellulosic biomass”. in Enzymatic conversion of biomass for

fuels production. M. E Himmel, J. O Baker and R. P. Overend, Eds. Washington DC: Amer. Chem. Soc, 1994, pp. 292-324.

[14] Mosier, M, Wyman, C, Dale, B, Elander, R, Lee, Y. Y, Holtzapple, M and Ladisch, M. 2005. "Features of promising technologies for pretreatment of lignocellulosic biomass". Bioresource Technology, 96, 673-686.

[15] Eggeman, T and Elander R. T. 2005. "Process and economic analysis of pretreatment technologies". Bioresource Technology, 96, 2019-2025.

[16] Wyman, C. E, Dale, B. E, Elander, R. T, Holtzapple, M, Ladisch, M. R and Lee, Y. Y. 2005. "Coordinated development of leading pretreatment technologies". Bioresource Technology, 96, 1959-1966.

[17] Hu, G, Heitmann, J. A and Rojas, O, J. 2008. "Feedstock pretreatment strategies for producing ethanol from wood, bark and forest residues". BioResources, 3, 270-294.

[18] Zhu, J. Y, Pan, X and Zalesny Jr, R. S. 2010. "Pretreatment of woody biomass for biofuel production: energy efficiency, technologies and recalcitrance". Applied Microbiology and Biotechnology, 87, 847-857.

[19] Hendriks, A.T.W.M and Zeeman, G. 2009. "Pretreatments to enhance the digestibility of lignocellulosic biomass". Bioresource Technology, 100, 10–18.

[20] Zhang, B and Shahbazi, A. 2011. "Recent developments in pretreatment technologies for production of lignocellulosic biofuels". Journal of Petroleulm and Environmental Biotechnology, 2, 108-115.

[21] Maurya, D. P, Singla, A and Negi, S. 2015. "An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol". Biotechnology, Doi. 10. 1007/813205-015-0279-4.

[22] Anwar, Z, Gulfraz, M, Asad, M. J. et al. 2012. "Bioethanol production from rice polish by optimization of dilute acid pretreatment and enzymatic hydrolysis". African Journal of Biotechnology, 11, 992-998.

[23] Conde-Mejia, C, Jimenez-Guterreza, A and EL-Halwagi, M. 2012. "A comparison of pretreatment methods for bioethanol production from lignocellulosic materials". Safety and Environment Protection 90: 189-202

[24] Kaar, W. E and Holtzapple, M. T. 2000. "Using lime pretreatment to facilitate the enzymic hydrolysis of corn stover". Biomass and Bioenergy, 18, 189-199.

[25] Rabelo, S. C, Filho, R. M, and Costa, A. C. 2008. "A comparison between lime and alkaline hydrogen peroxide pretreatments of sugarcane bagasse for ethanol production". Applied Biochemistry and Biotechnology, 148, 45-48.

[26] Xu, J, Chang, J. J, Shivappa, R. R. S and Burns, J. C. 2010. "Lime pretreatment of switchgrass at mild temperatures for ethanol production". Bioresource Technology, 101, 2900-2903.

[27] Fuentes, L. L. G, Rabelo, S. C, Filho, R. M and Costa, A. C. 2011. "Kinetics of lime pretreatment of sugarcane bagasse to enhance enzymatic hydrolysis". Applied Biochemistry and Biotechnology, 163, 612-625.

[28] Kumar, P, Barrett, D. M, Delwiche, M and Stroeve, P. 2009. "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production". Industrial Engineering Chemistry Research, 48, 3713-3729.

[29] Kumar, R, Mago, G, Balan, V and Wyman, C. E. 2009. "Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies". Bioresource Technology, 100, 3948-3962.

[30] Sundin, J and Hartler, N. 2000. "Precipitation of kraft lignin by metal cations in alkaline solutions". Nordic Pulp Paper Research, 15, 306-312.

[31] Torre, M, Rodriguez, A. R and Saura-Calixto, F. 1992. "Study of the interactions of calcium ions with lignin, cellulose and pectin". Journal of Agricultural and Food Chemistry, 40, 1762-1766.

[32] FAOSTAT. 2014. "Sweet potato production statistics". http://faostat.fao.org/ accessed on 12 January, 2015.

[33] Adeyosoye, O. I, Adesokan, I. A, Afolabi K. D and Ekeocha, A. H. 2010. "Estimation of proximate composition and biogas production from *in vitro* gas fermentation of sweet potato (*Ipomoea batatas*) and wild cocoyam (*Colocasia esculenta*) peels". African Journal of Environmental Science and Technology, 4, 388-391.

[34] Arva, H. R, Bhaskar, J. J, Salimath, P. V and Mallikarjuna. A. S. 2013. "Anti-diabetic effect of Elephant foot yam [*Amorphophallus paeoniifolius* (Dennst.) Nicolson] in streptozotocin-induced diabetic rats". International Journal of Biomedical and Pharmaceutical Sciences, 7, http://www.researchgate.net/publication/230757446

[35] Duru, C. C and Uma, N. U. 2002. "Post harvest spoilage of cormels of *Xanthosoma sagittifolium* (L) Schott". Bioscience Research Communications, 14, 277–283.

[36] Yahaya, I. A, Nok, A. J and Bonire, J. J. 2013. "Chemical studies on the peel of *Xanthosoma sagittifolium* (Tannia Cocoyam)". Pakistan Journal of Nutrition, 12, 40-44.

[37] The Wealth of India. "Raw Materials", vol. 6. New Delhi: CSIR, 1962, pp. 446-459.

[38] Sreenivas, K. M, Chaudhari, K and Lele. S. S. 2011. "Ash gourd peel wax: Extraction, characterization, and application as an edible coat for fruits". Food Science and Biotechnology, 20(2), 383-387.

[39] Upadhyay, V. P, Rajeswar Prasad, M, Srivastav, A and Singh, K. 2005. "Ecotools for urban waste management in India". Journal of Human Ecology, 18, 253-269.

[40] Anon. 2011. "Spezyme®Xtra-High performance α-amylase for starch hydrolysis". Product Information published by Genencor International, a Division of Danisco, Danisco US Inc, accessed on 17 May, 2010.

[41] Anon. 2014. "STARGEN™ 002: Granular Starch Hydrolyzing Enzyme for Ethanol Production", Product Information published by Genencor International, a Division of Danisco, Danisco US Inc, accessed on 22 December, 2014.

[42] Mithra, M. G and Padmaja, G. 2016. "Compositional profile and ultrastructure of selected root and vegetable processing residues subjected to steam and dilute sulfuric acid pretreatment". Current Biotechnology 7: doi: 10.2174/2211550105666160916124120.

[43] Shanavas, S, Padmaja, G, Moorthy, S. N, Sajeev, M. S and Sheriff, J. T. 2011. "Process optimization for bioethanol production from cassava starch using novel eco-friendly enzymes". Biomass and Bioenergy, 35, 901–909.

[44] Moorthy, S. N and Padmaja, G. 2002. "A rapid titrimetric method for the determination of starch content of cassava tubers". Journal of Root Crops, 28, 30-37.

[45] Goering, H. D and Vansoest, P. J. 1975. "Forage fiber analysis". USDA Agricultural Research Service, Washington.

[46] Updegroff. D.M. 1969. "Estimation of cellulose by anthrone reagent". Analytical Chemistry, 32, 420-423.

[47] AOAC. Official Methods of Analysis, 16th ed. Washington, DC: Association of Official Analytical Chemists, 1995.

[48] SAS. 2010. SAS Institute Inc, Cary, NC, USA.

[49] Chang, V. S, Nagwani, M and Holtzapple, M. T. 1998. "Lime pretreatment of crop residues bagasse and wheat straw". Applied Biochemistry and Biotechnology, 74, 135-159.

[50] Chang, V. S, Burr, B and Holtzapple, M. T. 1997. "Lime pretreatment of switchgrass". Applied Biochemistry and Biotechnology, 63-65, 3-19.

[51] Kim, S and Holtzapple, M. T. 2005. "Lime pretreatment and enzymatic hydrolysis of corn stover". Bioresource Technology, 96, 1994- 2006.

[52] Saha, B. C and Cotta, M. A. 2008. "Lime pretreatment, enzymatic saccharification and fermentation of rice hulls to ethanol". Biomass and Bioenergy, 32, 971-977.

[53] Pooja, N. S and Padmaja, G. 2015. "Enhancing the enzymatic saccharification of agricultural and processing residues of cassava through pretreatment techniques". Waste and Biomass Valorisation, 6, 303–315.

[54] Wanitwattanarumlug, B, Luengnaruemitchai, A and Wongkasemjit, S. 2012. "Characterization of corn cobs from microwave and potassium hydroxide treatment". International Journal of Chemistry, Biology and Engineering, 6, 354-358.

[55] Irfan, M, Syed, Q, Abbas, S, Sher, M. G, Baig, S and Nadeem, M. 2011. "FTIR and SEM analysis of thermo-chemically fractionated sugarcane bagasse". Turkish Journal of Biochemistry, 36, 322-328.

[56] Ghosh, K and Baghel, M. S. 2011. "A pharmacognostical and physiochemical study of *Benincasa hispida* with Ayurvedic review". International Journal of Research in Ayurveda and Pharmacy, 2, 1664-1668.

http://dx.doi.org/10.22161/ijeab/2.1.41 *ISSN: 2456-1878*

Performance evaluation and characterization of wetted soil parameters of improvised medi-emitters installed in a drip irrigation tomato field

Awe G.O.[1], Abegunrin T.P.[2*], Ojediran J.O.[2], Oyetoro O.O.[2]

[1]Department of Crop, Soil and Environmental Sciences, Faculty of Agricultural Sciences, Ekiti State University, Ado Ekiti, Nigeria.

[2]Department of Agricultural Engineering, Faculty of Engineering and Technology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

***Abstract*—** *Field study was conducted to evaluate the emission uniformity (EU), global coefficient of variation (CG_v), emitter flow variation (Q_{var}) and distribution uniformity (DU), and determine the wetted radius (r_w) on soil surface of improvised medi-emitters installed in a tomato field. Soil water content (SWC) at four layers was determined after different periods of irrigation. Radius of wetted soil surface was determined and predicted. Irrigation frequency had no significant effect on the average discharge rate of the medi-emitters throughout the growing cycle. Average Q_{var} and CG_v were significantly ($P=0.05$) influenced by the frequency of application while the EU and DU did not significantly ($P=0.05$) differ among the treatments. There were significant differences in the average values of SWC in different soil layers under the different periods of irrigation. Both the observed and calculated r_w on the soil surface were fitted with fourth order polynomial. The model performance parameters of MAE and RMSE between the calculated and observed radii were low, indicating good prediction. Medical infusion set can successfully replace the more expensive conventional emitters for drip irrigation system.*

***Keywords*— Medical infusion set, conventional emitters, wetted soil radius, soil water status.**

I. INTRODUCTION

One of the advocated strategies of averting the impending water crisis is the emphasis on increased water use efficiency from the irrigation sub-sector, and one way of ensuring efficient utilization of irrigation water is for farmers to switch over from the traditional surface flooding method and adopt highly efficient irrigation system, the drip irrigation systems. The drip irrigation system has become the best method of water application that has been used globally over other irrigation systems. Drip irrigation system supplies frequent and small amounts of irrigation water at single or many points to the field surface/subsurface within the plant root zone (Decroix and Malaval 1985; Youngs et al. 1999). According to Khan et al. (2014), drip irrigation system is one of the best methods with frequent, slow application of water either directly on the land or into the crop root zone rather than the entire land surface, which ensures optimum water content in the root zone.

In Nigeria, conventional pipes and emission devices used for most of the outstanding schemes of drip irrigations, though very efficient and adequate, are imported (Awe and Ogedengbe 2011). However, the devaluation of the local currency in the international market currently being experienced has make these items to be expensive, becoming unaffordable to rural and peasant farmers who make the bulk of agricultural sector. Consequently, of an estimated 71 million hectares of arable land, only about 7 per cent is being irrigated (Muhammad-Lawal et al. 2013) with the remaining area depending on rainfall. However, the high variability and uneven temporal distribution of rainfall in recent times have made rain-fed agriculture to be under threat. It was in line of this that the FAO (2010)listed Nigeria amongst the countries that are technically unable to meet their food demands from rain-fed farming. Therefore, the search for and use of locally available materials for irrigation are now promoted with a view to increasing productivity and ensuring food security.

Recently, the concept of affordable micro-irrigation systems has been identified as a commensurate drip technology for low-income farmers and these systems have posed momentous potential for efficient agricultural water use (Mofoke et al. 2004). For example, low-cost micro-irrigation systems in use today include the drum and bucket drip kits (Cornish and Brabben 2001), and the Nica

http://dx.doi.org/10.22161/ijeab/2.1.41 *ISSN: 2456-1878*

Irrigation (Anon. 2003) which apply water in pulses often more than once a day. In other to evaluate the efficiency and applicability of these systems, considerable studies have been conducted with success (e.g. Polak et al.1997; Bissrat et al.2001; Masimba 2003).

Recently too, medical infusion set, otherwise known as medi-emitter, used mainly in hospitals and clinics for transfusion purposes has been successfully adopted as emitters in drip irrigation. Mofoke et al. (2004) first used medi-emitters and reported satisfactory performance as drippers for a continuous-flow drip irrigation system installed in tomato plots in Awe and Ogedengbe (2011) employed medi-emitters in conjunction with bamboo laterals, the authors found that the locally sourced materials performed well with variation in medi-emitter discharge not more than 10.21 percent, low manufacturer's CV (3.35) and high statistical uniformity coefficient (98.33%) and distribution uniformity (97.69%). One shortcoming about these two studies is that the medi-emitters were calibrated to supply to the field in drops of water per minutes, in other words, they were not calibrated to supply water to the field at discharge rates that could be comparable to those of conventional emitters, therefore there is gap in knowledge on how the improvised systems perform over time.

Drip irrigation can potentially have high application efficiency and high distribution uniformity, which are very important in ensuring uniformly and high crop yields as well as preserving water quality, especially when both water and fertilizers are applied through the irrigation system. However, the uniformity and general performance of micro-irrigation systems are affected by several factors including manufacturer's coefficient of variation, grouping of emitters and emitter clogging, water temperature, water quality, among others (Frizzone 1997). The hydraulic of the system and topography of the field also cause variation in pressure head at individual outlet. According to Solomon (1984), as the pressure variation in drip irrigation system increases, both the uniformity and application efficiency tend to reduce, causing increase in water losses and leading to over- or under-irrigation. In addition, improper maintenance of irrigation system can cause the uniformity to decrease, resulting in increased water application to compensate for decreased application uniformity or reduced yields (Sammis and Wu 1985). Therefore, tests to evaluate performance, related to resistance and durability of irrigation system, defined through technical specification rules are required. The aim of evaluating the operation of irrigation systems is for better understanding of the adequacy and determination of the necessary procedures required for improving the system. According to Awe and Ogedengbe (2011), such evaluation should be carried out soon after the system installation in the field and periodically repeated until harvest. This is because drip irrigation systems are sensitive to operational conditions over time (Keller and Blisner 1990; Soccol et al. 2002). Therefore, the objective of this study was to evaluate the performance and determine the wetted radius on soil surface of improvised medi-emitters installed in a tomato field under different drip irrigation frequency.

II. MATERIALS AND METHODS

Description of study site

The experiment was conducted at the Teaching and Research Farm of Ladoke Akintola University of Technology, Ogbomoso southwest Nigeria. The field is located on Latitude 8.168343^{o}N, Longitude 4.269921^{o}E and Altitude 357 m. Ogbomoso is characterized by bimodal rainfall pattern, peaking in July and September with annual rainfall depth of about 1200 mm (Abegunrin et al. 2013) while the mean annual maximum and minimum temperature are 33 and 28^{o}C, respectively. The climate is cold and dry from November to March and then warm and moist from April to October, it could also be described as a hot humid tropical which falls in southern Guinea Savannah of Nigeria with mean relative humidity of about 74% all year round except in the months of December to February when it is low as a result of dry wind (harmattan) that blows from the north (Olaniyi et al.2010), while the soil of the site is classified as Hapludalf (SSS 2006), with the textural analysis showing sandy loam texture. Some physical and chemical properties of the 0-30 cm layer are presented in Table 1.

Table.1: Some soil physical chemical properties of the site before the experiment.

Soil depth, cm	pH	SOM %	BD g/cm^3	Sand	Silt	Clay	Texture
				----------%----------			
0-5	7.2	1.8	1.48	80.5	8.1	11.4	SL
5-10	6.8	1.2	1.62	77.6	12.0	10.4	SL
10-20	6.6	1.2	1.70	79.7	10.1	10.2	SL
20-30	6.2	1.0	1.73	76.3	13.2	11.5	SL

pH: level of alkalinity or acidity, SOM: soil organic matter, BD: bulk density, SL: sandy loam.

http://dx.doi.org/10.22161/ijeab/2.1.41 *ISSN: 2456-1878*

Installation of drip irrigation system

The experiment consists of two 6500 liters capacity tanks mounted at the upper end of the field. The feeder tank was positioned 3 m height above ground level and connected to the supply tank with 1.5 m height through a PVC pipe. The feeder tank continuously feed the supply tank with water at a constant head while the supply tank consists of a floating device to regulate the height of water. The floating device helps to ensure a constant pressure in the supply tank. The supply tank was connected to the main (diameter 25 mm) through a gate valve. Six laterals (made of garden hose), each 25 m long and diameter 15 mm, were laid at each side of the plot with 54 medi-emitters on each laterals. Twelve (12) medi-emitters were laid on each sub-plots, 6 to each side of the laterals with spacing of 0.33 m (Figure 1 and Plate 1). The medi-emitters were forced into the lateral of the drip line already on the field.

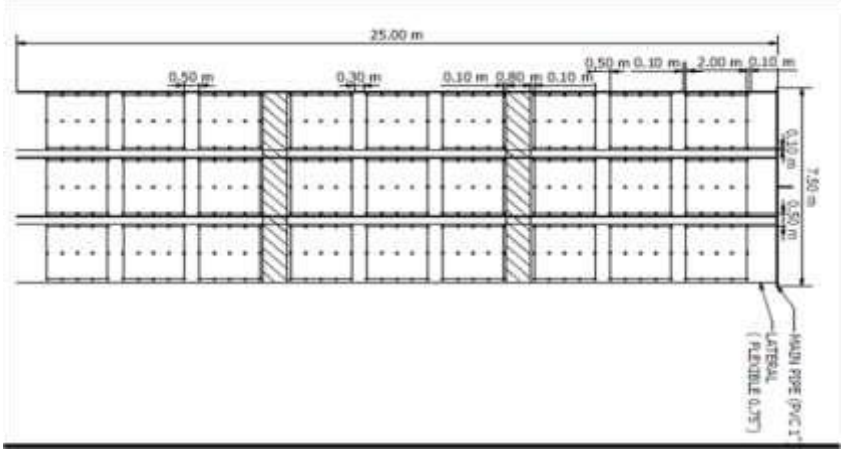


Fig.1: Experimental Field Layout

Plate.1: The drip irrigation setup showing the medi-emitters

Calibration of medi-emitters

The medi-emitters were calibrated on the field using a stop watch and a measuring cylinder of $1L$ capacity. The medi-emitters were fitted to the garden hose (laterals) while the control knobs were gradually adjusted so that water is allowed to flow to the measuring cylinder. The time taken to fill $1L$ measuring cylinder for each trial was noted and the procedure was repeated several times until a time was reached when 1litre was filled in 15 minutes. The position of the control knob at this point was marked. Several trials were made with the fixed position of the control knob to ensure accuracy and in each case, the discharge was $1L$ in 15 minutes which is equivalent to$4L/h$.

Performance evaluation of the medi-emitter

Water discharge from the emitters was used to evaluate the performance. This involves the direct process of collecting water in a plastic container, or catch can, measuring the water using a sensitive scale and converting it to volume on the assumption that 1 g = 1 cm^3. The collection time was set to 10 minutes, considered enough to obtain adequate volume (ASAE EP 458 1996). The performance parameters evaluated include: emission uniformity, global coefficient of variation, emitter flow variation and distribution uniformity as described below:

(i) The Emission uniformity (EU) was determined according to the procedure of Keller and Karmeli (1974).

$$EU = 100 \times \frac{Q_{min}}{Q_{ave}}$$

where EU= Field emission uniformity (%); Q_{min} = minimum discharge rate (L/h); Q_{ave} = average of all field data emitter discharge rates (L/h).

(ii)The emitter flow variation (Q_{var}) and coefficient of variation (C_v) was according to Keller and Karmeli (1974) as:

$$Q_{var} = \frac{Q_{max} - Q_{min}}{Q_{max}}$$

where Q_{var} = emitter flow variation (L/h); Q_{max} = maximum emitter flow rate (L/h); Q_{min} = minimum emitter flow rate (L/h)

(iii) Coefficient of global variation (CG_v) was calculated according to the equation

$$CG_v = \frac{S}{Q_{ave}}$$

where S = standard deviation of emitter flow rate (L/h); Q_{ave} = average emitter flow rate (L/h).

(iv) Distribution uniformity (DU) was computed according to Keller and Karmeli (1974):

$$DU = \frac{\bar{q}_{25}}{\bar{q}} * 100$$

where DU = distribution uniformity (%); $\bar{q}_{25}$= average of 25% lowest emitter rates (L/h); $\bar{q}$ = average emitter flow rate (L/h).

The performance indices were determined from spatial perspective considering all emitters in all the three drip irrigation frequency treatments after installation (a week after transplanting). These parameters were again evaluated for each treatment at mid-season and at harvest to reveal the system's change in discharge and distribution uniformity with time as suggested by Mofoke et al.(2004). The performance indices were compared with ASAE EP 405 (1985) standards for optimum operating system for drip irrigation systems.

Radius of water pool formed on the soil surface and soil water content

Twelve medi-emitters were randomly selected on the field and are numbered A1, A2, B1, B2, C1, C2, D1, D2, E1, E2 F1 and F2. Each of the emitter was opened to the calibrated discharge of 4 L/h. The emitters A1 and A2 were allowed to run for 15 minutes after which the knobs were closed. The wetted perimeter was measured by planimetry method, whereby the circumference of the wetted area was traced with flexible and tiny rope and the length was determined (Plate 2).

Plate.2. Determination of radius of wetted surface using planimetry method.

The diameter of the approximate circle formed was calculated using the equation

$$Circumference = \pi d$$

where d is the diameter of the circle, cm. The radius r was calculated as $r = d/2$.

At the end each experiment, soil samples (in triplicates) were collected from the 0-10, 10-20, 20-30 and 30-40 cm layers for the determination of gravimetric water content, θ_g. The soil volumetric water content, SWC, was computed from the product of θ_g and bulk density, BD.

Modeling of radius of water pool formed on the soil surface

In order to model the radius of circle of water entry at time 't', it is necessary to consider the infiltration rate (Subbaiah and Mashru 2013). These authors suggested the use of Kostiakov's equation ($I = At^n$) for the cumulative depth of infiltration in any soil. To apply this equation, infiltration tests were performed before transplanting of tomato seedlings.

The radius of the water pool formed on the soil surface (r_w) was given as:

$$r_w{}^2 = 0.955Q/a[2t^b + 3^b]$$

where Q is the emitter flow rate (cm^3 min^{-1}), $a = A * n$ and $b = n - 1$ are constants obtained from Kostiakov cumulative infiltration equation. The description of this equation can be found in Subbaiah and Mashru (2013). The calculated radii of water pool on soil surface (r_w) were plotted against time and a regression equation was obtained. The observed and calculated r_w values were compared using statistical parameters of mean absolute error (MAE) and root mean square error (RMSE).

Statistical analysis

Data obtained on performance evaluation and soil water content in the different layers were subjected to descriptive analysis and analysis of variance (ANOVA) at 5% level of probability. Statistical analyses were done in SPSS (IBM version 20).

III. RESULTS AND DISCUSSION

Performance evaluation of the medi-emitters

The descriptive statistics of the calibrated discharge rates of the medi-emitters installed in the tomato field under drip irrigation are presented in Table 2. Irrigation water supplied to the field at different frequency had no significant effect on the average discharge rates of the medi-emitters throughout the tomato growing cycle. The discharge rates from the medi-emitters ranged between 3.73 and 4.10 L/h; 3.57 and 4.07 L/h and 3.57 and 4.03 L/h shortly after transplanting, mid-season and harvest, respectively for the different drip irrigation regimes, with the mean discharge rate at par (3.91 L/h) for all treatments. The overall average calibrated discharge rate is slightly lesser (about 2.25% decrease) than the nominal discharge rate of 4 L/h from conventional drip emitters. The maintenance of the discharge rate throughout the growth cycle made it possible for supplying water towards meeting scheduled crop water requirements of the tomato crop.

Table.2: Descriptive statistics of the discharge rate of the medi-emitters installed in the tomato field.

	Max.	Min.	Mean	SD
Irrigation frequency	Shortly after transplanting			
F1	4.03	3.73	3.91a	0.083
F2	4.03	3.80	3.92a	0.054
F3	4.10	3.80	3.92a	0.062
	Mid-season			
F1	4.07	3.57	3.89a	0.103
F2	4.07	3.77	3.93a	0.067
F3	4.00	3.77	3.90a	0.067
	Harvest			
F1	4.10	3.57	3.89a	0.095
F2	4.03	3.60	3.92a	0.090
F3	4.00	3.80	3.91a	0.055

F1: irrigation every 7 days; F2: irrigation every 5 days and F3: irrigation every 3 days

Means followed by the same letter did not differ significantly at 5% level of probability by Fisher's LSD test.

Variation in discharge

Average discharge variation (Q_{var}) was significantly (P=0.05) influenced by the frequency of water application (Table 3). Shortly after transplanting, F1 and F3 treatments had significantly higher Q_{var} than F2 treatment. At mid-season stage, F1 treatment had the significantly highest Q_{var}(12.30) compared with other treatments. At harvest, similar result was obtained as F1 had the significantly highest Q_{var} (13.01). Except for F3 treatment, the variation in discharge increased with time.

Table.3: Performance evaluation of the medi-emitters at planting, mid-season and harvest of tomato.

	Q_{var}	EU	CG_v	DU
Irrigation frequency	Shortly after transplanting			
F1	7.44a	97.87a	2.13a	97.20a
F2	5.79b	98.63a	1.37b	98.32a
F3	7.32a	98.43a	1.57ab	98.11a
	Mid-season			
F1	12.30a	97.35a	2.65a	96.55a
F2	7.38b	98.30a	1.70b	98.04a
F3	5.83c	98.27a	1.73b	97.90a
	Harvest			
F1	13.01a	97.56a	2.44a	97.09a
F2	10.74b	97.70a	2.30a	97.05a
F3	5.00c	98.59a	1.41b	98.26a

F1: irrigation every 7 days; F2: irrigation every 5 days and F3: irrigation every 3 days

Means followed by the same letter did not differ significantly at 5% level of probability by Fisher's LSD test.

The significantly higher and increase in Qvar over time obtained from F1 and F2 treatments are attributed to certain phenomena taking place during the period (7 and 5 days, respectively) which the medi-emitters remained closed. For example, there may have been accumulation of dirts at the close point, causing partial plugging or the difficulty of the light hose to fully open as a result of elastic behaviour of the material. Thus, each time the emitters are reopened, about 2-3 minutes are needed before the system stabilizes, thus the higher variability. Mofoke et al. (2004) stated that the general variability in discharge could be attributed to major and minor losses occurring at the delivery pipe joints and fittings right from the supply tank to the emitters (e.g. inherent leakages at the point of immersion of emitters to the main line).

Coefficient of global variation

The coefficient of global variation (CG_v) in discharge describes the quality of the processes used to manufacture emission devices. The coefficient of global variation was significantly (P=0.05) affected by the frequency of water application. At the different periods of evaluation, the medi-emitters installed in F1 treatment had the significantly highest CG_v (Table 3). The average values of CG_v were generally low (not more than 2.65) and according to American Society of Agricultural Engineering (ASAE EP 1985) recommended classification of coefficient of global variation in discharge, these values are below the 10% threshold as 'good' for point source emitters. This showed that the adopted medical infusion set (medi-emitters), coupled with mechanism to control flow rate, can be employed as substitute for the conventional drippers for drip irrigation systems. This result agrees with the findings of Mofoke et al.(2004) and Awe and Ogedengbe (2011) on the adoption of medical infusion set as drip emitters.

Emission and distribution uniformity

Emission uniformity (EU) describes how evenly an irrigation system distributes the same depth of water to every unit area. On the other hand, distribution uniformity (DU) is an indicator of the magnitude of the system's distribution problems. Although F1 treatment had the lowest values (about 97%) of the uniformity coefficients, but they did not significantly differ from other treatments. According to the classification of irrigation system performance by ASAE EP 405 (1985), an EU rating of 90 - 95% is considered excellent and the system would only require regular maintenance, while a DU of 85% or greater

is considered excellent. In this study, the average values of both EU and DU monitored at different stages of the tomato growth cycle were above the maximum threshold for micro-drip irrigation system, indicating that the system performance was excellent. The high EU and DU values followed the trend of observed low coefficient of global variation in discharge (CG_v). The lower EU and DU obtained from F1 treatment is attributed to higher CG_v observed. These findings also agree with previous studies (Mofoke et al. 2004; Awe and Ogedengbe 2011). The EU values obtained are also comparable to those of Manisha et al. (2015) who studied the performance evaluation of conventional drip irrigation system at discharge rate of 4 L/h.

Spatio-temporal distribution of profile soil water content

The temporal variability of soil water content of the tomato field during the performance evaluation of the medi-emitters is presented in Fig 2.

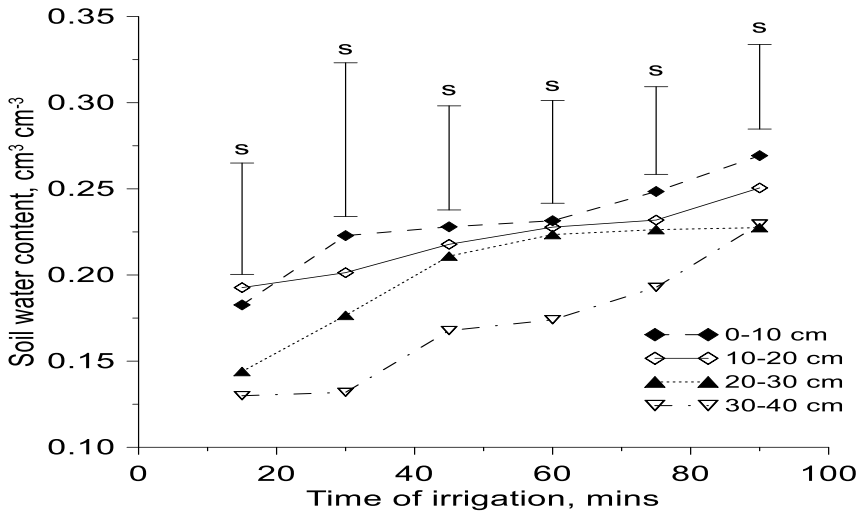


Fig.2: Temporal distribution of soil water content of the tomato field during the performance evaluation test of the medi-emitters. The vertical bars are the standard error
s: significant at 5% level of probability by Fisher's least significant difference test.

There were significant differences in the average SWC in the different soil layers under the different time of irrigation, with the 30-40 cm layer having the lowest SWC at all times. As expected, the SWC of each soil layer increases with time, with the 10-20 cm layer having the lowest amplitude while the 30-40 cm layer had the highest amplitude. The amplitude of the 0-10 cm surface layer was high compared to that of 10-20 cm layer. The high amplitude in the surface layer is attributed to evaporation since it is the layer that is exposed to the atmosphere. The SWC of the 0-10 cm surface layer was about 28.9% greater than that of 30-40 cm layer when irrigated for 15 minutes. However, at the end of 90 minutes of irrigation, the 0-10 cm surface layer had SWC higher than that of 30-40 cm layer by 14.6%, about half reduction. The high amplitude and high percent difference in the SWC of 30-40 cm layer at the initial stage compared with the surface layer is attributed to relative dryness in the subsurface layer when the evaluation was conducted, during this time, very few centimeters from the soil surface have been wetted (Fig 3). On the other hand, the rapid reduction in the gap between the SWC of the surface and subsurface layer at the end of 90 minutes is attributed to high water percolation and redistribution, in other words, high advance of wetting front in the soil matrix due to the sandy nature of the soil. Sandy-textured soils have greater macropores which facilitate rapid water movement and redistribution. The advance of the wetting front within the soil profile can be seen in the spatial distribution map shown in Fig 3.

http://dx.doi.org/10.22161/ijeab/2.1.41 *ISSN: 2456-1878*

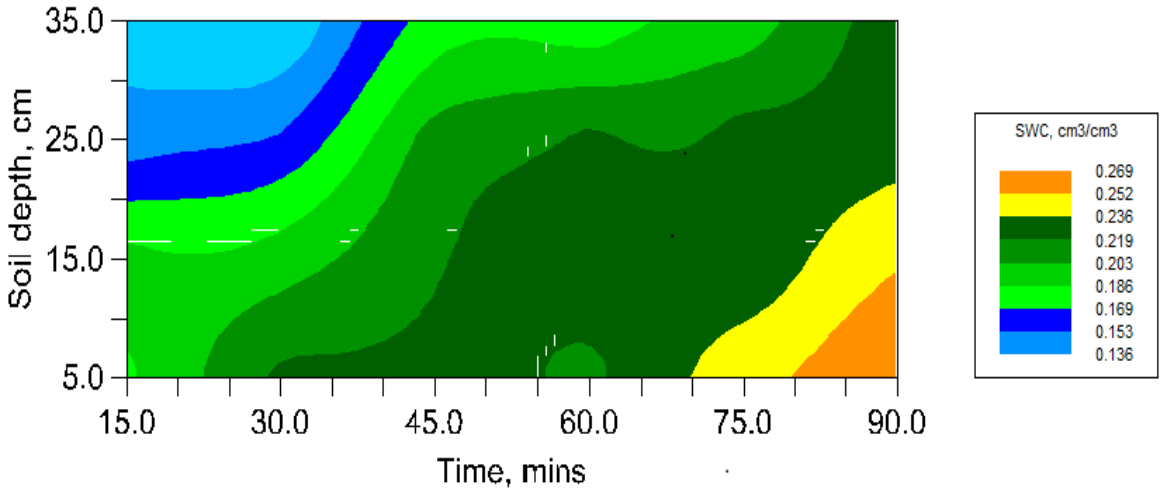


Fig.3: Spatial map showing the wetting front in the soil profile with time during the performance evaluation of the medi-emitters.

Cumulative infiltration and radius of water pool formed on the soil surface

The cumulative infiltration function of the tomato field shortly after installation of the drip irrigation system is shown in Fig 4.

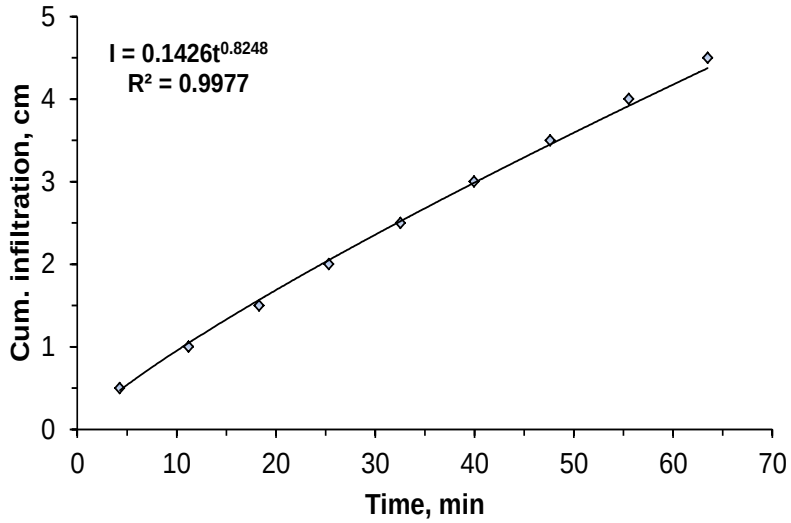


Fig.4: Cumulative infiltration of the field before the installation of the drip irrigation system.

The total cumulative infiltration was found to be about 50 cm. There was near perfect (R^2 = 0.9977) goodness of fit between the observed cumulative infiltration values and the Kostiakov model. For this soil, the model constants for the radius of the water pool on the soil surface, $r_w{}^2 = 0.955Q/a[2t^b + 3^b]$, a was 0.1176 while b was -0.1752.

The radius of wetted soil volume is an important parameter when deciding spacing between emitters for a given set of soil and crop conditions. It also a vital component in optimizing emitter spacing based on the geometry of the wetted soil volume and in the determination of length of laterals (Subbaiah and Mashru 2013). The temporal distribution of observed and calculated wetted radii on the soil surface by the medi-emitters is shown in Figure 5.

http://dx.doi.org/10.22161/ijeab/2.1.41 *ISSN: 2456-1878*

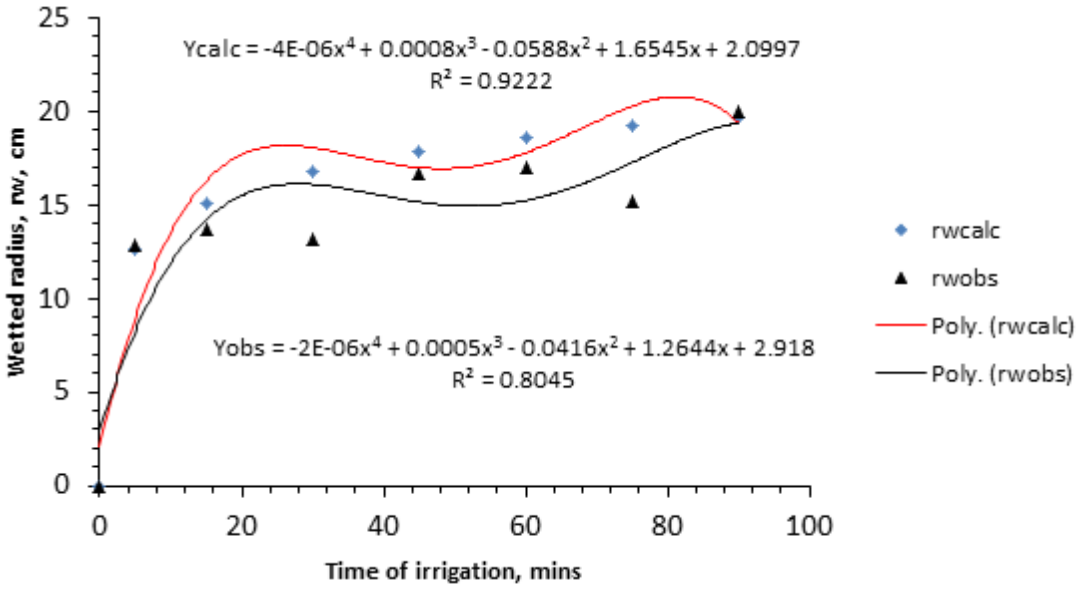


Fig.5: Calculated and observed wetted radius of the soil surface by the medi-emitters.

Increased duration of irrigation increased the wetted radius, in other words, the radial area of ponded water develops in the vicinity of the medi-emitters and expands with time. Both the observed and calculated radius of water pool on the soil surface as a function of time was fitted well with fourth order polynomial as also obtained by Subbaiah and Mashru (2013) who modeled the effect of three discharge rates on soil wetted radius of a point source surface trickle irrigation. The goodness of fit was 0.9222 and 0.8045 for the calculated and observed radii, respectively. These values were lower than those obtained by Subbaiah and Mashru (2013) who employed conventional drippers compared to the improvised medi-emitters used for this study.

In addition to goodness of fit, model validation parameters of mean absolute error (MAE) and root mean square error (RMSE) were used to compare observed and calculated r_w values and they were 0.2258 and 0.2573, respectively. The MAE shows the bias while the RMSE is an indication of the prediction accuracy (Chai and Draxler 2014). These values were low, indicating good prediction. In this study, only a single emitter discharge rate was evaluated, therefore we recommend further studies to include the effect of different medi-emitter discharge rates on wetted radius on the soil surface as well as depth of water pool in the subsurface. Nevertheless, this model can be used to determine the geometry of wetted bulb from surface drippers, whether conventional or improvised, for any operating discharge rate for a specific duration.

IV. CONCLUSIONS

The discharge rate of the medi-emitters did not vary throughout the tomato growing cycle and was almost at par compared to that of conventional emitter. According to American Society of Agricultural Engineering (ASAE 1985) recommendation, the evaluated parameters showed that the system performance was excellent. The different periods of irrigation significantly influenced the average values of SWC in the different soil layers. The model performance parameters of goodness of fit, MAE and RMSE between the calculated and observed radii showed that the mathematical model can be used to predict the radius of wetted soil surface under any surface drip irrigation system. The results of the study showed that the medical infusion set, with locally produced polyvinyl chloride pipes (PVC), can successfully replace the more expensive conventional emitters and delivery pipes for drip irrigation system. However, we recommend further studies to include the effect of different discharge rates on wetted radius on the soil surface as well as depth of water pool in the subsurface.

REFERENCES

[1] Abegunrin TP, Awe GO, Idowu DO, Onigbogi OO Onofua OE (2013) ‘‘Effect of kitchen wastewater on soil properties and growth parameters of cucumber (*Cucumis sativus*)’’. Journal of Soil Science and Environment. 4(7): 139-145.doi:10.5897/JSSEM2013.0412

[2] Anon (2003). Smart Water Solutions: examples of innovative, low-cost technology for wells, pumps, storage, irrigation and water treatment. - Netherlands Water Partnership (NWP). Delft, the Netherlands.
[3] ASAE- American Society of Agricultural Engineers (1985) Design, installation and performance of trickle irrigation systems, EP 405, pp.508.
[4] ASAE- American Society of Agricultural Engineers. (1996). Field evaluation of micro-irrigation systems, EP 458, 792-797.
[5] Awe GO, Ogedengbe K (2011) Performance evaluation of bamboo (*Bambusa vulgaris* Schrad)-pipe and medi-emitter in a gravity-flow drip irrigation system. Int. J. Agric. For., 1(1): 9-13.
[6] Bissrat G, Asghedom S, Mehari A (2001) Affordable micro-irrigation technology (AMIT). In: Eritrea Pre-feasibility study report, March-September 2001. Report of a consultative project between the Centre for Development and Environment (CDE), University of Bern, International Development Enterprises (IDE) and the College of Agriculture and Aquatic Science (CAAS), University of Asmara.
[7] Chai T, Draxler RR (2014). Root mean square error (RMSE) or mean absolute error (MAE)? Arguments against avoiding RMSE in literature. Geosic. Model Dev., 7: 1247-1250.
[8] Cornish G, Brabben T (2001) Field guide on irrigated agriculture for field assistants. International programme for technology and research in irrigation and drainage. FAO, Rome.
[9] Decroix M, Malaval A (1985) Laboratory evaluation of trickle irrigation equipment for field system design. Proceedings of the third International Drip/Trickle Irrigation Congress, Volume I, California, USA. pp. 325-338.
[10] FAO (2010) FAOSTAT Production Yearbook 2010.
[11] Frizzone JA (1997) Uniformidade e eficiencia da irrigacao. Departmento de Engenharia Rurual, ESALQ, Serie didatica, Piracicaba. 2-18.
[12] Keller J, Blisner RD (1990) Sprinkler and trickle irrigation. Van Nostrand Reinhold, New York. Pp 427-602.
[13] Keller J, Karmeli D (1974) Trickle irrigation design parameters. Transaction of ASAE, 7, 687-684.
[14] Khan GD, Ali A, Akbar F (2014) Assessment of coefficient of variation of emitters flow rate with respect to design, manufacturers and plugging in installed drip irrigation systems at selected sites of Peshawar valley. Adv. Life Sci. Tech., 19: 27-32.
[15] Manisha I, Sinha J, Tripathi MP (2015) Studies on hydraulic performance of drip irrigation under operating pressure. Int. J. Appl. Eng. Tech., 5(2): 1-6.
[16] Masimba B (2003) Drip irrigation helps smallholders. - In: "African Farming and Food Processing" July/August 2003 edition. (J. Phelan ed.). Alain Charles Publishing Ltd. London.
[17] Mofoke ALE, Adewumi JK, Mudiare OJ, Ramahan AA (2004) Design, construction and evaluation of an affordable continuous – flow drip irrigation system. Journal of Applied Irrigation Science. 39(2): 253-269.
[18] Muhammad-Lawal A, Adenuga AH, Jimoh O (2013). Determinants of adoption irrigation technology by small-holder farmers is selected Local Government Areas in Kwara State, Nigeria. Taraba Journal of Agricultural Research, 1(1): 21-27.
[19] Olaniyi JO, Akanbi WB, Adejumo TA, Akande OG (2010). Growth, fruit yield and nutritional quality of tomato varieties. Afric. J. Food Sci., 4(6): 398-402.
[20] Polak P, Bob N, Deepak A (1997): A low cost drip irrigation system for small farmers in developing countries. Journal of Applied Irrigation Science. 32(1), 105-112.
[21] Sammis TW, Wu IP (1985) Effect of drip irrigation design and management on fruit trees yield. Transactions of the ASAE American Society of Agricultural Engineers. 28(3): 832-838.
[22] Soccol OJ, Ullmann MN, Frizzone JA (2002) Performance analysis of a trickle irrigation subunit installed in apple orchard. Brazilian Archives of Biology and Technology. 45(4): 525-530.
[23] Soil Survey Staff (SSS) (2006). Keys to soil taxonomy. 10th ed. Washington, USDA-SCS, P. 332.
[24] Solomon KH (1984) Yield related interpretations of irrigation uniformity and efficiency measures. Irrig. Sci. 5:161-172.
[25] Subbaiah R, Mashru HH (2013) Modeling for predicting soil wetting radius under point source surface trickle irrigation. Agric. Eng. Int.: CIGR Journal, 15(3): 1-10.
[26] Youngs EG, Leeds-Harrison PB, Alghusni A (1999) Surface pounding of coarse-textured soils under irrigation with a line of surface emitters. J. Agric. Eng. Res., 73: 95-100.

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

Evaluation of Water Resources in Wadi El Natrun, Western Desert, Egypt

Mohamed kamel Fattah

Department of Evaluation of Natural Resources, Environmental studies and research Inst., University of Sadat City, Egypt.
mohamed.fattah@esri.usc.edu.eg

Abstract— *Groundwater of the Pliocene aquifer in Wadi El Natrun area represents the primary source of reliable water for drinking and agriculture uses. This research focuses on the study of the different sources of water in the study area and determines the origin and quality of this waterand also is interested in studying hydrogeochemical processes that affect them and the movement of water through the analysis and determine the activity of different elements, and also to changes in hypothetical salts with the direction of water flow in three sectors of the region and the statement of the most important geochemical processes that occur. Finally it is evaluated the suitability of the water for different purposes.*

The results show that, there is a zonation of chemical composition; this zonation is characterized by a change of cation species from dominantly Ca and Mg near the east to Na-dominated waters in the west. Mirroring this, anions change from HCO3 type to Cl and SO_4 type.The ions displays two grades of metasomatism along flow path, first the stage of mineralization(HCO3>Cl>So4) at the east then the advanced stage of mineralization (Cl>SO4 >HCO3) at the west. According to the ***(W.H.O.2005),*** *45 % of tested groundwater samples are permissible for drinking. Most groundwater samples of the Pliocene according to SAR andRSC are suitable for irrigation purposes under normal condition, but all surface water samples (lakes) are saline water,so it is not valid for drinking or irrigation purposes.*

Keywords—Water Resources, Pliocene Aquifer, Wadi El Natrun, Egypt.

1- INTRODUCTION

This research discusses the hydrogeo chemistry of groundwater in order to estimate the water quality variation, and to shed the light on the important indications about the history of various concentrations of major elements as well as groundwater recharge, discharge, and movement of groundwater in the study area. Water quality, hydro chemical coefficients and groundwater origin will be estimated. The hydro –geochemical characteristics are investigated through the discussion of both geochemical composition and distribution andthe geo- chemical classification of groundwater, also, it can provide a useful insight into the probable processes governing groundwater chemistry (Lyon and Bird, 1995), and (Soulsby et al., 1998.).To achieve this goal, six surface water samples representing the main lakes and thirty five groundwater samples representing productive water wells (Pliocene aquifer) were collected in November, 2015, (Figure 1).

1.1 -Geomorphological and geological setting

Wadi El Natrun occupies a portion of the Western Nile Delta region (Figure 1). It lies between longitude 30º 04´ and 30º 30´ E and latitudes, 30º 16´ and 30º 30´ N.The study area covers an area of about67608Feddans or 281 km2. (El- Abd, E. A, 2005).(Said, 1962), (Sanad, 1973),(El-Ghazawi, 1982), and (Abdel-Baki, 1983) studied the geomorphology and geology of the study area. They concluded that the study area comprises three geomorphological units. The Alluvial plains (young and old alluvial plains) which are characterized by an average gradient of 0.l m/km. The elevation varies from +12 m to +14 m for the young alluvial plains, and between 60 m and 20 m for the old alluvial plains. The lowest point in Wadi El-Natrun and Wadi El-Farigh depressions are -23m and -4m respectively. The Structural plains (depressions, folded ridges and structural plateaux) which have an elevation ranges between 110 m at Gebal Hamza and 200 m at Abu Roash (the ridges bounding Wadi El-Farigh). The tablelands which are differentiated into Maryut tableland marginal tableland. The sedimentary succession in the study area ranges in age from Late Tertiary which is differentiated into Oligocene at 400 m, Miocene at 200 m and Pliocene at 150 m to Quaternary at 300m. The study area also is affected by a number of faults having NW-SE and NE-SW trends.

In subsurface, the measured thickness of the Lower Pliocene at Wadi El-Natrun reaches about 100m and composed essentially of dark pyretic clays. This clay is also encountered in the area between Wadi El-Natrun and Nile Delta, underlying the Pleistocene deposits and overlying with unconformable surface the Middle and Lower Miocene succession.The Middle and Upper Pliocene

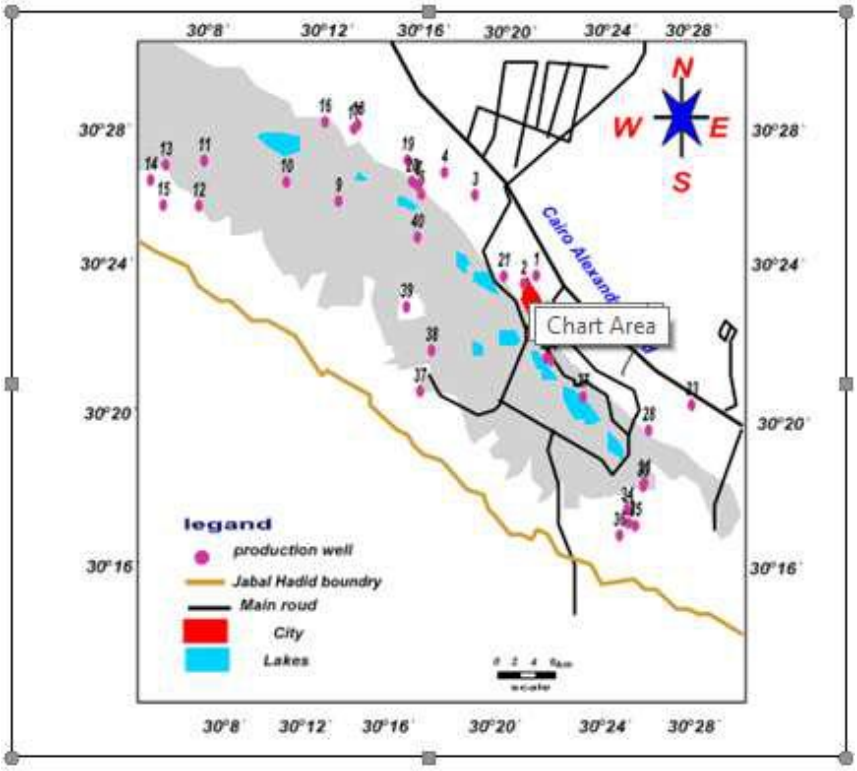
http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

strata, (Wadi El-Natrun formation) are largely restricted to the depression area; these are developed into gypseous clays and sands of typical brackish water origin. This portion of the Pliocene succession is differentiated into two series; Beni Salama Member at base and El-Muluk Member at top. The thickness of the Pliocene sediments in Wadi El Natrun varies from 240 m in the middle, 130 m in the northwest and 100 m in the southeast .The thickness of the Pliocene increases in the areas dominated by the graben-like structure and decreases in the area-dominated by horst one (El Fayumy, 1964).

1.2—Groundwater hydrology setting

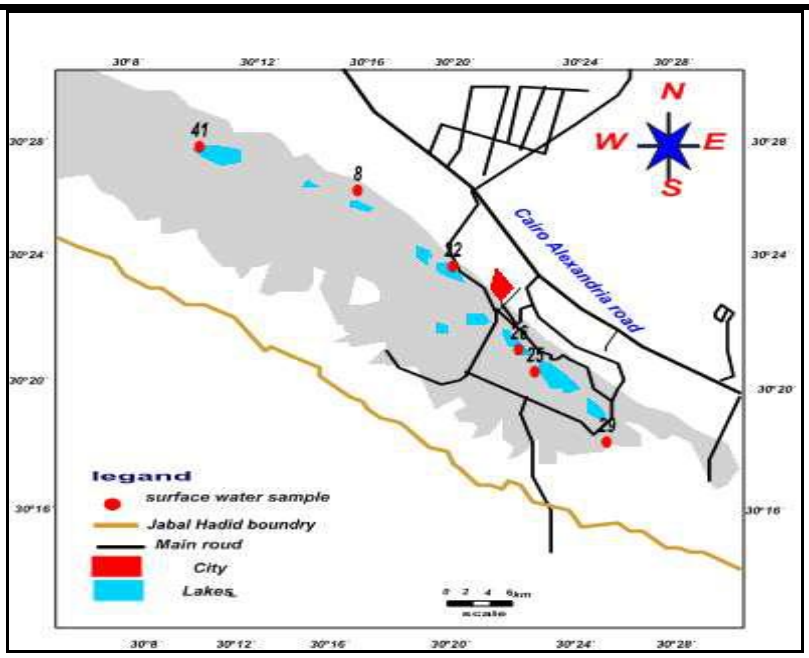
There are three main aquifers in the study area, namely; The Nilotic sand and gravel (Pleistoceneaquifer), Wadi El-Natrun sand and clay (Pliocene aquifer) and El-Moghraquartizitic sand (El-Sheikh, 2000) .The Pliocene sediments are mostly built up of clays underlying the Quaternary aquifer, and hence they represent the main aquiclude horizon in this area. Pliocene sediments in Wadi El Natrun are mainly affected by several faults which facilitate the connection between them and those of the Quaternary. These faults are trending NW-SE direction and bounding Wadi El Natrun from the east with downthrown side to the northeast. So the Quaternary aquifer is located in the front of the Pliocene one in the east. Also Wadi El Natrun is bounded from the south and west by clysmic NW-SE faults with downthrown side to the northeast. So the Miocene aquifer is located in the front of the Pliocene facilitating the hydraulic connection between them.

The depth to water of the Pliocene aquifer varies from about20 m to 30 m outside wadi El Natrun and less than 10 m to flowing in the surface inside Wadi El Natrun Figures (2 &3) . The Groundwater flows from northeast and southeast to southwest and northwest i.e. to Wadi El Natrun. Also local cones of depressions spread in the west and groundwater movements are mainly attributed to the over exploitation of ground water to irrigate the newly reclaimed lands. The Pliocene aquifer in wadi El Natrun is mainly recharged from the Quaternary and Miocene aquifers through the hydraulic connection. The water of the latter aquifers is laterally flowing towards Wadi El Natrun depression, which acts as natural discharging area. The recharge to the Pliocene aquifer, which equals the permissible extraction rate under any condition of future reclamation, was estimated by TRIWACO model to be 128040 m^3/day (Diab et al, 2002).Pliocene aquiferat wadi El Natrun receives other recharge from the southern portion of the Nile Delta through Wadi El Farigh and southwest of Wadi El Natrun depression (Abdel Baki, 1983).



(A)

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*



(B)

Fig.1:Location map of groundwater samples (A) and lakes (B)

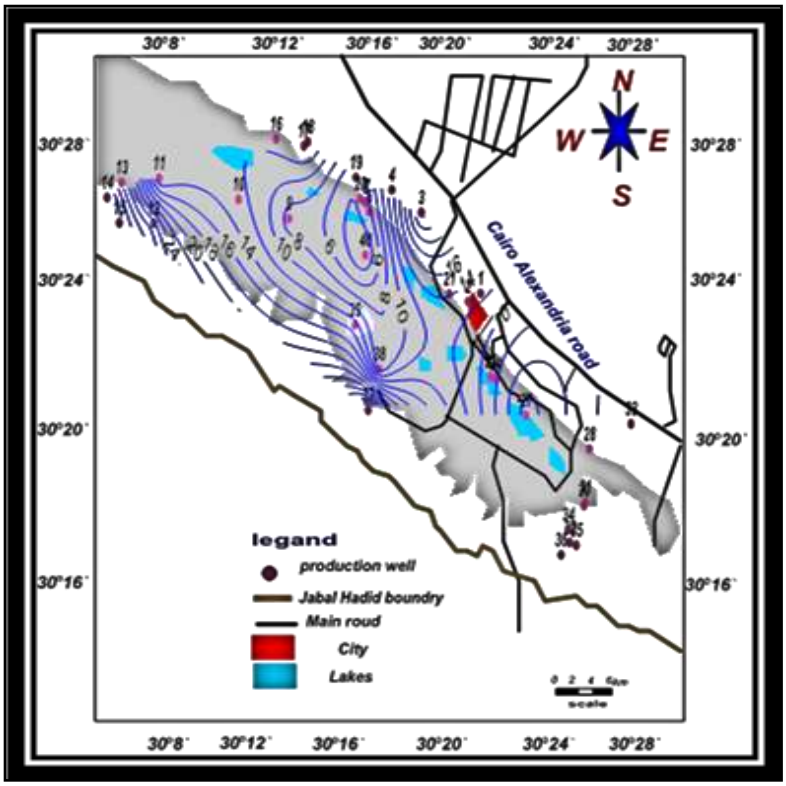


Fig.2:Depth to water contour map of Pliocene aquifer

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

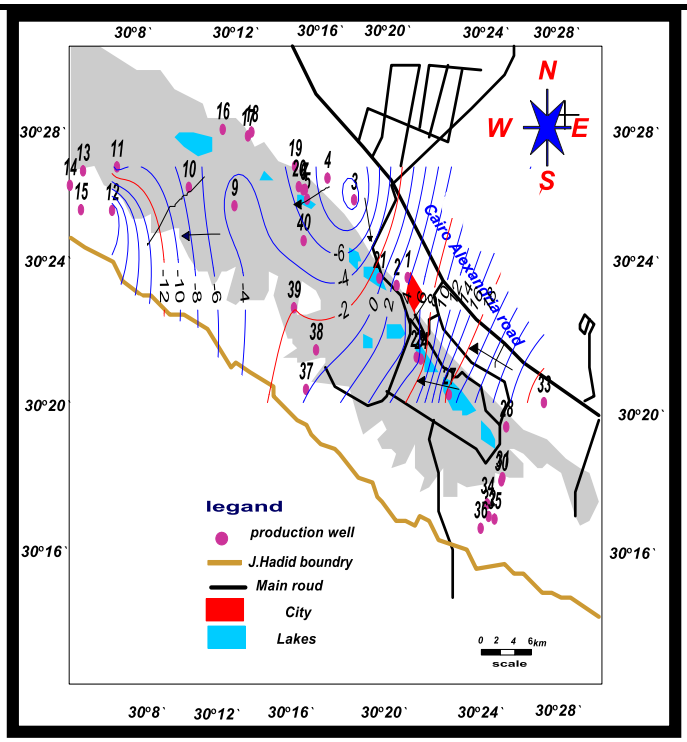


Fig.3: Water level contour map of Pliocene aquifer

2- MATERIAL AND METHODS

Complete chemical analysis of thirty five groundwater samples and Six surface water samples collected from the study area, The analyses conducted both in the field include total dissolved solids (TDS), measurement of Ph, electrical conductivity (EC), and in the laboratory concentrations of Ca^{+2}, Mg^{+2}, Na^{+} and K^{+} as cations, CO_3^{-2}, HCO_3^{-}, SO_4^{-2} and Cl^{-}as anions. Detailed Chemical analyses were carried out for the collected samples (Figure 1). Different methods were used for these analyses, ASTM, American Society for Testing and Material (2002), (Ca^{++}, Mg^{++}, CO_3, HCO_3, Cl) were measured volumetrically by titrimetric method. Sodium (Na^{+}) and potassium (K^{+}) were determined calorimetrically by means of Flame photometer. Sulphate (SO_4) is determined calorimetrically by means of Ultraviolet spectrophotometer screening method at wave length (690, nm). Also, a Global Positioning System (GPS) Garment 12 was used for location and elevation readings.

Tables (1 to 3 in appendix) include the concentration of major ions, salinity, total hardness, ions distribution, hydrochemical coefficients and hypothetical salt combinations for groundwater samples collected from the wells tapping the Pliocene aquifer in the study area.

3- RESULTS AND DISCUSSION

3.1 Hydrochemical characteristics

Thirty five groundwater samplesfrom the Pliocene aquifer and six surface water samples (Lakes) figure (1.A&B) were collected for chemical analyses (tables1&2, App. 1) will be utilized to determine water quality ,the geochemical evolution along the groundwater flow path and finally the suitabilityof these water for different purposes

3.1.1 pH values:

The pH value of water is related to its quality and affects, to a great extent, its suitability for different uses. The water pH is controlled by the amount of dissolved carbon dioxide (CO_2), carbonates (CO_3^{2-}) and bicarbonates (HCO_3^{-}) (Domenico, P.A., and Schwartz, F.W., 1990). The hydrogen ion concentrations (pH) in ground water for groundwater ofthe Pliocene aquifer, variesbetween 7.32 in well no. 4,and 9.96 in well no. 23. While surface water samples , the pH varies between 8.8 in sample (no. 42)(El Gaar lake) at the north west and 9.1 in sample (no. 26) (Rozitta lake) at south east.The values of pH for all studied water samples of Pliocene aquifer and lakes, reflect alkaline water conditions, The variations of pH value are mostly due to the chemical composition of the aquifer rocks.

3.1.2 Electric Coductivity (EC):

For the groundwater of the Pliocene aquifer, electric conductivityvaries between 0.472 m mohs/cm in well no.(30) at the south east , and 10.660 m mohs/cm in well no. 39 (Clay Quarries) at the middle part of wadi El Natrun.For

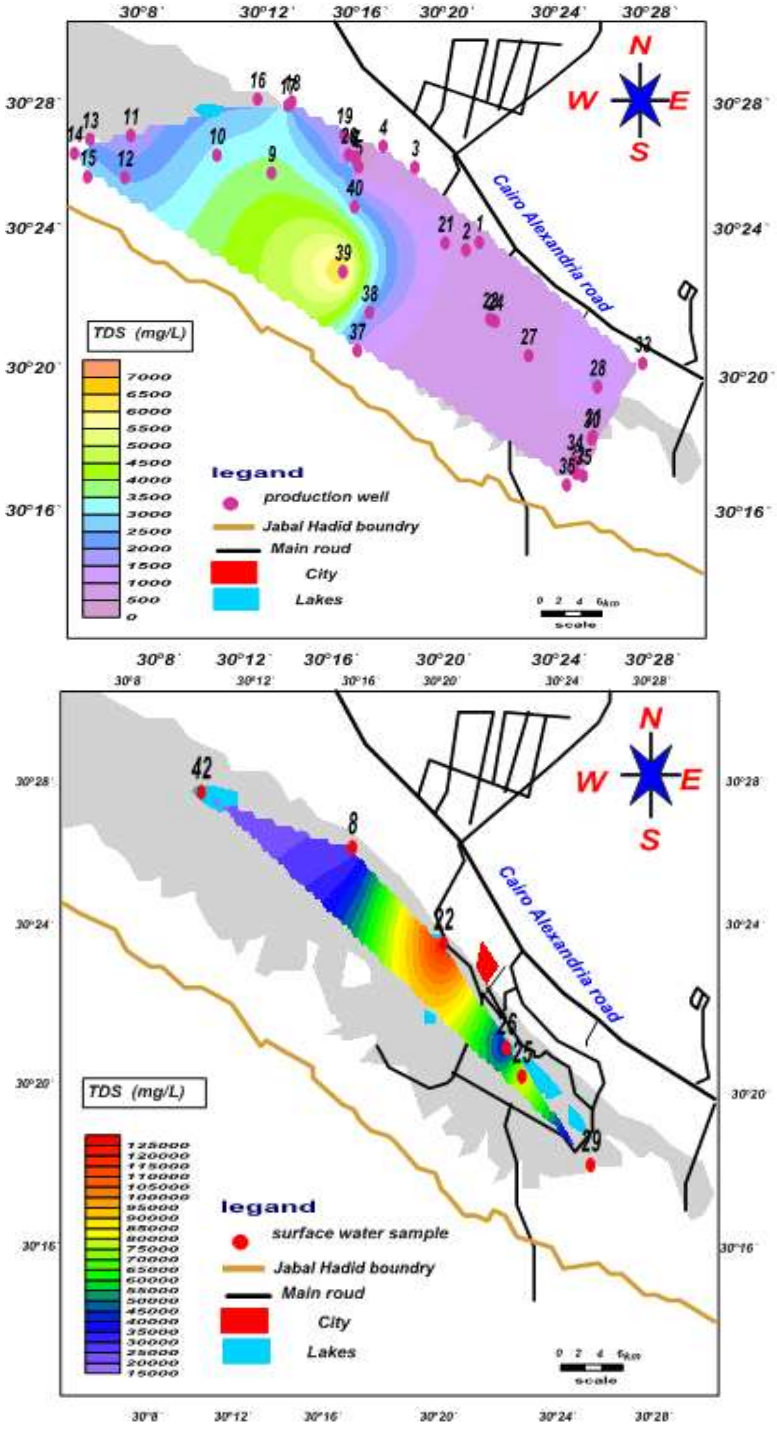
http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

surface water samples(lakes)in the study area , the electricconductivity varies between 18.9 m mohs/cm in sample no. 29(Malahet Bani Salama) , and 192 m mohs/cm in sample no. 22(El Hammra lake)

3.1.3 Total Dissolved Solids (TDS):

The salinity contents ranging from 300 mg/l in well no. 30 at the south east of Wadi El Natrun to 6822 mg/l in well no.39 (Clay Quarries) at the middle part of wadi el Natrun, the salinity increase from east to west in the direction of water flow. The groundwater of the Pliocene aquifer is mainly belongs to brackish saline water. The surface water samples of the lakes is mainly belongs to saline water with salinity contents ranging from 12096 mg/l in sample no.29 (Malahet Bani Salama) at south east to 122880mg/l in sample no.22 (El Hamra lake) .(Figure4)

(A)



(B)

Fig.4: Salinity distribution contour map of productive wells of the Pliocene aquifer (A) and lakes (B)atWadi-El Natrun

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

About **8**% of tested samples have salinity content less than **500** mg/l. **21** % of tested samples have salinity content ranging from 500-750 mg/l. 17 % of tested samples have contents ranging from 750-1000 mg/l. 54 % of tested samples have contents more than 1000 mg/l.According to theWorld Health Organization (W.H.O.2005) the maximum permissible limits of TDS in drinking water is 1000 mg/l. It's concluded that 45 % of tested samples are permissible, where 54% not permissible.

3.1.4 Distribution of hydrochemical elements in ground and surface water:

3.1.4.a- Major ions distribution in groundwater

Regarding major cations, sodium is mostly predominant cation followed by calcium and magnesium. Sodium concentration ranges from 50 mg/l (well No. 31) to 2360 mg/l (well No. 39). High sodium concentration is possibly due to leaching processes of clay and shale present in aquifer materials. Calcium concentration ranges from 12.8 mg/l (well No. 1) to 114 mg/l (well No. 39). Magnesium concentration ranges from 2.43 mg/l (well No. 4) to 21.39 mg// (well No. 33). Concerning major anions, chloride and sulphate are mostly predominantly over bicarbonate. Chloride concentration ranges between 40 mg/l (well No. 31) and 3000 mg/l (well No. 39). High values of Cl^- content is mainly attributed to dissolution of chloride–bearing deposits evaporates and clay minerals within the aquifer materials. . Sulphate concentration ranges from 49 mg/l (well No. 31) to 1600 mg/l (well No. 6). High sulphate concentration reflects dissolution of terrestrial deposits of gypsiferous shale and gypsum in the aquifer materials. Bicarbonate concentration ranges between 86 mg/l (well No. 32) and 320 mg/l (well No. 7).

3.1.4.b-*Major ions distribution in surface water (Lakes)*

Major cations in surface water samples (lakes)**,** sodium is mostly predominant cation followed by calcium and magnesium,Sodium concentration ranges between 4350ppm in sample no.29(MalahetBenisalama) to 44200 ppm in sample no. 22 (El Hammra lake) .High contents of sodium ion over the study area are due to active cation exchange and leaching processes of clays and salts present in aquifer material.calcium contents ranging from 70 ppm in sample no.29 (Malahet Beni Salama) at south east to 300 ppm in sample (no. 22)(El Hammra lake) at east. The high values of calcium concentration is mainly comes from dissolution of sediments rich in calcium. This may be due to leaching and dissolution processes of gypsum bearing of aquifer material. Magnesium contents ranging from 70 ppm in sample no. 29(Malahet Beni Salama) at south east to 200 ppm in sample no. 22 (El Hammra lake) at east. Potassium concentration ranges between 200 ppm in sample no. 29 (Malahet Beni Salama) to 540 ppm insample no. (26 Rozitta lake. Concerning major anions, chloride and sulphate are mostly predominantly over bicarbonate,chloride concentration ranging from 5500 ppm in sample no.29 (Malahet Beni Salama) to 54000ppm in sample(no. 22 El Hammra lake).The presences of chloride ion with high concentration in groundwater reflect the presence of evaporite salts rich in chloride. Chloride concentration generally increases from east to west with the direction of water flow.Sulfate contents ranging from 1450 ppm (in sample no. 41 El Gaar lake) at north to 20900ppm (in sample no. 22 El Hamralake) in east ,the high increase in sulfate contents was recorded at the north west part of the study area. This reflects effect of downward seepage of sanitary and agricultural waste water rich with sulfates, and to local terrestrial source of sulfate as gypsum.Bicarbonate has concentration ranging from170 ppm in sample no. Rozitta to 500 ppm in sample no22.(El Hammra lake). The highest value was recorded in east portion of the study area. The highest concentrations of bicarbonate reflect dissolution of carbonate rich sediments as well as the effect of Quaternary aquifer recharge at the east of the study area.

The relation between salinity (TDS) and major ions were statistically illustrated (Figure 5). This diagram shows correlation between salinity contents and concentration of ions of groundwater of the Pliocene aquifer. Sodium, sulphate and chloride show high correlation coefficients (R2) with salinity contents, the values of correlation coefficients (R2) of these ions are 0.8966, 0.8939 and 0.9464 respectively, this indicates that, the factors, which govern the distribution of salinity in the different localities, are the same factors controlling the distribution of sodium, sulphate and chloride. The factors controlling the distribution of such elements include upward leakage of of Miocene saline water and leaching processes of clay and lagonaal deposits present in aquifer materials. On the other hand, calcium and magnesium show low correlation coefficient (R^2) with salinity compared with that of sodium, sulphate and chloride, it is 0.2864 and 0.2564 respectively. Bicarbonate shows no correlation with salinity, it displays correlation coefficient (R^2) 0.0501.

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

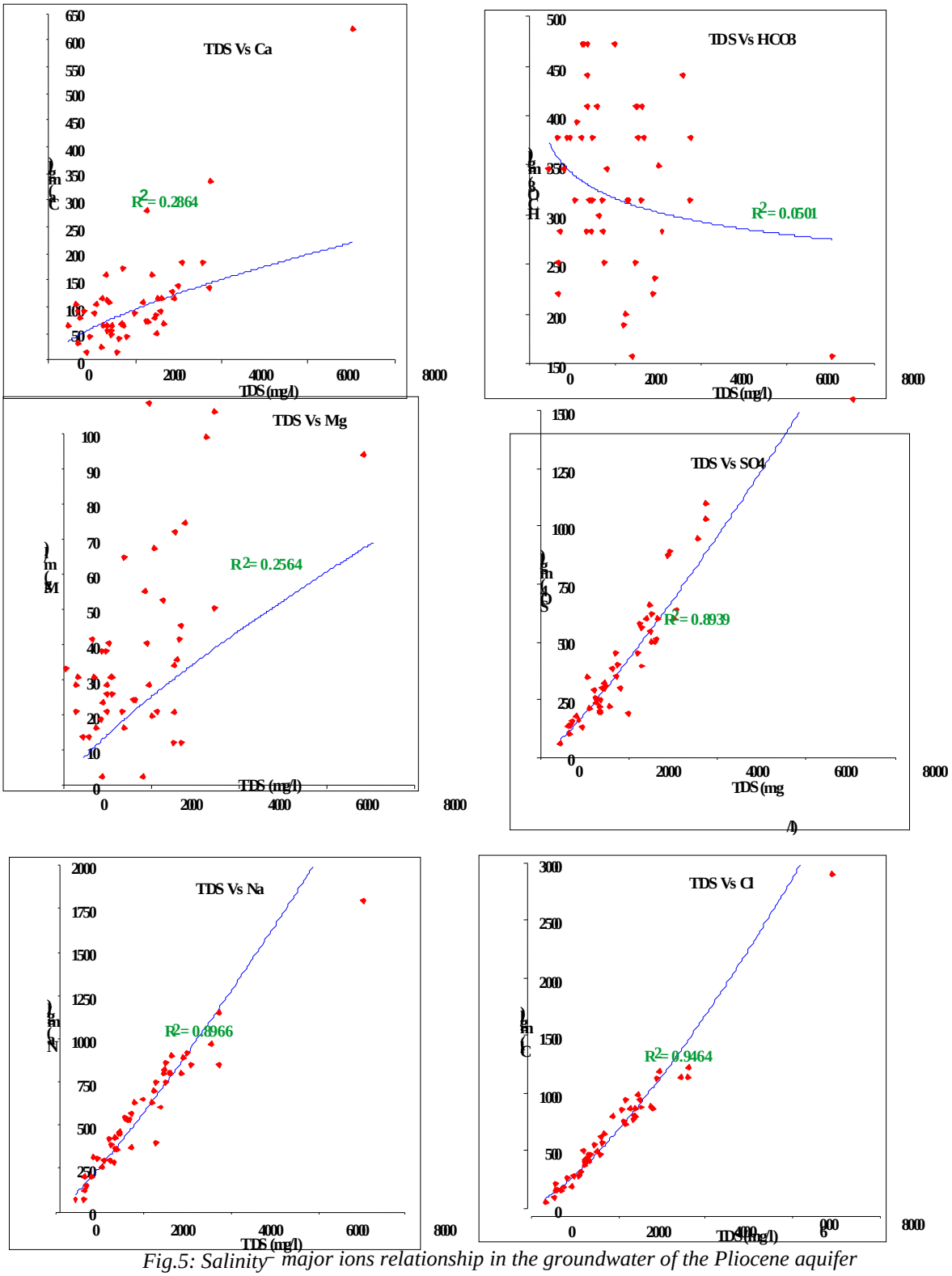


Fig.5: Salinity- major ions relationship in the groundwater of the Pliocene aquifer

3.2.-Ions dominance and water type of ground and surface water in the study area:

The cations and anions for the Pliocene aquifer are determined.

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

The sequences of the ions represent the variation of chemical composition of water, which is mainly controlled by lithology from recharging to discharge area also affected by presence of the fault, cracks and different structures,(Hem, J. D., 1989).

In this thesis water type defined by the following

1- **Schoeller's.** 2- **Piper**

3.2.1 According to Schoeller's diagram (1962): Firstly for ground water:

.

The majority of groundwater samples have Ion sequences ordering **(Na^+>Ca^{++}>Mg^{++}) , Cl^-> SO_4^{--}> HCO_3^{-}) - (HCO_3^- > Cl^-> SO_4^{--})** .Dominance of sodium, calcium and magnesium (Na^+, Ca^{++}, Mg^{++}) as cation, chloride and sulphate (Cl^-, $So4^{--}$) as anion in the majority of samples reflect the predominance of sodium and calcium (Na^+, Ca^{++}). The dominant salts are Sodium Colrid and Sodium Sulphate water types.From figures**(6&7)**. The prevailing hypothetical salts combinations are NaCl and Na_2SO4 water type followed by NaHCO3

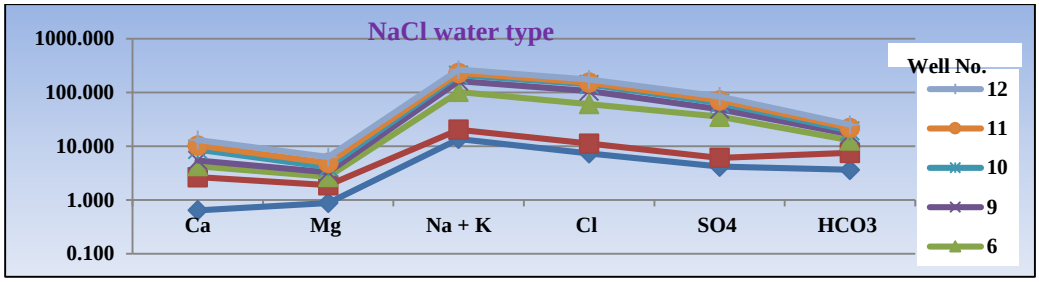


Fig.6: Sodium Chloride facies of groundwater Pliocene aquifer in Wadi El-Natrun

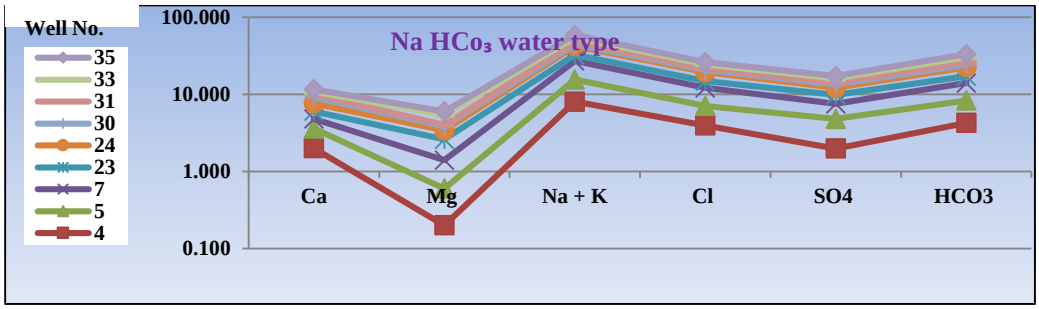


Fig.7: Sodium Bicarbonates facies of groundwater Pliocene aquifer in Wadi El-Natrun

Secondary for surface water:

Ion sequences ordering of surface water samples have cations and anion sequence ordering (Na^+>Ca^{++}>Mg^{++}) ,(Na^+> Mg^{++}>Ca^{++}) and -(Cl^-> $SO4^{--}$> $HCO3^{-}$).

Dominance of sodium, calcium and magnesium (Na^+) as cation and chloride and sulphate (Cl^-, $So4^{--}$) as anion in the majority of samples reflect the predominance of sodium (Na^+). Dominant hypothetical salts are Sodium Chlorid and Sodium Sulphate water types **(Figure8)**

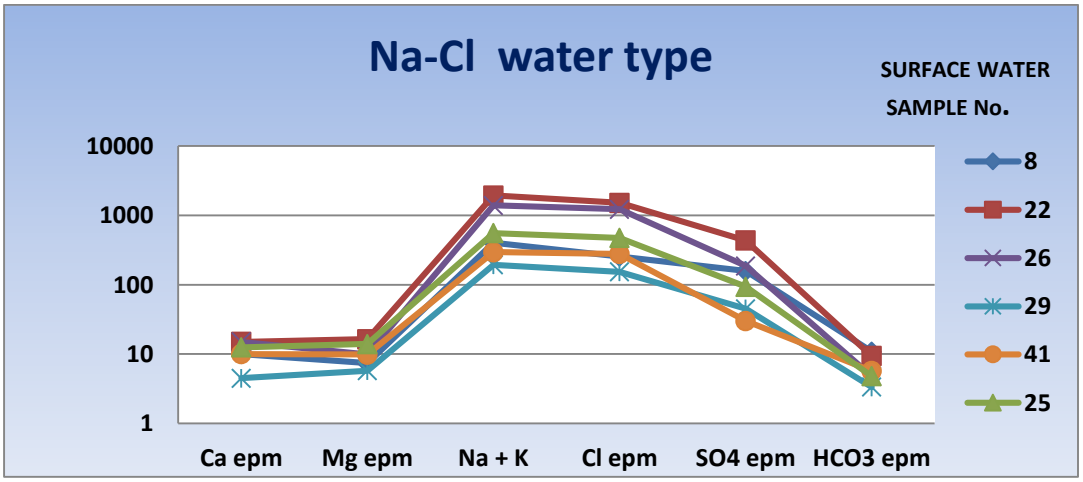


Fig.8:Sodium Chloride facies of Surface water in Wadi El-Natrun

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

3.2.2 Classification of groundwater and surface water According to Piper (1953):

The geochemical classification of groundwater is based on ion relationships, the most common are the tri linear diagram of (**Piper ,1953).** Piper's triangle diagram for groundwater wells consequently show that **(Figure9),** most groundwater samples and all surface watersamples (Six samples) lie in the upper triangle and has secondary salinity properties, where $SO_4^{--} + Cl^- > Na^+ + K^+$.So, the hypothetical salts are Ca and Mg chlorides and sulphate. Few water samples (represent the eastern part)lies in the lower triangle and the primary alkalinity proportion where $\mathbf{CO3^{--} + HCO_3^- > Ca^{++} + Mg^{++}}$ and the hypothetical salts are Na and K carbonate and bicarbonate.

(A) **(B)**

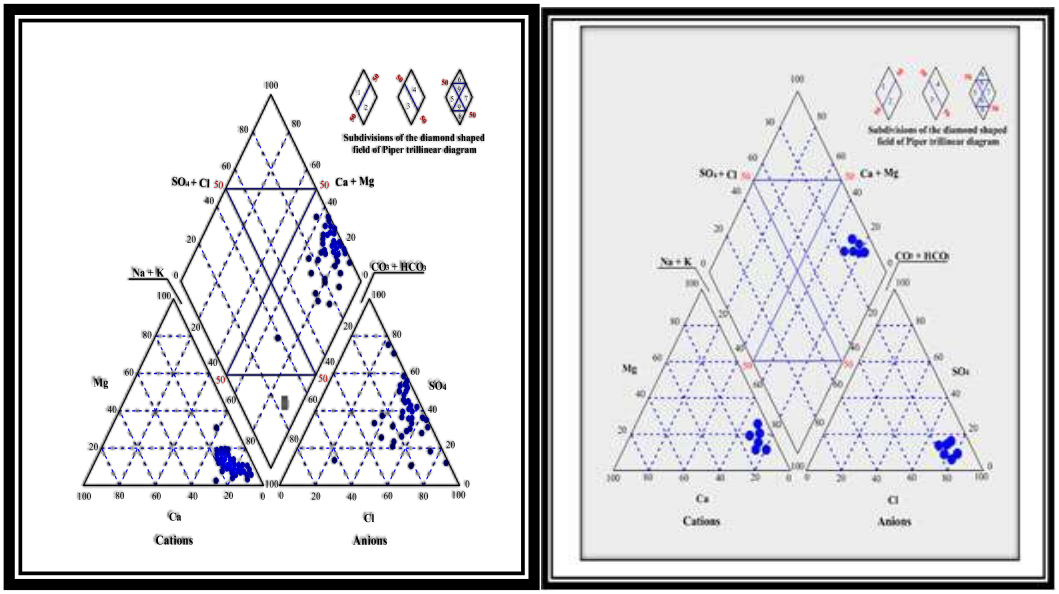


Fig.9: Piper triangle diagram for groundwater samples (A) and lakes (B) of the Pliocene aquifer.

3.3-Hydrochemical coefficient ratios

The relationship between the different major ions ratios is helpful in detecting the previous hydrochemical processes affecting water quality. The hydrochemical coefficients are used as a tool for detecting the origin of groundwater and helped in discovering the previous hydrochemical processes affecting water quality such as leaching, mixing and ion exchange.

The hydrochemical coefficients (rNa/rCl, rSO4/rCl,rCa/rMg) and (rHCO3/rCl) revealed good information about the origin of groundwater and helped in detecting the hydrochemical processes affecting water quality. The values of rNa/rCl for the analyzed water samples range between 1.02 and 2.86 in groundwater and between 1.03 and 1.54 in the lakes. These values indicate predominance of sodium over chloride reflecting meteoric water origin. The increase in the concentration of Na+ ion than Cl- inthese water samples is mainly attributed to the dissolution of sodium bearing silicates. The values of hydrochemical coefficient rSO4/rCl range between 0.39 and 1.26 between 0.11 and 0.29 in the lakes. High value of this coefficient is mainly due to the dissolution processes of local terrestrial sulphate minerals present in aquifer materials. The hydrochemical coefficient rCa/rMg shows high values varying from 0.70 to 12.85, between 0.78 and 1.25 in the lakes.which is more related to rainwater value (3.08) than normal sea water (0.21). This confirms the meteoric water origin of groundwater of the Pliocene aquifer in the study area.The value of the hydrochemical coefficient ($rHCO_3/rCl$) in the groundwater varies between 0.04 (well No. 39) at the west of the study area to 1.5 (well No.2) at east with an average of 0.48 in ground water wells. The majority of samples have value more than unity which indicates predominance of bicarbonate over chloride and leaching process of carbonate bearing minerals as well as precipitation of chloride minerals at the east and vice versa at the west where the chloride salts are the dominant

The value of the Hydrochemical coefficient ($rHCO_3/rCl$) in the surface water samples varies between 0.006 at sample No.22 (El Hammra lake) at the east of the study area to 0.2 at sample No. 41 (El Gaar lake) at north with an average of 0.01 in surface water samples, where the chloride salts are the dominant. More confirmation of this concept is the occurrence of NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $Mg(HCO_3)_2$ and $Ca(HCO_3)_2$ in groundwater .The presence of marine salts of NaCl, Na_2SO_4 and $MgSO_4$ and $CaSO_4$ is mainly due to the flushing of salt water by fresh water through local heavy infiltration of rainwater in the past pluvial times and the long –term contact time between rock matrix and

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

groundwater also, due to the dissolution of these salts encountered in the Quaternary and Pliocene water bearing sediments, return flow of irrigation water and salt water encroachment as a result of over pumping specially at the middle and western portion.

3.4- Water/ Rocks interaction

Water/ Rocks interaction focuses on the interactions that occur between groundwater and the solid phases comprising the unsaturated and saturated zone, these solids phase consist of inorganic material (minerals and amorphous compounds) and organic materials. Solid are important to the geochemistry of the system because they are the primary sources and sinks of dissolved constituents. The primary water rocks interaction described in this work are adsorption /desorption and mineral precipitation / dissolution process (Deutsch, W. J. 1997).,(Fattah K.,M. 2012), (Fattah and Abdelrazak, 2014)

3.4.1-Adsorption /desorption

Adsorption is the removal of a dissolved species from solution by its attachment to the surface of a solid, desorption is the release of the species back into the solution. Adsorption /desorption will be discussed in term of ion exchange which primary impact the major cation in solution. Adsorption /desorption will be discussed through

a-Hypothetical salts b- Cation Exchangeprocess c- The cation exchange index

3.4.1.a- **Hypothetical salts combinations**

Hypothetically, ions of strong acids (Cl^- and SO_4^{2-}) form chemical combination with alkali (Na^+ and K^+) and the rest of acid radicals combine with alkaline earths (Ca^{2+} and Mg^{2+}). If the cations of alkali and alkaline earths are surplus in groundwater, they well combine with anions of the weak acids (CO_3^{2-} and HCO_3^-).

The hypothetical salts assemblages of groundwater of Pliocene aquifer have the following assemblage:

Assemblage I:

NaCl, Na_2SO_4, $NaHCO_3$, $Mg(HCO_3)_2$ and $Ca(HCO_3)_2$

(Wells no. 1,2,3,4,5,6,7,9,11,14,15,18,20,21,23,24,27,31,33,35) (57.5% of the total samples).

Assemblage II: NaCl, Na_2SO_4, $MgSO_4$, $Mg(HCO_3)_2$, $Ca(HCO_3)_2$

(Wells no.12,19,30,34) (11.5% of the total samples).

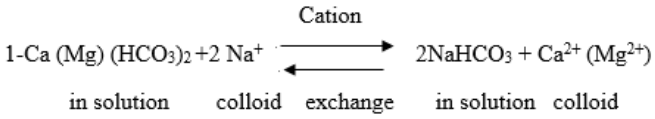
Assemblage III: NaCl, Na_2SO_4, $MgSO_4$, $CaSO_4$, $Ca(HCO_3)_2$

(Wells no.10,13,16,17,28,32,36,38,39,40) (31% of the total samples).

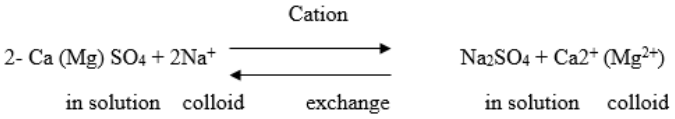
Most groundwater samples of the Piocene aquifer are characterized by the following assemblages of salt combination. Assemblages I (three bicarbonate salts) and II (two bicarbonate salts) reflects the effect of pure rain water or fresh surface water on groundwater and also reflect recent recharge (from Quaternary aquifer). Assemblage III (three sulphate salts), and assemblage II(two sulphate salts) (42% of the total groundwater samples) reflect the effects of leaching and dissolution of evaporation deposits and represent intermediate stages of chemical development. Regarding hypothetical salt combinations of surface water (Lakes) in the study area, all surface water samples have the following assemblage: NaCl, $Na2SO_4$, $MgSO_4$, $CaSO_4$, $Ca(HCO_3)_2$.(100% of the total surface water samples). Three sulphate salts, reflect the effects of leaching and dissolution of evaporation deposits and represent intermediate stages of chemical development. The presence of three types of sulphate salts with high concentrations which reflects the effect of old marine salt pollution (marine facies groundwater type) with possible contribution of cation exchange phenomena resulting from the presence of the clay intercalated with aquifer in the study area.

3.4.1.b- Cation Exchange process

Cation Exchange process is a type of adsorption /desorption phenomenon that applied principle to material with a porous lattice containing fixed charge**.** Clay minerals are the most common ion exchangers in the soil and aquifer environments. The fixed charge on clay minerals is a result of substitution of Al^{3+} for Si^{4+} in the tetrahederal clay lattice sites and Fe^{2+} and Mg^{2+} for Al^{3+} in the octahederal lattic sites, the presence of the lower charged cation in the structure results in a net negative charge on the surface of the clay this evident that, the groundwater types affected by leaching and dissolution of terrestrial salts (assemblages of hypothetical salts combination I,2,3 and 4), are possibly accompanied by cation exchange process. This process is related to clay minerals assemblage, dominated by kaolinite, palygorskite, chlorite, vermiculite and illite (hydrous mica) as well as amorphous inorganic materials .As measured in the laboratory. The cation exchange process leads to an increase or a decrease in the water salinity, temporary hardness (equation 1) and permanent hardness (equation 2) as follows:



http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*



Several indices are used for the identification of water that have undergone cation exchange processes (Matthess, G., 1982),(Appelo CAJ and Postma D.2005):.

The alkali number is expressed as 100(Na+K)/(Cl, me/l), an increase or decrease of the alkali number is mainly attributed to cation exchange that takes place under three conditions as follows:

a- Up to alkali number 100, alkalis in their halogens in solution replace Ca and Mg on the surface of clay minerals in aquifer matrix.

b-From 100 to 120 alkaline earths (Ca and Mg) in their sulfates and part of their carbonate in solution replace alkalis on the surface of clay minerals in aquifer matrix.

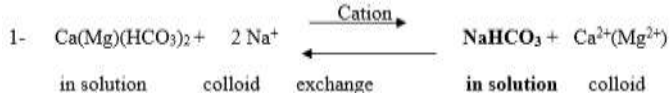
c- Above 120, alkaline earths (Ca and Mg) in their carbonate in solution replace alkalis on the surface of clay minerals in aquifer matrix.

From tables (1 in appendix I), most groundwater samples (94%) of the Pliocene aquifer display an alkali number above 120 also most of groundwater types have the assemblages of salt combination I and II which reflect the effect of leaching and dissolution of terrestrial salts (continental facies groundwater) with some contribution of cation exchange process, forming hypothetical salt combination as follows:

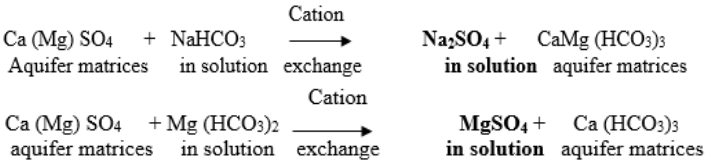
Assemplage I NaCl, Na_2SO_4, $NaHCO_3$, Mg HCO_3, $Ca(HCO_3)_2$

Assemplage II NaCl, Na_2SO_4, $MgSO_4$, Mg HCO_3, $Ca(HCO_3)_2$

In this case, alkaline earths (Ca^{2+} and Mg^{2+}) in their carbonate in solution replace alkalis on the surface of clay minerals in aquifer matrix. As a result of cation exchange process, the increase of Na^+ concentration and decrease in Ca^{2+} and Mg^{2+} concentration in solution and appearance of **$NaHCO_3$** salt. This is in agreement with the following cation exchange equation (1):



The absence of both $NaHCO_3$ and Mg $(HCO_3)_2$ and appearance of **Na_2SO_4** and **$MgSO_4$** salts in some groundwater samples, may be result from cation exchange after simple dissolution of $Ca(Mg)SO_4$ salts from the aquifer matrices or the sourrounding area and the catchements areas through heavy local precipitation in the past times that leads to **the replacement of $NaHCO_3$ and Mg$(HCO_3)_2$ salts by Na_2SO_4 and Mg$(HCO_3)_2$** salts and according to the following reactions:

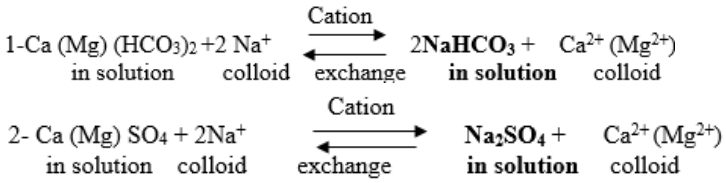


On the other hand, minor groundwater samples (6%) of the Pliocene aquifer have an alkali number that ranges from 100 to 120. These groundwater types are related to the salt combination assemblages I and 2 which reflect the effect of leaching and dissolution of terrestrial salts (continental facies groundwater) combination I and II as follows:

Assemplage I NaCl, Na_2SO_4, $NaHCO_3$, Mg HCO_3, $Ca(HCO_3)_2$

Assemplage II NaCl, Na_2SO_4, $MgSO_4$, Mg HCO_3, $Ca(HCO_3)_2$

In this case, alkaline earths (Ca^{2+} and Mg^{2+}) in their sulfates and part of their carbonate in solution, replace alkalis on the surface of clay minerals in aquifer matrix. This leads to appearance of Na_2SO_4 and disappearing of $CaSO_4$ As a result of cation exchange process. , the increase of Na^+ concentration and decrease in Ca^{2+} and Mg^{2+} concentration in solution, lead to a slight decrease in salts causing temporary hardness in form Ca $(HCO_3)_2$ which is in equilibrium with P_{CO2} and permanent hardness in the form of both $CaSO_4$ and $MgSO_4$, while causing a slight increase in water salinity but no change in pH and HCO3- content because each mole of Ca^{2+} or Mg^{2+} adsorbed is replaced by 2 moles of Na^+. The loss of Ca^{2+} and Mg^{2+} decreases the degree of water saturation with respect to both carbonate and gypsum minerals, leading to ion activity products (IAP) of carbonate and gypsum minerals became less than that of the solubility product constant (K_{SP}), (Freeze, R.A. and Cherry, G.E. 1979). .. This is supported by the finding that groundwater samples are unsaturated with respect to gypsum (sulphates minerals). This case is quite coincident with the following cation exchange equations (1 and 2):



The disappearance of both $NaHCO_3$ and Mg $(HCO_3)_2$ salts in the groundwater samples are due to the same already mentioned reasons.

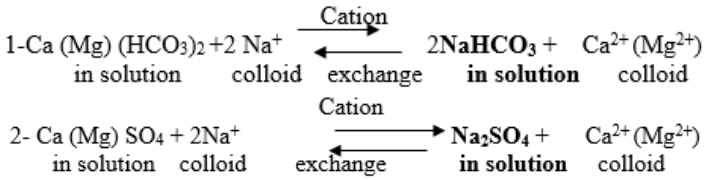
3.4.1.c-The Cation exchange index

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

For further elucidation of the data, the cation exchange index is employed where cation exchange index = **rCl – r(Na+K)/ rCl**

This ratio has either negative or positive values. The negative value means that, alkaline earths (Ca and Mg) in water replace alkalis (Na^+ + K^+) on the surface of clay minerals in aquifer and vice versa in case of positive value, Glynn PD, Plummer LN (2005) .

All groundwater samples of the Pliocene aquifer have negative values of cations exchange index, regardless of water salinity. (tables 1 to 3, in appendix I). This means that alkaline earths (Ca and Mg) in water replace alkalis (Na^+ and K^+) on the surface of clay minerals in aquifer matrices. Combination of cation exchange index, alkali number and hypothetical salts data reveals that, most groundwater of the Pliocene aquifer (continental facies groundwater) are characterized by an alkali number above 100, negative values of cation exchange index and terrestrial salts (I and II assemplages). This case is quite coincident with the following cation exchange equations (1 and 2):



The disappearance of both $NaHCO_3$ and $Mg(HCO_3)_2$ salts in the groundwater samples are due to the same already mentioned reasons.

In brief, one can conclude that in most cases, cation exchange phenomenon in the Pliocene aquifer is playing an important role in the hydrogochemistry of the study area and in the relation of water rocks interaction.

3.5.- Spatial variation in groundwater chemistry:

Spatial variation in groundwater using toexplain geochemicalevolution, geochemical variation and water-rock interaction of the local groundwater in the study area through the Pliocene aquifer.Geochemical profiles was performed for three groundwater paths (South, Centre and North path 1 (A-A\), path 2(B-B\) and path 3 (C-C\) Figure(10)

3.5.1. Along the east–west in southern part ;(Profile A – A\ cross section)

This profile passes through Three productive wells (33,27,37) representing the Pliocene aquifer (Figure11). This hydrochemical profile is developed along the Pliocene aquifer in the study area from east to west direction, where the general flow of groundwater is in the same direction.

From this profile (Figure11) the following points could be concluded:

1-The total dissolved salts (TDS) increase in the west direction in the same direction of groundwater flow to reach their maximum values at well No. 37 (**1280** mg/l) in middle part of study area.

2- The behavior of different ions concentration is developed along the profile where there is increasing in each of Na, Ca and Cl, in west direction while the content of each of Mg, SO4 and HCO3 decrease in the same direction.

3- There is superiority of Na^+ over Cl^- in all groundwater sample, indicating leaching and dissolution of terrestrial salts.

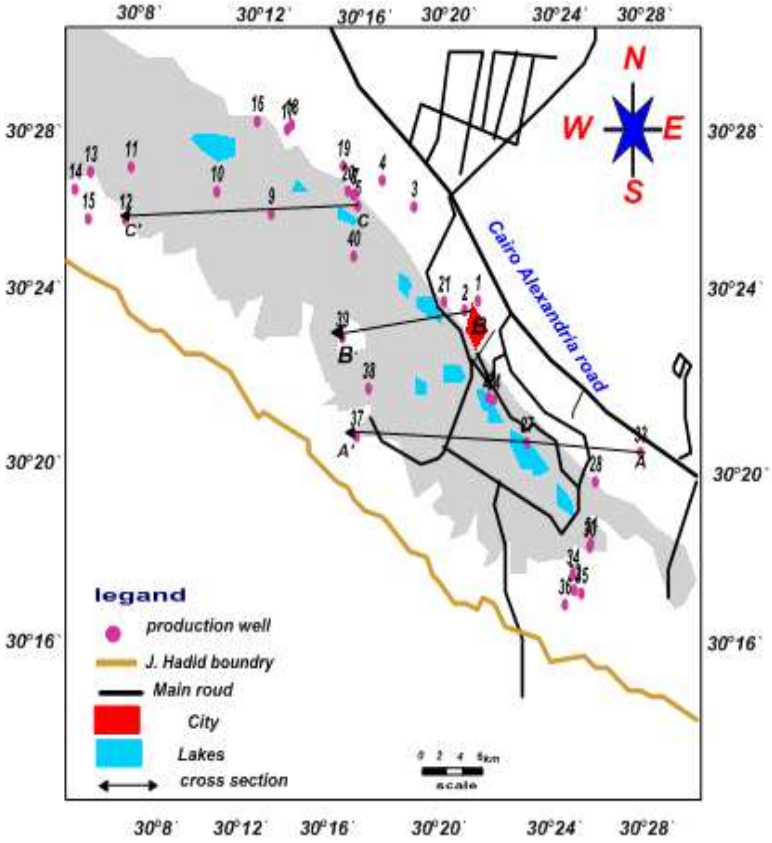
4-Carbonate contents represent about **24** % from TDS at the east part (well 33) while it reach to about 5% from TDS at the west.

5- The assemblage I of salt combination KCL, NaCl, Na_2SO_4, $NaHCO_3$,$Mg(HCO_3)_2$ and $Ca(HCO_3)_2$ is dominant in ground water samples in the east direction, while the assemblage II of salt combination KCl, NaCl, Na_2SO_4, MgSo4, CaSO4, $Ca(HCO_3)_2$(Well NO.37)is dominant in ground water samples in the west direction.

6- The ions displays two grades of metasomatism along this profile, first the stage of mineralization (HCO3>Cl>So4) at wells No. (33 , 27) then the advanced stage of mineralization (Cl>SO4 >HCO3) at well no 37. These agree with the general gradient of chemical evolution of groundwater.

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

(A)



(B)

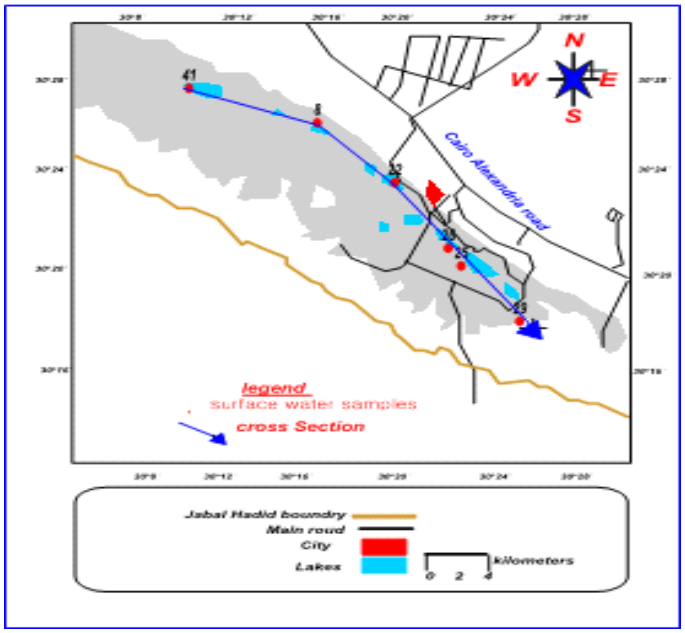


Fig.10: Profiles selected along the groundwater wells (A) and lakes (B)

3.5.2-Along the east – west in middle part ; (Profile B – B\ cross section)

This profile passes through three wells (2,21,39) (Figure12). The total salinity of the groundwater along this profile is (**410** mg/l at well No.2) then increases to(1206 mg/l at well No21) then increases in the west direction to reach their maximum value at well NO.39 (**6822** mg/l). Bicarbonate content represents 35 % from TDS at (well

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

No.2) then gradually decreases to **9** % from TDS at(well No.21) then it decreases to **4**% from Tds at well No 39/. While the concentration of SO_4 and Cl are generally increase in the west direction along this profile. Concerning the metasomatic change of water chemistry in horizontal direction, it is clear that the groundwater of the Pliocene aquifer in this profile has change in groundwater composition, one can notice that KCl, $Mg(HCO_3)_2$, $Na_2SO_4$and $NaHCO_3$ salts has changed behavior between increasing in well no 2 and decreasing in well 39 , this behavior may relate to recharge from the Quaternary aquifer in well no. 2 while NaCl increases in west direction in well no. 39 this agree with the general gradient of chemical evolution of groundwater. The assemblage of salts combination (I) KCl, NaCl, Na_2SO_4 NaHCO3,$Mg(HCO_3)_2$ and $Ca(HCO_3)_2$ is the dominant in groundwater samples at wells No (2,21). The assemblage of salts combination (II) KCl,NaCl, Na_2So_4 $MgSO_4$, $CaSO_4$ and $Ca(HCO_3)_2$.at well No39. The ions displays two grades of metasomatism along this profile, the first grade is a less advanced stage of mineralization (HCO_3 > SO_4 > Cl) at east part and the second grade is a more advanced stage of m ineralization(Cl> SO_4> HCO_3) at west part. These agree with the general gradient of chemical evolution of groundwater.

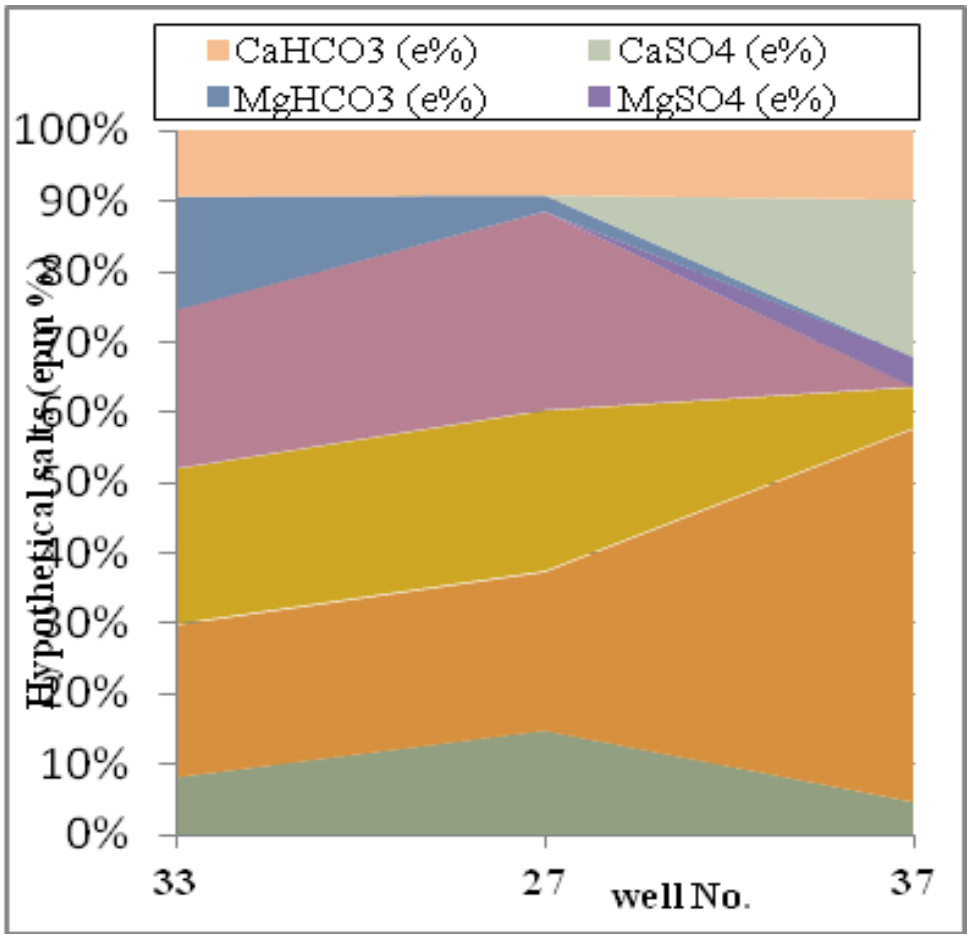


Fig.11: Hydrochemical cross section A - A'

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

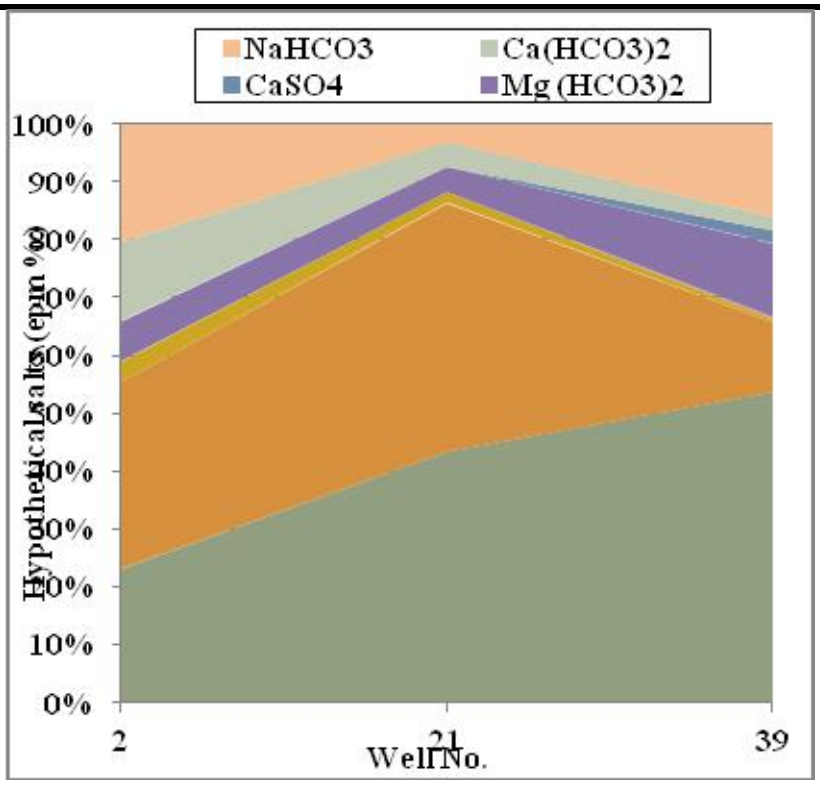


Fig.12: Hydrochemical cross section B - B'

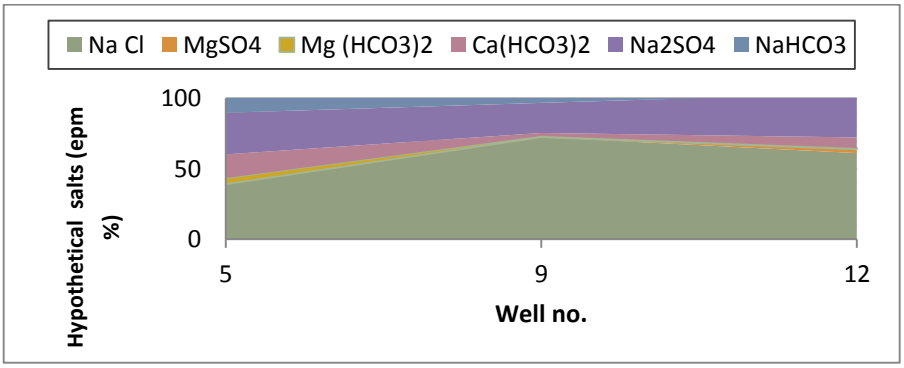


Fig.13: Hydrochemical cross section C - C'

3.5.3.Along the east – west in nortern part ;(Profile C – C\ cross section)

This profile passes through three productive wells (5,9,12) representing the Pliocene aquifer (Figure13). This hydrochemical profile is developed along the Pliocene aquifer in the study area from east to west direction, where the general flow of groundwater is in the same direction.

From this profile the following points could be concluded:

-The total dissolved salts (TDS) are increased from (691 mg/l) at well NO. 5 at east to reach their maximum values at well No.9 (**3712**mg/l) then it decreases o(2432 mg/l at well no. (12) in west direction. Bicarbonate contents represent about **33** % from TDS at eastern part (Well No. 5) then it decreases to 5% at Well No. (9) thenit increases to 7% at well no.12 in west direction. The concentration of sulphate and chloride are generally increase groundwater flow direction along this profile follow the geochemical evolution system reported by(Burdon**1958),**(Back, W. and Hanshaw, B. B. 1979): this suggestion is agreement with the fact that there is recharge and groundwater flow from east to west direction. Concerning the metasomatic change of water chemistry in horizontal direction[21] Glynn PD, Plummer LN (2005), it is clear that the groundwater of the Pliocene aquifer in this profile has change in groundwater composition, one can notice that NaCl, increased towards west direction, while KCl, Na_2SO_4,$NaHCO_3$ $Ca(HCO_3)_2$ and $Mg(HCO_3)_2$ salts decrease in the same direction. The

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

assemblage of salts combination(I) KCL, Nacl, Na_2SO_4, $NaHCO_3$, Mg $(HCO_3)_2$ and$Ca(HCO_3)_2$is the dominant in groundwater samples in east direction well no (5,9) .while the assemblage of salt combination (II) KCL, Nacl, Na_2SO_4,$MgSO_4$, $Mg(HCO_3)_2$ and $Ca(HCO_3)_2$ is dominant in groundwater samples in the west direction well no(12). The ions displays different grades of metasomatism along this profile, the first grade is a less advanced stage of mineralization ($HCO_3 > Cl > SO_4$) at the east point well (NO. 5).The second behavior is ($Cl > SO_4 > HCO_3$) at wells no (9,12) These indicated that evolution degree reaches the maximum.

3.6- Evaluation of groundwater:

3.6.1- Evaluation of groundwater for drinking.

According to the **World Health Organization (W.H.O.2005)** the maximum permissible limits of TDS in drinking water is **1000** mg/l. It's concluded that **45** % of tested groundwater samples are permissible, while 55 % not permissible

3.6.2- .Evaluation of groundwater for irrigation

3.6.2.a-According to U.S. Salinity Laboratory Staff (1954):

Groundwater samples collected from the Pliocene aquifer in the study area were evaluated according to U.S. Salinity Laboratory Staff (1954)based on the Sodium Adsorption Ratio (SAR), and the specific conductance (Figure14). The recommended classification of water for irrigation according SAR ranges are shown in (Table 3 in App.). **SAR = $Na^+/[(Ca^{++} + Mg^{++})/2]^{1/2}$** .Where, the concentrations of these cations are expressed in epm. According to the U.S. Salinity Laboratory diagram Fig(14). As recorded above, it clears that: **24** % of groundwater samples of the Pliocene aquifer fall in the good water class (C2-S1) and class (C2-S1) which good for soils of medium permeability for most plants. 51% fail in the moderate water class (class (C2-S2) and class (C3-S2) which Good with coarse grained permeability soils, unsatisfactory for highly clayey soils with low leaching and Satisfactory for plants, having a moderate salts tolerance on soils of moderate permeability with leaching. 20% fail in the intermediate and 6% fail in bad water class Suitable only with good drainage and satisfactory for salt tolerant crops on soils of good permeability with special leaching.

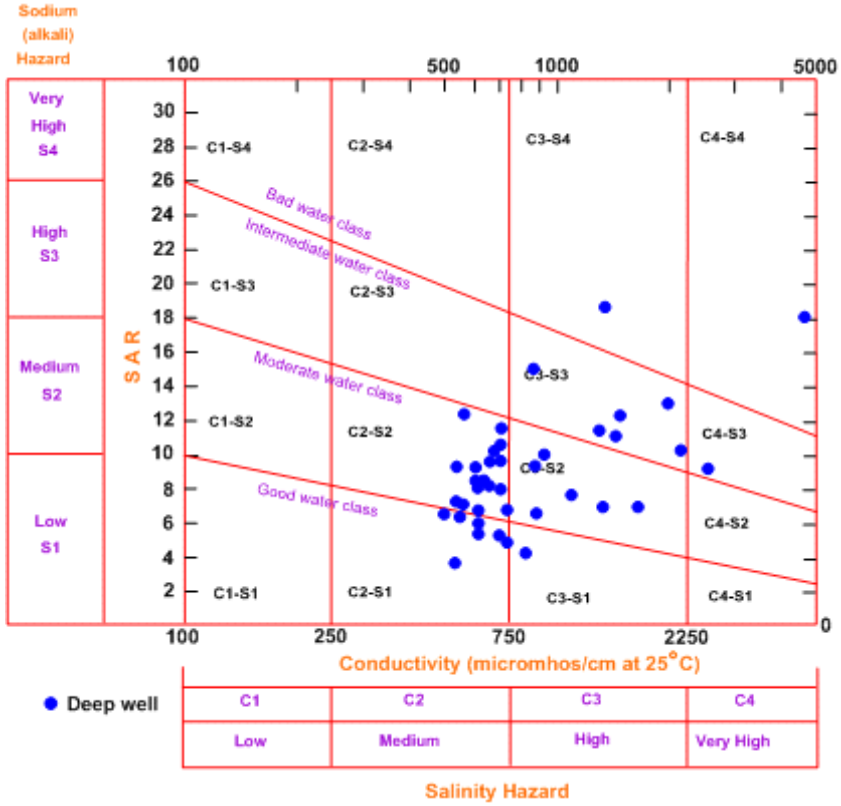


Fig.14: Water classification for irrigation (according to US Salinity Lab. 1954

3.6.2.b- Evaluation of groundwater according to Residual Sodium Carbonate (RSC):

When the sum of carbonate and bicarbonate is in excess of calcium and magnesium, there is almost complete **precipitation** of the latter **(Eaton, 1950)**. This can cause an increase in the proportionate amount of sodium, and so, the effect on the soil is high. The term Residual Sodium Carbonate is defined as follows: **RSC = $(CO_3^{--} + HCO_3^-) - (Ca^{++} + Mg^{++})$inepm.(Richards ,1954)** stated that, water having more than **2.5**epm RSC is not suitable for irrigation, water containing (**1.25-2.5 epm**) RSC is

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

marginal, and water have less than **1.25**epm RSC is probably safe. According to the calculated RSC of the Pliocene groundwater samples, (Appendix1, table 3), **48.5**% of groundwater samples safe for irrigation purposes, 28.5 % of groundwater samples are marginal and this water is suitable for irrigation in this area under normal condition. and 23% from the samples is not suitable for irrigation according to(RSC).

3- CONCLUSIONS

Using geological, hydrological and hydrogeochemical knowledge's a clear understanding of the origin of hydrogeology, hydrogeochemistry and water resources patterns in the Pliocene aquifer at Wadi El Natrun area.Depth to water of the Pliocene aquifer varies from about 20 m to 30 m outside wadi El Natrun and less than 10 m to flowing in the surface inside Wadi El Natrun . The Groundwater flows from northeast and southeast to southwest and northwest i.e. to Wadi El Natrun..The groundwater flow, recharge and geochemical evolution in the study area are complex and effected by the geological sequence, there is a zonation of chemical composition,. This zonation is Characterized by a change of cation species from dominantly Ca and Mg near the east to Na-dominated waters in the west. Mirroring this, anions change from HCO3 type to Cl and SO_4 type. From spatial variation in groundwater chemistry,the assemblage (I) of hypothetical salt combination KCL, NaCl, Na_2SO_4, $NaHCO_3$,$Mg(HCO_3)_2$ and $Ca(HCO_3)_2$ is dominant in ground water samples in the east direction, while the assemblage (II) of hypothetical salt combination KCl, NaCl, Na_2SO_4, MgSo4, CaSO4, $Ca(HCO_3)_2$ is dominant in ground water samples in the west direction. The ions displays two grades of metasomatism along this profile, first the stage of mineralization (HCO3>Cl>So4) at wells No. (33 , 27) at the east then the advanced stage of mineralization (Cl>SO4 >HCO3) at well No.(37) at the west. These agree with the general gradient of chemical evolution of groundwater.The regional geochemical variation in the study area from east to west is principally controlled by heterogeneity of aquifer composition complex of groundwater flow patterns beside the geochemical evolution processes (leaching, dissolution and precipitation) along groundwater flow path.According to the **World Health Organization (W.H.O.2005)** the maximum permissible limits of TDS in drinking water is **1000** mg/l. It's concluded that **45** % of tested groundwater samples are permissible, while 55% are not permissible, most of groundwater samples according to SAR (45.5%) of the sample are excellent, 17% are good, 13.5% are fair and 24% are poor for irrigation which reflects suitability of most groundwater in the study area for irrigation purposes under normal condition. According to the calculated RSC of the Pliocene groundwater samples, (Appendix1, table 3), **48.5**% of groundwater samples safe for irrigation purposes, 28.5 % of groundwater samples are marginal and this water is suitable for irrigation in this area under normal condition, while 24% from the samples is not suitable for irrigation according to(RSC). All surface water samples are saline water(lakes) so it is not valid for different purposes ,because of the high saline (TDS) content, so surface water in the study area is not available for drinking or irrigation purposes.

REFERENCES

[1] Abdel Baki, A. M A. (1983):"Hydrogeological and Hydrogeochemical studies in the area west of Rosetta branch and South El Nasr canal", Ph. D. Thesis, Faculty of Science, Ain Shams Univ. 156 p.

[2] ASTM, American Society for testing and material (2002). In "water and environmental technology". annual book of ASYM standars, Sec. 11, Vol. 11.01 and 11.02, West Conshohocken, U.S.A.

[3] Appelo CAJ and Postma D.(2005). Geochemistry, groundwater and pollution.A.A. , Second edition, Amesterdam, the Netherlands, Balkema 649 p.

[4] Back, W. and Hanshaw, B. B. (1979): "Major geochemical processes in the evolution of carbonate aquifer system". Elsevier Scientific Publishing Company, Amesterdam, The Netherlands.

[5] Burdon, D.J. (1958). Metasomatism of groundwater at depth, UNESCO course on Hydrology, Desert Institute, Cairo, Egypt.

[6] Deutsch, W. J. (1997). Groundwater Geochemistry: fundamentals and application to contamination. Lewis puplishers, Boca Raton, New York. 221p.

[7] Diab, M.Sh., Gad, M.I., Abd-el Baki, and El Sheihk, A.A. (2002): Study the effect of new reclamation projects on the groundwater system in the area northwest Wadi El Natrun and south El Nasr by using mathematical modeling. Final program and Abstracts at the Third International Conference, Groundwater level control Inside Urbanized Areas, Fac. Eng., Mansoura Univ., El Mansoura, December, 17 – 19, 2002.

[8] Domenico, P.A., and Schwartz, F.W., (1990): Physical and chemical hydrogeology: John Wiley and Sons, New York, 824 p.

[9] El- Abd, E. A. (2005): The Geological impact on the water bearing formations in the area south west Nile

Delta, Egypt., Ph.D. Thesis, Geol. Depart. Fac. Sci; MenoufiaUniv; 319 p.

[10] El-Fayoumy, I.F.; (1987):"Geology of the quaternary succession and its impacts on the groundwater reservoir in the Nile Delta region, Egypt. submitted to the bull. Fac. of Sci. Menoufia Univ.

[11] El Ghazawi, M.M. (1982):Geological studies of the Quaternary –Neogeneaquifers in the area northwest Nile Delta. M.Sc. Thesis, Fac. Sci, El AzharUniv, Cairo, Egypt,170 p.

[12] El Sheikh, A.E. (2000): Hydrogeology of the area north and west of Wadi El Natrun. M. Sc. Thesis, Fac. Sci., Minufia Univ., Shibbin El Kom, Egypt, 177p.

[13] Eaton, F.M. (1950): "Significance of carbonates in irrigation water". Soil Sci., Vol. 69, No.2, pp.123-133.

[14] Fattah, K. M. (2012): Hydrogeochemical evaluation of the Quaternary aquifer in El Sadat city, Egypt. Arabian Journal for science and engineering, Springer, vol.37, pp.121-145.

[15] Fattah, M. K. and Abdelrazek, S. A.(2014): "The Improvement of the Quality of Irrigation Water Contaminated with Heavy Metals in the Borg El Arab, Egypt". Journal of Water Resource and Protection, 6, P. 1703-1715.

[16] Freeze, R.A. and Cherry, G.E. (1979).Groundwater. Prentice - Hall, Englewood Cliffs, New Jersey, U.S.A.460 p.

[17] Glynn PD, Plummer LN (2005) Geochemistry and understanding of groundwater systems. Hydrogeology J. volume 13:pp 263–287.

[18] Hem, J. D., (1989): Study and interpretation of the chemical characteristic of natural water. U.S. geol. Surv., Water supply paper 2254, 3rd edition, 264 p.

[19] Lyon WB, Bird DA. (1995) : Geochemistry of the Madeira River Brazil: comparison of seasonal weathering reactions using a mass balance approach. Journal of South American Earth Science ; Volume 8, Number 1. Elsevier pp:97-101 .

[20] Matthess, G., (1982): The Properties of Groundwater." John Wiley & Sons, New York.

[21] Piper,A.M.(1953).A graphic representation in the geochemical interpretation of groundwater analyses. American Geophysical Union Transactions, U.S.A.,volume 25, pp 914-**923.**

[22] Richards, L.A. (1954): Diagnosis and improvement of saline and alkaline soils. U. S. Agric. HANDBOOK, No. 60, U. S. Dept. Agric. Washington D. C., pp.69 – 82.

[23] Said, R. (1990): The geology of Egypt For the Egyptian gen. pet .cor. Conoco Hurghada Inc., and repsol exploration, Balkema, Rotterdam, Netherlands.

[24] Sanad (1973): "Geology of the area between Wadi El-Natrun and theMoghra Depression", Ph. D. Thesis, Faculty of Science, AssuitUniv.,Assuit, Egypt, 184 p.

[25] Schoeller, R. (1962): Les eauxSouterraines, Msson and Cie, Paris, 642pp.

[26] Soulsby C, Chen M, Ferrier RC, Helliwell RC, Jenkins R, Harriman R.(1998). Hydrogeochemistry of shallow groundwater in an upland Scottish catchment. Hydrological Process;12, pp 1111-1117.

[27] U.S. Salinity Lab. Staff. (1954): "diagnosis and improvement of saline and alkaline soils". U.S. Dept. Agric., Handbook No. 60.

[28] World Health Organization (WHO) (2005): International standards for drinking Waters. 5th Edition, Vol.1 Geneva, Switzerland. 130P.

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

Appendix (1) , Table (1) : Chemical analyses of groundwater samples of the Pliocene aquifer

Sample No.	EC mmohs	TDS	PH	Cations (ppm)				Anions (ppm)			
				K	Na	Mg	Ca	Cl	SO4	HCO3	CO3
1	1.64	1050	8.36	40	290	10.7	12.8	261	200	205	**8**
2	0.64	410	8.32	8	102	4.84	16	55	90	145	**0**
3	0.94	602	8.25	13	143	12.15	40	135	90	170	**32**
4	1.07	688	7.32	7	180	2.43	40	140	95	260	**0**
5	1.08	691	7.75	12	165	4.86	32	110	135	215	**16**
6	8.35	5344	7.8	38	1850	9.72	32	1750	1400	309	**8**
7	1.4	896	8.28	19	252	9.72	24	180	130	320	**12**
9	5.8	3712	8.23	3	1390	7.29	24	1600	644	176	**28**
10	4.98	3187	8.03	44	1061	9.72	64	1230	670	160	**0**
11	2.41	1542	8.31	26	450	9.72	32	414	400	160	**12**
12	3.8	2432	8.04	28	757	17.01	56	770	623	168	**12**
13	2.8	1792	8.18	25	540	7.92	64	573	470	108	**4**
14	4.8	3072	7.59	36	1020	2.43	40	1100	616	220	**0**
15	5.18	3315	7.94	37	1134	7.29	32	1258	650	210	**0**
16	3.53	2259	7.99	20	742	4.96	64	644	756	150	**0**
17	5	3200	7.8	28	1020	17.01	72	1027	900	145	**0**
18	1.9	1216	8.23	15	394	4.86	16	347	340	96	**8**
19	2.52	1613	7.55	20	487	9.72	64	550	300	240	**0**
20	1.2	768	7.5	14	200	4.86	32	145	152	220	**12**
21	1.88	1206	7.48	13	380	9.72	16	296	380	100	**16**
23	0.83	531	7.56	3	122	14.58	24	95	110	170	**10**
24	1.32	848	7.5	86	175	9.72	32	160	110	250	**36**
27	0.98	627	7.48	50	147	2.43	16	115	95	210	**0**
28	2.66	1664	7.55	32	502	14.58	48	590	383	100	**0**
30	0.47	302	7.5	6	51	4.86	32	50	61	100	**0**
31	0.49	313	7.55	7	70	2.43	16	40	36	130	**6**
32	0.12	763	7.5	64	123	21.39	51.2	150	272	86	**0**
33	0.97	621	7.48	27	130	16.52	16	90	90	249	**0**
34	0.14	930	7.55	17	250	19.44	32	300	200	111	**0**
35	0.64	414	7.56	7	94	7.8	16	60	60	160	**0**
36	0.12	800	7.5	12	195	3.4	62.4	250	128	150	**0**
37	2.00	1280	7.55	36	271	10.7	128	410	310	100	**10**
38	3.16	2022	7.5	20	621	14.6	64.1	673	540	102	**0**
39	10.6	6822	7.48	32	2360	6.8	144	3000	1100	227	**0**
40	4.14	2650	7.55	7	617	11.66	96	831	360	110	**0**

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

Appendix (1)

Table (2) : Chemical analuses of surface water (Lakes) at Wadi El natrun

EC mmohs	TDS	PH	Cations (ppm)				Anions (ppm)			
			K	Na	Mg	Ca	Cl	SO4	HCO3	CO3
2.3	27070	9	540	9080	90	200	9100	7600	400	68
.92	122880	9	500	44200	200	300	54000	20900	500	36
4.4	34816	9	450	12300	170	250	16800	4500	170	60
.33	85120	9	400	31900	120	300	34650	9000	260	16
8.9	12096	9	200	4350	70	90	5500	2200	205	0
8.2	18048	9	300	6600	120	200	9888	1450	316	16

Appendix (1)

Table.3: Residual Sodium Carbonate (RSC) and Sodium adsorption ratio (SAR):

Well NO.	TDS(PPm)	EC(mmos/cm)	RSC	SAR
W1	1050	1.640	1.84	14.47
W2	410	0.640	1.18	5.73
W3	602	0.940	0.86	5.08
W4	688	1.075	2.06	7.46
W5	691	1.080	2.05	7.96
W6	5344	8.350	2.67**	73.44*
W7	896	1.400	2.26	10.96
W9	3712	5.800	1.09	63.71*
W10	3187	4.980	1.38	32.65*
W11	1542	2.410	0.22	17.86
W12	2432	3.800	1.05	22.72
W13	1792	2.800	1.9	26.3*
W14	3072	4.800	1.39	41.46*
W15	3315	5.180	1.24	47.72*
W16	2259	3.530	1.14	24.02
W17	3200	5.000	2.62**	28.05*
W18	1216	1.900	0.37	22.12
W19	1613	2.520	0.17	14.97
W20	768	1.200	2.01	8.87
W21	1206	1.885	0.58	18.48
W23	531	0.830	0.39	4.84
W24	848	1.325	0.88	7.18
W27	627	0.980	2.44	9.47

http://dx.doi.org/10.22161/ijeab/2.1.42 *ISSN: 2456-1878*

W28	1664	2.660	1.95	16.27
W30*	302	0.472	0.36	2.22
W31	313	0.490	1.33	2.07
W32	763	1.192	2.92**	3.64
W33	621	0.970	2.64**	3.29
W34	930	1.453	1.38	3.74
W35	414	0.647	1.18	3.61
W36	800	1.250	0.94	6.50
W37	1280	2.000	5.31**	6.18
W38	2022	3.160	2.74**	18.19
W39	6822	10.660	4.04**	52.09*
W40	2650	4.140	3.96**	15.81

http://dx.doi.org/10.22161/ijeab/2.1.43 *ISSN: 2456-1878*

Economy of Production and Labor Requirement in Major Field Crops of Kavre, Nepal

Bibek Dhital

HASERA Agriculture Research and Training Center, Nepal

Abstract— *Economic analysis is found as the major aspect of measurement of efficiency of a farm. In most cases, this part is lagging in Nepalese farmers. With the objective to find benefit cost ratio of growing different crops, identify profitable crops and estimate labor requirement for cultivation, this case study was performed. The scope of this case study is it helps farmers in selecting the crop comparing the profit and labor available. This study was done as a case study in Kavre district, Nepal. From this research, potato (B: C=2.44) and onion (B: C=1.95) were found the most profitable crops and wheat and maize the least. Labor requirement for onion was highest 643 men/ha and wheat was the lowest i.e. 142 men/ha.*

Keywords— Benefit Cost ratio, Economic analysis, Hill farming, Labor, Nepal, Profit.

I. INTRODUCTION

Agriculture is the major source of Nepal's economy contributing about 33% in GDP (Karki, 2015) and is major source of food, income and employment for 65.7 % of population (Karki, 2015). Those crops which are a part of everyday meal or occupy an important part in socio-economic life of farmers are major crops. Ecologically Nepal consists of three major geographical division viz. Terai, mid hills and high hills. Terai is considered as the grain basket of the country as it harvests most of the countries staple food where rice followed by maize and wheat are the major cereal crops (NARC, 2013). In mid and higher hills cropping pattern is mainly dominated by maize (Tripathi and Jones2010). In Kavre, in terms of cereals, maize accounts the highest production followed by rice and wheat (MoAD, 2013). Potato is major cash crop followed by onion and mustard is grown as an oil seed crop (MoAD, 2013).Combination of livestock, forest and crop is typical in Nepalese agriculture system (Tripathi and Jones2010). Due to topographical disadvantage traditional methods of agricultural practices and animal power are still major in the hills and high hills but in Terai mechanization is seen (Shrestha, 2012).

The importance of agriculture sector and its overall development is directly linked with the objectives of meeting basic needs of the people (Karki, 2015). Increasing farm production and farmers' income is primarily dependent on farm planning (Karki, 2015). 65.7% of population is dependent on agriculture for livelihood and 60% of these farmers are subsistence farmers because of small land holding (Karki, 2015).Due to higher competition and agri-business challenges (Ghimire, 2008), credit and labor deficiency (Maharjan et al 2013) return to small land holding farmers is decreasing. Economic analysis of farming system will help them and the concerned development facilitators to make proper decisions required for further improvement (Karki, 2015). It helps in determination of successfulness and sustainability of a farm and farming practices.

Economic analysis of agricultural crops includes estimation of cost incurred during cultivation and the monetary output we obtain from our harvest. In mathematical term, profit or loss is expressed as Benefit Cost ratio (B: C) which is the ratio of gross return to total cost of cultivation (Adhikari, 2011). In agriculture, crops and cropping practice with B: C higher than 1.5 is regarded as economically viable for farmers.

Farmers of Nepal are mostly illiterate and are farming in rural context with very less extent of mechanization (Ghimire, 2008). Majority of farmers of Kavre donot know the economic situation of their cropping pattern and practice. The major objective of this study is to find the benefit cost ratio, unit cost of production of different crops grown in Kavre district. This study also aimsto find the most profitable crop and to estimate the labor requirement for growing different crops. This study will help farmers and developmental organization involved to plan their farming pattern according to the economic status and labor availability, as labor and economics are the two of the major factors responsible for crop production.

II. METHODOLOGY OF STUDY

This study was conducted as a case study. This case study involved site selection, data collection, literature review, data analysis etc.

Site Selection:

Pathlekhet VDC (Ward no 1, 3,4) , Pankhalmunicipality (Ward no. 11,13)and Kasikhandamunicipality(Ward no. 6, 3)of Kavre districtwere selected as the site for the study. Pathlekhetis 41KM and Kasikhanda municipality is 51Km east of Kathmandu in BP highway and Pankhal lies

http://dx.doi.org/10.22161/ijeab/2.1.43 *ISSN: 2456-1878*

20KM from Dhulikhel on Araniko highway. Pathlekhet is at 1100 to 1400masl, Pankhal is at 700-1100 and Kasikhanda is a bit lower i.e. 950-1200masl. Farmers with average land holdings of about 0.3-0.5 Ha (Survey result) are dominant in the district. Major occupation of most of the people (>80%) being agriculture, it occupies an important position in social and economic life. Being in hilly area, mechanization is very poor (Kasikhandaand Pankhallittle more mechanized than pathlekhet).In pathlekhet, sill animal are the major draft power. Few mini tillers/power tillers are seen in the fields with road access. For intercultural operations like weeding, irrigation harvesting, threshing manual labor is the only power to depend on. In some family paddle thresher is seen but farmers don't prefer paddle thresher as the quality of straw is low. About 90% of the fields are rain fed and remaining 10% is irrigated. Due to low mechanization and transport facility, cost of production seems higher as compared to mechanized part of the country.

Data Collection:

The method applied for the collection of data was interview with key informants (leader farmers, Agricultural officers)and with with local farmers growing different crops. The data collected was primarily quantitative by using standard open ended questionnaire. Cost was calculated as a function of labor, manure, fertilizer, machinery/tools, food and other inputs. income was estimated by calculating the market value of economic yield i.e. grain and straw in rice and wheat, grain in maize and mustard, bulb in onion and tuber in potato. Pretesting was done with 5 respondents of pathlekhet VDC. A total of 35 respondents were interviewed. Separate data was collected for separate parcel of land (for those farmers having more than one parcel). Respondents were selected at random. Data collection was done in 2015.

III. RESULT AND DISCUSSION

Major crops and cropping pattern:

Difference in cropping patter in lowland (Khet) and upland (Bari) is common.In Khet land farmers practice rice based cropping pattern where most of the farmers grow 2 season rice, rainy and spring. Apart from rice, maize, wheat, potato, onion is popular. While in Bari land there is maize based cropping pattern. In Bari wheat, mustard, potato onion and vegetables are grown along with maize. In general, major crops for Kavre district are Rice, Maize, Wheat, Onion, Mustard and Potato.

Table.1: Major crops grown in village as per the % of farmers involved. (Very small scale i.e. kitchen garden, backyard garden aren't considered).

Crop	% of farmers involved
Rice	**95%**
Maize	**100%**
Wheat	**60%**
Onion	**70%**
Mustard	**60%**
Potato	**98%**

(Source: - Survey Research)

In terms of number of farmers involved, maize occupies the 1st position. It is so as maize can be grown in both Khet and Bari land. For rice, about 5% farmers only have Bari land and have no area suitable for rice. In case of wheat, as potato harbors more return per unit area (Table 2) and growing season of wheat and potato overlap in Khet land, more farmers are involved in potato than wheat. Also because of harvesting and threshing difficulty in wheat, farmers tend to grow other crop instead of wheat. Onion requires more labor per unit area (Table 4), so onion is cultivated by fewer farmers. For mustard, because of low productivity and farmers prioritizing for staple crops, only farmers with bigger land holding tend to grow mustard.

Table.2: Cost of production and value of output, Benefit: Cost and variance between respondents of rice, maize, wheat, onion, mustard and potato of Kavre district.

(In case of rice and wheat, both grain and straw are considered)

Crop	Average Cost per Hectare (NRs.)	Average Income per Hectare (NRs)	Benefit : Cost (B:C)			Coefficient of variation (In cost)
			Min	Max	Average	
Rice	162380.00	185746.00	1.0493	1.723	1.1439	20.19%
Maize	114623.20	106250.00	0.8	1.035	0.9269	21.32%
Wheat	59268.00	72811.42	0.92	1.554	1.228	14.54%
Onion	390613.00	762000.00	1.02	2.22	1.9507	18.46%
Mustard	44997.00	68294.00	1.182	1.960	1.5177	10.61%
Potato	243846.6	562983.2	1.49	3.29	2.44	32.20%

(Source: - Survey Research)

Study shows that, potato and onion are the most profitable crop followed by mustard, rice,wheat and maize. Even though rice, wheat and maize are less profitable, maize followed by rice and wheat are the crops grown by majority of farmers as it is the staple food. In case of rice, transplanting, weeding and harvesting cover the major fraction (50-60%) of the total cost. Use of transplanters, weeding and harvesting machines might be a way to increase B:C of rice. Similarly, in maize fertilizer covers the major fraction of cost (30-40%) and Stover of maize doesn't give any return. Use of improved variety can help to enhance B: C of maize.

Table.3: Unit cost of production and market value of output of rice, maize wheat, onion, mustard and potato of Kavre district.

(In case of rice and wheat only grains is considered)

Crop	Unit cost of production Cost/Kg (NRs)			Market value of output Price/Kg (NRs)
	Min	Max	Average	
Rice	30.94	45.94	36.84	35-45
Maize	24.21	31.22	27.27	27-32
Wheat	23.87	53.63	40.61	24-26
Onion	20.97	31.32	25.09	60-130
Mustard	17.62	45.07	32.38	55-60
Potato	9.47	22.1	14.4	20-40

(Source: - Survey Research)

Table.4: Average labor requirement (men/ha) for cultivation of rice, maize, wheat, onion, mustard and potato of Kavre district and variance between respondents.

Crop	No. of labor/Ha	Variance between respondents
Rice	360	12.59%
Maize	173	12.75%
Wheat	142	26.94
Onion	643	10.37%
Mustard	256	12.27%
Potato	210	11.09%

Source: Survey Research

Rice (360 man days/ Ha) after onion (643 man days /Ha) is the most labor requiring crops in mid-hills. Though onion requires high labor, because of its high market value (Table 3) and higher return per unit area (Table 2), it is popular among farmers as a cash crop.

IV. DISCUSSION

This obtained result of benefit cost ratio is supported by different scientists. According to Adhikari2011,Ghemire 2013 andMoAD 2013, BC ratio of rice is 1.15, 1.42 and 1.293 respectively. Timsina, 2011 and MoAD, 2013 found BC ratio of potato to be 2.9 and 1.79 respectively. Paudel, 2008, Ghemire, 2013 and MoAD, 2013found BC ratio of Maize as 1.03, 1.1 and 1.18 respectively. Mishra, 2010 estimated the maximum BC ratio of Mustard as 2 and Dhakal, 2015 estimated the average BC ratio of Mustard as 1.43. Ghemire, 2013 and MoAD said wheat can give average benefit cost ratio of 1.38 and 1.275 respectively.Bhandari 2015 reported maximum BC ratio of onion as 2.48.

ACKNOWLEDGEMENT

I would like to offer my sincere thanks to HASERA Agriculture Research Centre, Mr.Govinda Sharma, all the farmers involved in providing the information, and all others who indirectly helped in completion of this study.

REFERENCES

[1] B Mishra, RN Chaudhary. 2010. Sustainability of productivity in rice-mustard sequential cropping system through integrated nutrient management for Terai condition of Nepal. Agronomy Journal of Nepal, (Agron JN) Vol. 1: 2010.

[2] NB Bhandari. 2015. Ministry of Agriculture Development. Average Production cost and Profit Margin of Pulse, Oilseed, Spice and Commercial Crops. Available at: http://www.doanepal.gov.np/downloadfile/Pulses%20book_1444370865.pdf

[3] BP Tripathi, JE Jones. 2010. Biophysical and socio-economic tools for assessing soil fertility: A case of western hills, Nepal. Agronomy journal of Nepal. V (1). Available at: http://www.nepjol.info/index.php/AJN/article/view/7536

[4] KP Timsina, K Kafle, S Sapkota.2011. Economics of potato (Solanumtuberosum L) production in Taplejung district of Nepal. Agronomy Journal of Nepal (Agron JN) Vol. 2: 2011

[5] MoAD. 2013. Statistical Information on Nepalese Agriculture. Available at: http://www.moad.gov.np/uploads/files/YearBook%202013.pdf

[6] NARC. 2013. Annual Report. NARC Publication Serial No. 0006-2013/14. Available at: http://narc.gov.np/publicaton/pdf/book/CPDD%20Annual%20Report_2069-70.pdf

[7] NB Bhandari, D Bhattarai. M Aryal. 2015. COST, PRODUCTION AND PRICE SPREAD OF CEREAL CROPS IN NEPAL : A time series analysis. MoAD. Available at: http://www.doanepal.gov.np/downloadfile/abstract%20book%20setup_1444370508.pdf

[8] P Paude. A Matsuoka. 2008. The System and Profitability of Community-Based Maize Seed Production in Nepal, A Case Study of Chitwan District. Available at: https://www.jstage.jst.go.jp/article/arfe/45/1/45_1_167/_pdf

[9] R. Adhikari, 2011. Economics of organic rice production. The journal of agriculture and environment vol:12. Available on: www.nepjol.info/index.php/AEJ/article/download/7569/6152,

[10] S Ghemire, SM Dhungana, V Krishna. N Teufel and DP Sherchan. 2013. Biophysical and Socioeconomic characterization of cereal production system of central Nepal. CIMMYT Working paper 9. Available at: https://books.google.com.np/books?id=W2HtOnlaBbcC&pg=PA10&lpg=PA10&dq=benefit+cost+ratio+wheat+nepal&source=bl&ots=fQ5C-RAOX-&sig=A_YHwxyaodaxUyLBMJ_NiBhe794&hl=en&sa=X&ved=0ahUKEwjglZObg5bRAhXBP48KHS6mAEIQ6AEISTAG#v=onepage&q=benefit%20cost%20ratio%20wheat%20nepal&f=false

[11] SC Dhakal, PP Regmi, RB Thapa, D.B Khatri-Chhetri. 2015. Resource use efficiency of mustard production in Chitwan district of Nepal. Int J ApplSciBiotechnol, Vol 3(4): 604-608.

[12] Shree Ram Ghimire. 2008. Environmental concern in Nepalese agriculture. The journal of agriculture and environment. Vol (9). Available on: http://ncf.org.np/upload/files/570_en_6shree_ram.pdf

[13] Shreematshrestha, 2012. Status of agriculture mechanization in Nepal. Available athttps://www.researchgate.net/publication/228534387

[14] Yogendra Kumar Karki. 2015. Nepal Portfolio Performance Review (NPPR). MoAD Available at: http://mof.gov.np/ieccd/pdf/Agriculture_NPPR-2015.pdf

http://dx.doi.org/10.22161/ijeab/2.1.44 *ISSN: 2456-1878*

Evaluation of the euglycemic effect of oral administration of *S. rebaudiana* B. cultivated in Mexico in normoglycemic and induced-diabetic rats

Aranda-Gonzalez Irma[1], Ortiz-Andrade Rolffy[2], Moguel-Ordonez Yolanda[3], Betancur-Ancona David[4*]

[1]Facultad de Medicina, Universidad Autónoma de Yucatán, Avenida Itzáez No. 498 x 59 y 59A Col. Centro. Mérida, Yucatán, México. C.P. 97000.
[2]Facultad de Química, Universidad Autónoma de Yucatán. Calle 43 SN x 96 Paseo de las Fuentes y 40 Inalambrica. Mérida, Yucatán, México. C.P. 97069.
[3]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo experimental Mocochá, Carretera Mérida-Motul, Km. 25, C.P. 97454, Mocochá, Yucatán, México
[4]Facultad de Ingeniería Química, Universidad Autónoma de Yucatán, Periférico Norte Km. 33.5, Tablaje Catastral 13615, Col. Chuburná de Hidalgo Inn. Mérida, Yucatán, México. C.P. 97203.

Abstract— *Stevia rebaudiana is a plant widely used as sweetener and food supplement. There are some reports of the euglycemic effect of S. rebaudiana extracts, which has been attributed mainly to stevioside for the results obtained by other authors. However, studies with extracts lack the precise quantification of glycosides and it is known that several agricultural and environmental factors affect the steviol glycosides content. The aim of this study was to evaluate the euglycemic effect of oral administration of extracts of S. rebaudiana varieties cultivated in Mexico in normoglycemic and induced-diabetic Wistar rats and quantify their glycosides content. Aqueous leaves extracts of Criolla and Morita II varieties of S. rebaudiana were used to quantify glycosides by a validated HPLC method or lyophilized for in vivo experiments. Hypoglycaemic effect was evaluated in normoglycemic fasting rats, subjected to intragastric administration of Criolla or Morita II (100 or 200 mg/kg) and glucose measured at time 0, 1, 3, 5 and 7 hours. The antihyperglycemic effect was first evaluated with Streptozotocin / Nicotinamide-induced diabetic rats following the methodology previously described, and further evaluated with glucose load in normoglycemic rats. Control groups were distilled water and glibenclamide (5 mg/kg). Stevioside content was 2.08 ± 0.11 and 5.22 ± 0.23 (g/100 g of dry leaves) in the Morita II and Criolla variety, respectively. Acute administration of both varieties of S. rebaudiana had no euglycemic effect in normoglycemic or induced-diabetic rats (p> 0.05) at any of the doses tested.*

Keywords— Antihyperglycemic, Hypoglycemic, glucose, Stevia rebaudiana Bertoni, Steviol glycosides.

I. INTRODUCTION

Stevia rebaudiana Bertoni is a plant native of Central and South America whose leaves contains steviol glycosides with high sweetening capacity. [1] The steviol glycosides are compounds derived from steviol, which is glycosylated at C-19 and C-13 replacing the carboxyl hydrogen with glucose, xylose or rhamnose. [2]

In 2010, the Joint FAO/WHO Expert Committee for Food Additives (JECFA) identified nine steviol glycosides: Rebaudioside A, Rebaudioside B, Rebaudioside C, Rebaudioside D, Rebaudioside E, Rebaudioside F, Stevioside, Dulcoside A and Steviolbioside, [3] however there are many more steviol related-molecules which have been reported elsewhere. [4] In *Stevia* varieties usually cultivated, Rebaudioside A and Stevioside are the glycosides found in greater quantities whereas other glycosides are found in lesser amounts. [1, 5]

Since 2008 the Food and Drug Administration in the United States (FDA) granted the status of "Generally Recognized As Safe" (GRAS) to Rebaudioside A for use as a sweetener and in the same year, the JECFA and The Food Standards of Australia and New Zealand (FSANZ) established the acceptable daily intake (ADI) to steviol glycosides at 0-4 mg/kg bw/day (based on steviol equivalents). [6] The FDA does not allow the use of crude whole-leaf extracts of *S. rebaudiana*, and only isolated steviol glycosides with a purity of at least 95% are

permitted. [7] However, in the local market dried leaves of *S. rebaudiana* can be found to prepare homemade extracts or to sweeten hot beverages and even as capsules to be consumed as food supplements.

Some studies have shown that *S. rebaudiana* extracts have an antihyperglycemic activity in diabetic rats, [8-10] and subsequent studies have attributed this to stevioside as it increases insulin production and lowers blood glucose in rats. [11-13] However, those studies performed with extracts of *S. rebaudiana* have not quantified total steviol glycosides including stevioside, and it is known that several factors contribute to the amount and type of glycosides in *Stevia rebaudiana*, such as plant variety, weather conditions, soil and extraction method. [14,15]

In a previous work, we evaluate the effect of acute administration of 10 mg/kg of extract of *S. rebaudiana* Morita II grown in Mexico, without finding any hypoglycemic effect; [16] however the dose used could be very conservative and do not represent regular consumption as it was administered intraperitoneally.

Since regular consumption of *S. rebaudiana* could be as crude whole-leaf extracts or food supplement, which glycoside content is unknown, the aim of this study was to evaluate the euglycemic effect of oral administration of extracts of *S. rebaudiana* varieties cultivated in Mexico in normoglycemic and induced-diabetic Wistar rats and quantify their glycosides content.

II. MATERIALS AND METHODS

1. Materials and reagents

1.1 Reagents

Standards of Rebaudioside B (ASB-00018227), Rebaudioside C (ASB-00018228), Rebaudioside D (ASB-00018229), Steviolbioside (ASB-00019349) and Dulcoside A (ASB-00004949) were purchased from Chromadex (Irvine, CA, USA), and Stevioside (Sigma S3572) and Rebaudioside A (Sigma 01432) were purchased from Sigma-Aldrich (USA). The standards were lyophilized under vacuum pressure of 133 x 10^{-3} bar at -40 ºC (Labconco, Kansas City, MO, USA), suspended in HPLC grade water, filtered with 0.45 um membrane and frozen at -20 °C until use. Acetonitrile and HPLC grade water were purchased from J.T. Baker (Phillipsburg, NJ, USA). Streptozotocin, Nicotinamide and Glibenclamide were purchased from Sigma-Aldrich (St. Louis, MO) and commercial Metformin was used (Glucophage ®).

1.2. Experimental animals

Wistar rats (200-250 g) were purchased from Regional Research Center "Dr. Hideyo Noguchi" at the Universidad Autónoma de Yucatán. The animals were kept under controlled temperature (24 °C) with light-dark cycle. Food and drinking water was administered *ad libitum*. All procedures were conducted in accordance with the Official Mexican Standard "Norma Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio". [17]

1.3. Plant material

First cut leaves of two varieties of *Stevia rebaudiana* were collected. Morita II variety was grown in experimental field from INIFAP in Tizimín, Yucatán, México, whereas Criolla variety was grown in a farm field located in Quintana Roo, México. Leaves obtained were dried in the shade and powdered in Willey mill mesh of 1 mm and protected from light for later use.

2. Procedures

2.1. Aqueous extracts of *S. rebaudiana* Bertoni

Five hundred milligrams of leaves of each *S. rebaudiana* variety was extracted three times with 5 mL of HPLC grade water each time in a boiling water bath (100 ºC) for 30 min; extracts were cooled to room temperature and subsequently centrifuged for 10 minutes at 2,500 *x g* and 10 °C. The supernatant was transferred to a 25 mL volumetric flask and filled to capacity after the last extraction. [18] Extracts were filtered through a membrane filter (0.45 μm) to remove any solid residue before HPLC analysis or lyophilized (133 x 10^{-3} bar and -40 ºC) for *in vivo* tests on rats.

2.2. Diabetes Mellitus induction

DM Induction was performed in Wistar rats after 12 hours of fasting. Intraperitoneal injection of 65 mg/kg of streptozotocin dissolved in citrate-phosphate buffer (0.1 M, pH 4.5) was performed 15 minutes after administration of 120 mg/kg (i.p.) of nicotinamide. [19] Two weeks later, the blood glucose was measured from the tip of the tail and only animals with glucose ≥200 mg were included. [20]

2.3. Evaluation of hypoglycemic effect in normoglycemic Wistar rats

After 16 hours of fasting, lyophilized extracts of *S. rebaudiana* Criolla, Morita II (100 and 200 mg/kg), distilled water (2.5 mL) or glibenclamide (5 mg/kg) were administered to rats through an intragastric tube. Lyophilized extracts and glibenclamide were weighted at corresponding dose and suspended in 2.5 mL of distilled water immediately before being administered. Glucose was measured in the blood of the tip of the tail using a commercial glucometer Optimun Xceed (Abbott) at time 0 and 1, 3, 5 and 7 hours. [21] Glucose value at 0 h was considered as basal and percentage of change in glucose at each time were later calculated.

2.4. Evaluation of antihyperglycemic effect in induced-diabetic Wistar rats

The evaluation of the antihyperglycemic effect was made

only with the highest dose (200 mg/kg) of *S. rebaudiana* Criolla or Morita II. In induced-diabetic rats, procedure was repeated. After 16 hours of fasting, distilled water, glibenclamide and *S. rebaudiana* Criolla or Morita II were administered to rats through an intragastric tube and glucose measured at time 0 and up to 7 hours. [21] Glucose value at 0 h was considered as basal and percentage of change in glucose at each time were later calculated.

2.5. Evaluation of antihyperglycemic effect in normoglycemic Wistar rats by a glucose load

This evaluation was also performed with the highest dose (200 mg/kg) of *S. rebaudiana* Criolla or Morita II. In normoglycemic rats and after 16 hours of fasting, a load of glucose (2 g/kg) was administered through an intragastric tube 10 min before treatments. Treatments administration was set as 0 h and glucose was measured in the blood of the tip of the tail at time 0, 0.5, 1, 2, 3 and 4h. [20] Glucose value at 0 h was considered as basal and percentage of change in glucose at each time were later calculated.

2.6. Chromatographic conditions and quantification of steviol glycosides

According to JECFA (2010), [3] liquid chromatography was performed using an Agilent 100 HPLC system with a UV-Vis detector set to a wavelength of 210 nm. Chromatographic method was carried out using a Luna C18(2) 100A (Phenomenex Co., Ltd., CA, USA) column (250 mm length, 4.6 mm internal diameter and 5 μm particle size) with mobile phase of 32:68 (v/v) acetonitrile and buffer sodium phosphate 10 mmol/L (pH 2.6), and isocratic flow at 1 mL/min. The results were analyzed with the Clarity program version 2.7.3.498 (2000-2009). Standard solutions were prepared at concentration ranges of 100-500 μg/mL for Rebaudioside A and Stevioside whereas the rest of glycosides at 25–150 μg/mL. Each concentration was injected a total of six times onto the HPLC equipment. Quantification of the steviol glycosides content was based on peak area, from linear regression curves of the standards. [22, 23]

3. Statistical analysis

The analysis was performed using the statistical package Statgraphics, by t-test or ANOVA Student. Differences were considered significant with a p-value <0.05.

III. RESULTS AND DISCUSSION

Single oral administration of both varieties of *S. rebaudiana* at any dose tested, had not hypoglycemic effect in normoglycemic rats (Fig. 1). In this assay, as can be seen in Figure 1, there is a slight decrease in the percentage of change in glucose at 5 hours in all the experimental groups. As this phenomenon also occurred in the control group it could be attributed to the period of fasting reached at that time (21 hours approximate) or also be the result of mild stress caused by repeated measurement of glucose and handling of animals. [24] As expected, the group treated with glibenclamide had a marked hypoglycemia from the first hour, reaching the peak (approximately -60%) at 3 hours and remaining constant up to 7 hours.

As in normoglycemic rats, administration of *S. rebaudiana* varieties in induced-diabetic rats had no antihyperglycemic effect (Fig. 2). No variety of *S. rebaudiana* at the dose tested had any effect on glucose. As expected, glibenclamide exerted his glucose lowering effect but it is north worthy that the effect of glibenclamide was more powerful in normoglycemic rats compared to induced-diabetic rats, as glucose lowering was greater (-60 vs. -40%) and in a shorter time (1 vs. 5 hours).

Finally, as seen in figure 3, any of the varieties at the highest dose had an antihyperglycemic effect in normoglycemic rats subjected to glucose load. The only effect was in the group treated with glibenclamide, with a similar response (-60%) at 3 h as observed in single administration (Fig. 1).

The steviol glycoside content of *S. rebaudiana* B. varieties grown in the Yucatán is presented in Table 1.

It is noteworthy that the quantification of glycosides was obtained from a previously validated HPLC method, which chromatographic data and validation parameters can be consulted elsewhere. [22, 23] In this regard, all steviol glycosides are at lower concentration than previously reported, [22, 23] possibly attributed to a different batch. Comparing the varieties Morita II and Criolla, there are no differences in the content of minor glycosides, except for Rebaudioside B and Rebaudioside D; however, the most important difference are the content of Stevioside and Rebaudioside A. Morita II variety had higher content of total glycosides as compared to the Criolla variety (16.74 vs. 9.65 g/100 g) with a higher content of Rebaudioside A (4.5 times more). It is known that Stevioside is 250-300 times sweeter than sugar but with a slightly bitter aftertaste, while Rebaudioside A has more sweetener capacity (350-450 times more than sugar) without bitter taste. [1] The Rebaudioside A to Stevioside ratio is a commonly measure used of sweetness quality, [4] and as expected Morita II had a better profile with a ratio of 5.9, unlike Criolla which ratio was 0.52. Taking into account the total content of glycosides, particularly Rebaudioside A and its sweetness quality, Morita II variety cultivated in Mexico is suitable for the food industry.

http://dx.doi.org/10.22161/ijeab/2.1.44 *ISSN: 2456-1878*

The quantification of steviol glycosides in *S. rebaudiana* extracts through a validated chromatographic method, allowed to set the steviol glycoside content administered in 100 and 200 mg/kg of lyophilized extracts. Steviol glycoside administration expressed in mg/kg is detailed in table 2, considering the highest dose used (200 mg/kg).

There are studies that have evaluated the hypoglycemic effect of *S. rebaudiana* extracts, with varying results. In Wistar rats with alloxan-induced diabetes, chronic administration had hypoglycemic effect, [8, 9] but the administration in normoglycemic Wistar rats had no antihyperglycemic effect during a glucose tolerance test. [25]. Aqueous extract of *S. rebaudiana* administered (400 mg/kg) during 28 days to Sprague-Dawley rats with streptozotocin-induced diabetes had an antihyperglycemic effect through PPAR-γ mechanism. [10]

However, studies by Misra *et al* (2011) [9] as well as the Kujur *et al* (2010) [8] used extracts with another polarity and not aqueous. On the other hand, Assaei *et al* (2016) [10] although they used and aqueous extract and elucidated a mechanism, used a higher dose of extract without quantification of steviol glycosides and therefore, no comparisons can be made.

Stevioside is the molecule that has been attributed the euglycemic effects of *Stevia* as has been shown to inhibit gluconeogenesis and increase production of insulin *in vivo* [11-13] and *in vitro.* [26]

It has been reported that acute administration of a dose of 200 mg/kg of stevioside in normoglycemic Wistar rats, increases the plasma insulin concentration but has no effect on glucose tolerance during the test. [11] In this context, the administration of 100 mg/kg *of S. rebaudiana* is equivalent to 4.58 mg/kg of stevioside in Morita variety, while in Criolla variety it is equivalent to 15.21 mg/kg of stevioside (Table 2) which in both cases are much less than that the dose used by Jeppesen *et al* (2002). [11]

Administration of stevioside in diabetic rats at doses of 20 mg/kg for 14 days, [27] 25 mg/kg for 6 weeks, [12] or 30 mg/kg for 3 weeks [13] has shown antihyperglycemic effect during oral or intravenous glucose tolerance tests. In these studies the dose of stevioside are higher than that used in this study, administration was for several days and with a different diabetes model.

Nevertheless, high amounts of stevioside do not represent the usual consumption of this steviol glycoside as a sweetener since its sweetening power has been calculated as 300 times sweeter than sugar. [1] That is, the sweetness of even the small amount of 1 mg/kg of stevioside would be equivalent to using 18 grams of sugar for a 60 kg person.

The administration of Criolla variety extracts, despite its relative high content of stevioside had no effect on blood glucose levels in normoglycemic and induced-diabetic rats, suggesting that the presence of this compound in varieties grown in southeast Mexico is not sufficient to achieve an *in vivo* effect on glucose. However it cannot be ruled out that chronic consumption could have a different effect.

It is known that *S. rebaudiana* leaves also contain protein, fats and carbohydrates, diterpenes, flavonoids, tannins, among other phytochemicals, [28] not quantified in this project and that might have some effect on the regulation of glycaemia. Further studies are needed to fully characterize the extracts in addition to glycosides content.

The administration *S. rebaudiana* extracts had no effect on glucose in normoglycemic or diabetic-induced Wistar rats. The relevance of the results are that the diabetes model used in this study was more close to clinical reality, since it is considered Type 2 diabetes unlike alloxan, [29] whose model is considered type 1 diabetes; type 2 diabetes model is more relevant since this type affects 90-95% of people with diabetes. [30] Furthermore, the intragastric administration of *S. rebaudiana* resembles oral consumption through food, infusions (as aqueous extract) or even food supplements. This might suggest that the consumption of *S. rebaudiana* extract varieties cultivated in southeast Mexico is safe to be consumed by healthy or diabetic individuals, however further studies are needed.

IV. FIGURES AND TABLES

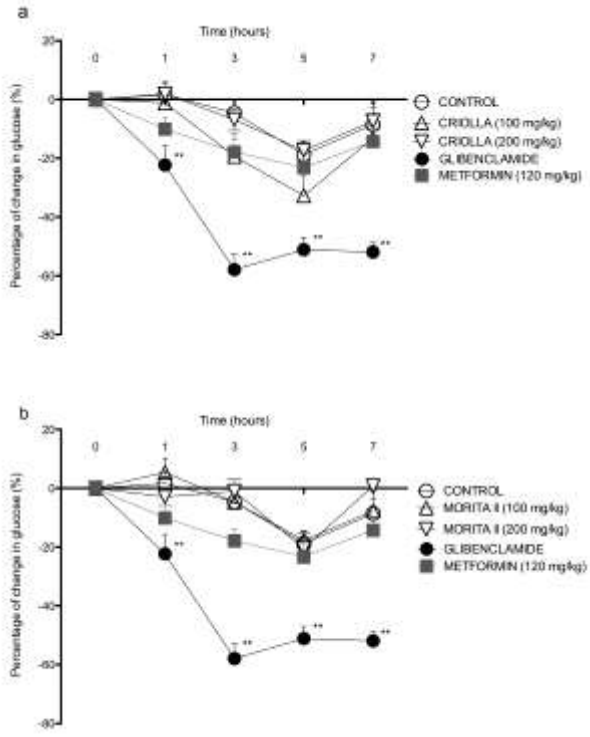


Fig.1: Evaluation of hypoglycemic effect of S. rebaudiana extracts in normoglycemic rats. Each point represents the

http://dx.doi.org/10.22161/ijeab/2.1.44 *ISSN: 2456-1878*

*average of variation in the percentage of glucose after administration of extracts from Criolla (a) or Morita II (b) variety of 5 animals ± SEM. Statistical significance by One-way ANOVA and post-hoc Tukey HSD vs controls denoted by ** p <0.01.*

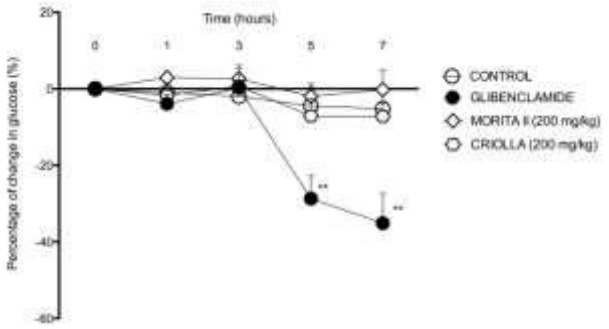


*Fig.2: Evaluation of antihyperglycemic effect of S. rebaudiana extracts in induced-diabetic rats with induced diabetes mellitus. Each point represents the average of variation in the percentage of glucose after administration of extracts from Criolla or Morita II variety of 5 animals ± SEM. Statistical significance by One-way ANOVA and post-hoc Tukey HSD vs controls denoted by ** p <0.01.*

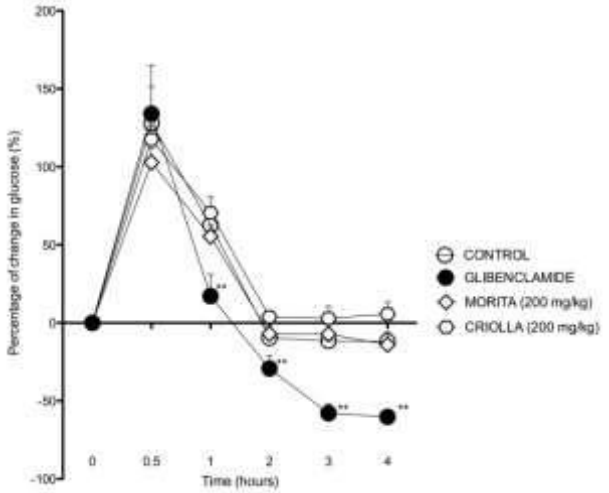


*Fig.3: Evaluation of antihyperglycemic effect of S. rebaudiana extracts in normoglycemic rats with glucose load. Each point represents the average of variation in the percentage of glucose after administration of glucose load and extracts from Criolla or Morita II variety of 5 animals ± SEM. Statistical significance by One-way ANOVA and post-hoc Tukey HSD vs controls denoted by ** p <0.01.*

Table.1: Contents of glycosides in g/100 g of dried leaves of S. rebaudiana Morita II and Criolla

Steviol glycoside	Morita II Mean ± D.E.	Criolla Mean ± D.E.
Dulcoside A	0.11 ± 0.000	0.13 ± 0.03
Rebaudioside B	0.46 ± 0.01 **	0.16 ± 0.03 **
Rebaudioside C	0.81 ± 0.07	0.56 ± 0.17
Rebaudioside D	0.67 ± 0.04 *	0.49 ± 0.06 *
Steviolbioside	0.32 ± 0.15	0.38 ± 0.11
Rebaudioside A	12.29 ± 0.27 **	2.71 ± 0.08 **
Stevioside	2.08 ± 0.11 **	5.22 ± 0.23 **

Values are expressed as mean ± standard deviation. The significant differences found in the same row are denoted denote by *p <0.05, **p <0.01 (Student's t-test).

Table.2: Steviol glycosides dose (mg/kg) administered in 200 mg/kg of lyophilized extract of Stevia rebaudiana B.

Steviol glycosides	Dose (mg/kg) according *to Stevia rebaudiana* variety	
	Morita II	Criolla
Dulcoside A	0.48	0.76
Rebaudioside B	2.03	0.93
Rebaudioside C	3.57	3.26
Rebaudioside D	2.95	2.86
Steviolbioside	1.41	2.21
Rebaudioside A	54.14	15.79
Stevioside	9.16	30.42
TOTAL	**73.74**	**56.23**

Values are expressed as mean of steviol glycoside content.

V. CONCLUSION

The highest dose orally administered (200 mg/kg) of lyophilized *Stevia rebaudiana* extract contains 30.42 and 9.16 mg/kg of stevioside and about 56 or 74 mg/kg of total steviol glycosides in Criolla and Morita II varieties, respectively, with no acute euglycemic effect in normoglycemic or induced-diabetic rats. These results suggest that it is unlikely that the use of *S. rebaudiana* leaves as sweetener or food supplement could decrease glucose in normoglycemic or diabetic people since the acceptable daily intake of glycosides is 4 mg/kg.

VI. ACKNOWLEDGEMENTS

The authors acknowledge the funding provided by the Fondos Fiscales-INIFAP and CONACyT for the scholarship provided to Aranda-González for her postgraduate studies.

REFERENCES

[1] Goyal SK, Samsher, Goyal RK. Stevia (*Stevia rebaudiana*) a bio-sweetener: a review. Int J Food Sci Nutr. 2010;61(1):1-10.

[2] Chaturvedula VS, Upreti M, Prakash I. Diterpene glycosides from *Stevia rebaudiana*. Molecules. 2011;16(5):3552-62.
[3] JECFA. Steviol glycosides. In: Compendium of Food Additive Specifications. FAO JECFA Monographs; Roma, 2010, pp. 17-21.
[4] Ceunen S, Geuns JM. Steviol glycosides: chemical diversity, metabolism, and function. J Nat Prod. 2013;76(6):1201-28.
[5] Jackson AU, Tata A, Wu C, Perry RH, Haas G, West L, et al. Direct analysis of Stevia leaves for diterpene glycosides by desorption electrospray ionization mass spectrometry. Analyst. 2009;134(5):867-74.
[6] FDA. Rebaudioside A: GRAS assessment. In: Center of Food Safety and Applied Nutrition. FDA: USA, 2008, pp. 1-40.
[7] Abdel-Rahman A, Anyangwe N, Carlacci L, Casper S, Danam RP, Enongene E, et al. The safety and regulation of natural products used as foods and food ingredients. Toxicol Sci. 2011;123(2):333-48.
[8] Kujur RS, Singh V, Ram M, Yadava HN, Singh KK, Kumari S, et al. Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetic rats. Pharmacognosy Res. 2010;2(4):258-63.
[9] Misra H, Soni M, Silawat N, Mehta D, Mehta BK, Jain DC. Antidiabetic activity of medium-polar extract from the leaves of *Stevia rebaudiana* Bert. (Bertoni) on alloxan-induced diabetic rats. J Pharm Bioallied Sci. 2011;3(2):242-8.
[10] Assaei R, Mokarram P, Dastghaib S, Darbandi S, Darbandi M, Zal F, et al. Hypoglycemic Effect of Aquatic Extract of Stevia in Pancreas of Diabetic Rats: PPARgamma-dependent Regulation or Antioxidant Potential. Avicenna J Med Biotechnol. 2016;8(2):65-74.
[11] Jeppesen PB, Gregersen S, Alstrup KK, Hermansen K. Stevioside induces antihyperglycaemic, insulinotropic and glucagonostatic effects in vivo: studies in the diabetic Goto-Kakizaki (GK) rats. Phytomedicine. 2002;9(1):9-14.
[12] Jeppesen PB, Gregersen S, Rolfsen SE, Jepsen M, Colombo M, Agger A, et al. Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism. 2003;52(3):372-8.
[13] Jeppesen PB, Dyrskog SE, Agger A, Gregersen S, Colombo M, Xiao J, et al. Can stevioside in combination with a soy-based dietary supplement be a new useful treatment of type 2 diabetes? An in vivo study in the diabetic goto-kakizaki rat. Rev Diabet Stud. 2006;3(4):189-99.
[14] Jarma-Orozco A, Araméndiz-Tatis H, Cleves-Leguízamo. Phenotypic stability and plant densities of stevia (*Stevia rebaudiana* Bert.) genotypes in the Caribbean Region of Colombia. Acta Agronómica. 2011;60(2).
[15] Kolb N, Herrera JL, Ferreyra DJ, Uliana RF. Analysis of sweet diterpene glycosides from *Stevia rebaudiana*: improved HPLC method. J Agric Food Chem. 2001;49(10):4538-41.
[16] Aranda-Gonzalez I, Barbosa-Martin E, Toraya-Aviles R, Segura-Campos M, Moguel-Ordonez Y, Betancur-Ancona D. Safety assessment of *Stevia rebaudiana* Bertoni grown in southeastern Mexico as food sweetener. Nutr Hosp. 2014;30(3):594-601.
[17] Diario Oficial de la Federación. NOM-062-ZOO-1999. Especificaciones técnicas para el Cuidado y Uso de los Animales de Laboratorio. Norma Oficial Mexicana, México, 2001.
[18] Woelwer-Rieck U, Lankes C, Wawrzun A, Wüst M. Improved HPLC method for evaluation of the major steviol glycosides in leaves of Stevia rebaudiana. Eur Food Res Technol. 2010;231:581-8.
[19] Masiello P, Broca C, Gross R, Roye M, Manteguetti M, Hillaire-Buys D, et al. Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes. 1998;47:224-9.
[20] Ortiz-Andrade R, Torres-Piedra M, Sánchez-Delgado JC, García-Jiménez S, Villalobos-Molina R, Ibarra-Barajas M, et al. Acute and sub-chronic effects of Cochlospermum vitifolium in blood glucose levels in normoglycemic and STZ-Nicotinamide-Induced Diabetic rats. Rev Latinoamer Quím. 2009;37(2):122-32.
[21] Ortiz-Andrade RR, Rodriguez-Lopez V, Garduno-Ramirez ML, Castillo-Espana P, Estrada-Soto S. Anti-diabetic effect on alloxanized and normoglycemic rats and some pharmacological evaluations of *Tournefortia hartwegiana*. J Ethnopharmacol. 2005;101(1-3):37-42.
[22] Aranda-Gonzalez I, Moguel-Ordonez Y, Betancur-Ancona D. Validation of HPLC-UV method for determination of minor glycosides contained in *Stevia rebaudiana* Bertoni leaves. Biomed Chromatogr. 2015;29(5):733-8.
[23] Aranda-González I, Moguel-Ordoñez Y, Betancur-Ancona D. Determination of Rebaudioside A and Stevioside in Leaves of *S. rebaudiana* Bertoni Grown in México by a Validated HPLC Method. AJAC. 2015;6:878-85.
[24] Ladriere L, Malaisse-Lagae F, Fuhlendorff J, Malaisse WJ. Repaglinide, glibenclamide and glimepiride administration to normal and

hereditarily diabetic rats. Eur J Pharmacol. 1997;335(2-3):227-34.

[25] Fujita Y, Wideman RD, Speck M, Asadi A, King DS, Webber TD, et al. Incretin release from gut is acutely enhanced by sugar but not by sweeteners in vivo. Am J Physiol Endocrinol Metab. 2009;296(3):E473-9.

[26] Jeppesen PB, Gregersen S, Poulsen CR, Hermansen K. Stevioside acts directly on pancreatic beta cells to secrete insulin: actions independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K+-channel activity. Metabolism. 2000;49(2):208-14.

[27] Raskovic A, Mikov M, Skrbic R, Jakovljevic V, Vasovic V, Posa M, et al. Effect of stevioside and sodium salt of monoketocholic acid on glycemia in normoglycemic and diabetic rats. Eur J Drug Metab Pharmacokinet. 2008;33(1):17-22.

[28] Lemus-Mondaca R, Vega-Gálvez A, Zura-Bravo L, Ah-Hen K. *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. Food Chemistry. 2012;132:1121-32.

[29] Szkudelski T. Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. Exp Biol Med. 2012;237:481-90.

[30] ADA. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33 Suppl 1:S62-9.

http://dx.doi.org/10.22161/ijeab/2.1.45 *ISSN: 2456-1878*

Insecticidal effects of eudesmanes from *Pluchea sagittalis* (Asteraceae) on *Spodoptera frugiperda* and *Ceratitis capitata*

A. Sosa[1], M. Costa[1], A. Salvatore[4], A. Bardon[1,3], S. Borkosky[1], N. Vera[2*]

[1] Instituto de Química Orgánica, [2]Instituto de Estudios Farmacológicos, [3]INQUINOA-CONICET, Facultad de Bioquímica, Química y Farmacia, UNT, Phone: 54-381-4247752, Ayacucho 471 (4000), Tucumán, Argentina

[4] Facultad de Agronomia y Zootecnia, UNT, Phone: 54-0381-4364147, Florentino Ameghino s/n. Bº Mercantil. Campo Experimental (4105), El Manantial, Tucumán, Argentina

[*]Correspondence: N. Vera. Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán. Ayacucho 471. Tucumán (4000).

Abstract— *Eudesmanoids play an important role in the protection of plants against herbivores. Pluchea sagittalis (Lamarck) Cabrera (Asteraceae) is widespread in tropical South America and contains compounds that provide protection against phytophagous insects. In the present work we isolated seven sesquiterpenoids with eudesmane skeletons that were evaluated for their insecticidal activities against Spodoptera frugiperda and Ceratitis capitata, pests that cause serious damage to crops in the Argentine northwest. The Eudesmanes were incorporated at different concentrations to the diet of Spodoptera frugiperda. In the choice test, larval feeding behavior was altered. The eudesmanes **1**, **5** and **7** showed the highest activity with feeding election indexes (FEI) of 50, 50, and 72 %, respectively at 200 µg/g of diet. When tested for insecticidal activity using neonate larvae with the nochoice artificial diet bioassays, eudesmane **1** was the most toxic in the larval stage (LD_{50} 177.80 mg/g of diet). Compounds **5** lowered the percentage of adult emergence and produced the most malformations (72%) compared with control. Drastic effects were observed in the oviposition deterrence activity against C. capitata. The maximum oviposition deterrence (87 %) was recorded with eudesmane **5** at dose 30 µg/cm^2 of artificial fruit. Finally, eudesmanes **6** and **7** showed significant larval and pupal mortality against the first generation larvae of viable eggs oviposited by females fed with the treated diet (100 µg / g artificial diet).*

Keywords— sesquiterpenoids, antifeedant, insect growth regulation, oviposition deterrence.

I. INTRODUCTION

It is well accepted that plant natural products may constitute new sources of insect pest control. The species belonging to the genus Pluchea (Asteraceae) consist of approximately 90 herbaceous species that grow in several countries of South America, *i.e.* Paraguay, Argentina and Brazil (Bremer & Anderberg 1994). Previous chemical investigations of the genus have shown the presence of eudesmane-type sesquiterpenoids (Ahmad *et al*. 1990; 1991; Guilhon & Müller 1996; 1998 a,b; Mahmoud 1997) as well as monoterpenes, lignan glycosides, triterpenoids, (Chakravarty & Mukhopadhyay 1994) and flavonoids (Ahmed *et al*. 1987; Scholz *et al*. 1994). *Pluchea sagittalis* (Lam.) Cabrera is popularly known as "Lucera," "Yerba Lucero" or "Quitoc. Pharmacological studies demonstrated that aqueous and dichloromethane extracts obtained from *P. sagittalis* have a wide spectrum of anti-inflammatory, antioxidant (Pérez-Garcia *et al*. 1996; 2001), antinociceptive and gastroprotective activities (Figueredo *et al*. 2011). Nevertheless, there is little information about their action on insects. Insecticidal and antifeeding effects have been reported for other sesquiterpenes against different insect species (Wang *et al*. 1991; Gonzalez *et al*. 1993; Dadang *et al*. 1996). The results of previous works by Céspedes *et al*. (2001) and Alarcon et al. (2013) reported feeding deterrent and insecticidal activities of certain eudesmane-type sesquiterpenoids against *S. frugiperda* (Lepidoptera: Noctuidae) larvae and *Drosophila melanogaster*, respectively. In a previous paper, we reported the isolation and identification of twelve eudesmane-type sesquiterpenoids from a Bolivian collection of *P. sagittalis*, together with their antifeedant activity at 100 µg / g of diet against *S. frugiperda*, under chosen conditions (Vera *et al*. 2008).

Objectives of the present study were to evaluate the insecticidal activity and sublethal effects of eudesmanes isolated from *P. sagittalis* against fall armyworm *S. frugiperda* and *Ceratitis capitata* (Wiedemann) (Diptera

Tephritidae), known as mediterranean fruit fly. For both insect pests, the bioassays were conducted under laboratory conditions.

II. MATERIALS AND METHODS

Plants and compounds.

P. sagittalis (Lamarck) Cabrera was collected at the flowering stage in December of 2013 in Tarija, Bolivia. Structures of tested compounds are shown in Figure 1. Compounds **1-7** were isolated from aerial parts of the plant and were purified in sufficient amount to be used in the bioassays. Identification of the eudesmanes **1-7** was accomplished by spectroscopic methods (IR, ^{1}H-NMR, ^{13}C-NMR, and MS) and direct comparison with authentic samples (Vera *et al*. 2008). Previous to the bioassays, purity of eudesmanes was checked by HPLC (Gilson with a differential refractometer, Middleton, USA) in Beckman C18 and C8 columns (250 x 10 mm i.d., 5 µm particle size), using MeOH : H_2O mixtures as eluents.

Bioassays

Insect rearing

S. frugiperda larvae were obtained from our laboratory population. The larval diet consisted of a mixture of yeast (3 g), boiled and milled bean (250 g), wheat germ (12.5 g), agar agar (12.5 g), ascorbic acid (1.5 g), methyl p-hydroxybenzoate (1.5 g), formaldehyde (4 ml of a 38% water solution), and water (500 ml).

The colony of *C. capitata* used in the bioassays was derived from the laboratory of the Estación Experimental Agroindustrial Obispo Colombres (EEAOC). It was initiated with pupae of infested fruits obtained from northwestern Argentina cultivating oranges. Adults were fed on a diet prepared with an aqueous solution of a mixture of sucrose and hydrolyzed protein (3: 1 ratio). The brood chamber was maintained at 24 ± 2 °C, 60 ± 10% relative humidity and a photoperiod of 12L: 12D.

Antifeedant test against S. frugiperda (*Choice test*)

Antifeedant tests against *S. frugiperda* larvae were carried out as follows (Vera *et al*. 2008). A portion of artificial diet was mixed with acetone and, after solvent removal in vacuum this portion was employed as control diet. Another portion was mixed with an acetone solution of each test compound, in order to reach 25, 50, 100 and 200 µg/g of treatment per g of diet. After solvent evaporation, control and treated diets were placed in test tubes (20 replicates) in which third instars larvae were placed between both portions of diet to be kept at 27°C and 60 ± 15% relative humidity. When 50% of control diet had been eaten, control and treated diets were removed from the tubes and weighted accurately. To evaluate the feeding behavior a "Feeding election index" was calculated as FEI = (1 – T/C) 100, where C and T represent the amounts of control and treated diets eaten, respectively.

Antifeedant test against S. frugiperda (*No choice Test*)

A portion of artificial diet was mixed with acetone and, after solvent removal in vacuum; this portion was employed as control diet. Another portion was mixed with an acetone solution of each test compound (treatment), in order to reach 25, 50, 100 and 200 µg/g of treatment per g of diet. After solvent evaporation, control diet was placed in a test tube and a larva was introduced. The same amount of treated diet was placed in a different test tube with a larva inside. Larvae were allowed to eat an after the 50% of control diet had been eaten, control and treated diets were removed from the tubes and weighted accurately. The experiment was carried out in 20 replicates. To evaluate the feeding behavior under no choice conditions, the FEI was calculated (Vera *et al*. 2006).

Insecticidal bioassay against S. frugiperda

The insecticidal bioassay activity against larvae of *S frugiperda* was carried out as follows (Vera *et al*. 2006). Control and treated diets were placed in different test tubes (20 replicates for treated and 20 for control experiments) in which 2nd instar larvae were placed to be kept at 27 °C and 60 ± 15% relative humidity until emergency of the 1st generation of adults. Larval developmental periods as well as mortality rates were recorded for treatments containing eudesmanes (25, 50, 100 and 200 µg/g of diet) and control experiments. The larval period duration (days), larval and pupal mortality percentage and the number of malformed adults were registered.

Food consumption and utilisation

Ten days after the beginning of the experiment, the larval weight and diet eated were determined again, in order to record the relative consumption rate (RCR), relative growth rate (RGR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD) and approximate digestibility (AD) during the early larval instars (Waldbauer 1968; Farrar *et al*. 1989). The developmental indices were calculated as follows:

RCR = I/BaT

RGR = DB/BaT

ECI = (DB/I)*100

AD = [(I) F)/I]*100

ECD = [DB/(I) F)] * 100

where

I = weight of food consumed;

Ba = arithmetic mean of insect weight during the experiment ¼
[(PF-PI)/log (PF/PI)];
PF = larvae final weight (mg);
PI = larvae starting weight (mg);
T = feeding period in days;
DB = change in body weight;
F = weight of faeces produced during the feeding period

Oviposition-Deterrent test

Artificial fruits (oviposition substrates) were prepared by boiling a mixture of peach juice (500 ml), agar (15 g), and sodium benzoate (one teaspoonful) as preservative. This agar solution was poured into cylindrical molds, allowed to gel, and sliced. The agar cylinders were then wrapped in PVC film to avoid dehydration. The surface of the wrapped cylinder was pricked with a needle and treated with an acetone or methanol solution of the sample to be tested. An amount of 30 µg/cm^2 of the test compound was deposited. Control cylinders were impregnated only with the solvent that was then removed in vacuum. Three groups of *C. capitata* adults were selected from the EEAOC laboratory colony. Each group, consisting of seven male–female pairs, was placed in a small cage and covered with voile (a light, almost transparent cloth made of silk). Two agar cylinders (sample and control) were placed over the voile, and females oviposited on one or the other according to their preference (Fig. 2). After 4 days, eggs were gently rinsed from the agar and counted.

The oviposition index was defined as IO% = (1-T/C) x 100, where T is the number of eggs laid in the treated artificial fruit, and C is the number of eggs deposited in the control fruit (Socolsky *et al*. 2008).

Insecticidal bioassay with C. capitata adult flies

Assays were performed by adding compounds to artificial diet of the adult flies at a concentration of 100 µg / g (sample weight / weight of artificial diet). This diet is offered to adult flies (F1) of *C. capitata* (300 flies per trial). They are kept in cages until seen 2 days after the beginning of intercourse and then are allowed to oviposit.

In this assay were evaluated:
1- The volume of oviposited eggs.
2- The viability of eggs.
3- The mortality of adult flies.
4- The larval mortality F2 (1st generation of neonates from parents subjected to treatment).
5- The pupal mortality F2.

The experiment was performed in triplicate, using an identical but without the addition of substances, as a target system. The experience takes place at a controlled temperature (24 ± 2 °C) and r.h. (60 ± 10) and photoperiod L12:D12 (Salvatore *et al*. 2004).

Statistical analysis

The results are reported as mean ± SEM. The differences in the mean values were evaluated by analysis of variance (ANOVA). The Tukey test was used for all pair wise multiple comparisons of groups. In all statistical analysis, $P > 0.05$ was considered not significant. The LD_{50} values for each activity were calculated using a probit analysis software program based on percentage of mortality obtained at each concentration of the samples (MINITAB *Release* 14 for Windows).

III. RESULTS

During our screening program on biological activities of plants from Argentine and Bolivia in a preliminary trial (Vera *et al*. 2008), the eudesmanes of *P. sagittalis* displayed antifeedant activity at 100 µg/g of diet on *S. frugiperda*. Based on this information, we carried out several studies with eudesmanes **1 - 7** (Figure 1), in a concentration range 25-200 µg/g of diet.

Antifeedant activity

The antifeedant activities of eudesmanes **1-7** against *S. frugiperda* are shown in Table 1. *S. frugiperda* larvae ate significantly less when fed a diet treated with compounds **1**, **5** and **7** at 200 µg/g of diet (Feeding election indexes FEI, of 50, 50, and 72%, respectively). Eudesmane **5**, was the only one with an inhibition percentage higher than 25% at the lowest dose tested 25 µg/g diet. All eudesmanes have a concentration dependent deterrent activity. In the no-choice feeding assay none of the tested compounds displayed antifeedant effects against *S. frugiperda* (Table 1).

Insecticidal bioassay against S. frugiperda

A significant growth reduction was observed in the larvae fed on the treated diet containing eudesmanes **1-3**, **5** and **7**, even at the lowest concentration. Growth inhibition of about 60% relative to control, was observed in the treatment with compound **7** at all the concentrations tested (Table 3). A marked decrease in the daily consumption of diet was observed in treatments with eudesmanes **1**, **3**, **5** and **7** at the four concentrations tested. At doses of 100 µg and 200 µg/g, diet intake inhibition percentage reached 85% relative to control. A significant reduction ($P < 0.01$) in ECI and ECD was also observed in the compounds at 100 and 200 µg/g except for eudesmanes **4** and **6** that showed no significant differences compared to control. No significant differences ($P < 0.01$) on DA were observed in any of the eudesmanes at 100 µg/g of diet (Table 2).

The larvae were fed during the course of the experiment. Eudesmanes **1**-**7** were incorporated to the larval diet at 25-200 µg/g and larval development and mortality were recorded. Our results are outlined in Table 2. Eudesmane **1** was the most toxic in the larval stage with 57 % larval mortality at 200 µg/g of diet. Products **1** and **5** also showed high percentages of pupal mortality with 33 and 30%, respectively, at the highest dose tested (200 mg/g diet). Adult insects that survived the treatment showed higher rates of malformation. Compound **5** was the most active at 200 µg/g diet with a 50% of malformation in the wings which prevented them from reproducing. Also, the duration of larval and pupal cycle, compared to that of the control, were affected by the treatments at doses of 100 and 200 µg/g of diet (Table 3).

Oviposition Deterrent test

The results are summarized in Table 4. All tested compounds (**1-7**) produced oviposition deterrence. At a dose 30 µg/cm^2 of artificial fruit the eudesmanes **1** and **5** were the most active with percentages of 84.18 and 87.52 of inhibition, respectively. (Table 4)

4.4 Insecticidal bioassay with C. capitata adult flies

Only the major eudesmanes (**6** and **7**) were evaluated. As shown in Table 5, the treatments did not produce mortality in adult insects that ingested the treated diet. The viability of oviposited eggs by females who ingested treatments was not affected. However, higher percentages of F2 neonate larvae mortality were observed (F2: the first generation larvae of viable eggs oviposited by females consuming the treated diet), 51% for eudesmane **6** and 54% for eudesmane **7** at 100 µg/g artificial diet. F2 pupal mortality was 33 and 29% for **6** and **7** respectively (Table 5).

IV. DISCUSSION

Botanicals are a rich source of organic chemicals on earth. Discovery of novel toxins and/or antifeedants from plant extracts has been recently emphasized as a potential method for the development of ecologically safe pesticides (Wheeler *et al*. 2001). Antifeedants offer first line of crop protection against notorious insects. Any substance that reduces food consumption by an insect can be considered as an antifeedant or feeding deterrent (Isman 2002). In general, antifeedants have profound adverse effects on insect feeding behavior (Hummelbrunner LA & Isman MB). Antifeedants can be described as allomone substances which inhibit feeding and do not kill the insect pests directly, but rather limit its developmental potential considerably and act as a phagodeterrent or phagorepellent over test as well as permanent insect pests feeding on the plant (Lakshmanan *et al*. 2012). Plant substances acting as antifeedants are found in all the compound groups of secondary plant metabolism. However, the most effective insect feeding inhibitors come from terpenoids, alkaloids, saponins and polyphenols (Koul 2005). In a previous study carried out by our group, we determined that sesquiterpenes eudesmane-type produced a significant deterrence of the intake in *Spodoptera frugiperda* larvae when the diet choice tests were performed at 100 µg / g diet (Vera *et al*. 2008). The results of our bioassays showed that eudesmanes 1-**7** produced inhibition of feeding in a concentration range between 25-200 µg / g of diet and the effect is concentration dependent for all eudesmanes tested in the choice test. Compound **7** was the most active, reaching a value of inhibition at 200 µg / g of diet seven times greater than the one at 25 µg / g of diet. However, when evaluating the feeding behavior of the insect without giving it the choice, we observed that none of the eudesmanes inhibited the insect from feeding, even at the highest dose used (200 µg / g diet). This difference in dietary behavior may be due to a suppressive effect of food where the reduction of food intake occurs after initial consumption. Similar results were presented by Macleod *et al*. (1990), who used two active ingredients isolated from *M. azedarach* (meliatoxin A2 and meliatoxin B1), which inhibited feeding when the larvae of *Spodoptera litura* were able to choose. However, in tests without this option Meliatoxin B1 did not reduce the intake. Other extracts also showed increased anti-food activity on *S. littoralis*, when the caterpillars had access to the control over non-choice trials (Sadek 2003).

The nutritional indices help us to be able to approach the mode of action of these compounds. The EUs 1, 3, 5 and 7 produced a significant reduction in larval growth (RGR) even at 25 µg / g diet. In relation to consumption, there is a marked decrease in RCR for treatments with EUs 1, 3, 5 and 7 at all doses evaluated. A significant reduction in ECI and ECD were observed for all compounds at 100 and 200 µg / g, except for EUs 4 and 6 that did not show significant differences with the control (table 2). Energy diversion to other metabolic pathways, such as those involved in the detoxification of allelochemicals, may be the cause of the decrease in efficiencies (Koul & Isman 1991; Hernández & Vendramim 1997). This decrease causes inhibition of larval growth and is considered as a chronic post-ingestive toxic effect (Wheeler & Isman 2001; Sadek 2003) by several authors. As the diet concentration of the EUs increases, feed intake, growth and feed conversion efficiency reduction were observed suggesting that these compounds have a toxic effect on *S. frugiperda*, producing larval, pupal mortality and high percentages of malformations in adults.

The EUs 18 and 21 produced the greatest *Ceratitis capitata* inhibition of oviposition with% IO of 84 and 88%, respectively. That is to say, these substances caused a reduction of more than 80% in the number of eggs deposited in the treated artificial fruit in comparison with the artificial fruit control. These compounds exhibit an activity similar to naringin, isolated from *Elaphoglossum spathulatum* (IO = 74%, 6 µg / cm^2), one of the natural products evaluated in our work group that produced the greatest inhibition of oviposition in *C. capitata* (Socolsky 2003), which would be promising for the control of this pest. These results constitute the first antecedent of the study of EUs as inhibitors of oviposition in *C. capitata*.

Although the number of tested eudesmanes was reduced to establish a relationship between structure and biological activity, our results show how small structural changes may modify the mode of action of these compounds. Eudesmanes that had a hydroperoxide (**5**) or formoxi groups in their structures (**1** and **7**) were the most active, while the eudesmanes possessing an acetyl group in position 4 lost such activity noticeably. Additionally, an α,β-unsaturated system played an important role in antifeedant activity, as seen when comparing compound **2** with compound **3**, that showed a low activity (Table 1). These results are in agreement with those published in previous works by other authors and our research group (Srivastava *et al*. 1990; .Faini *et al*. 1997; Vera *et al*. 2008; Alarcon *et al*. 2013). Eudesmanes highly oxygenated has a marked influence on biological activity and the individual biological activities of sesquiterpenoids depend on the different functional groups present and the pattern of oxygenation. Similar results were published by Gonzalez *et al*. (1993); Céspedes *et al*. (2001); Guan *et al*. (2005); and Alarcon *et al*. (2013). All eudesmanes assayed have trans-fused decalin architecture differently from the sesquiterpenes used by Miyazawa *et al*. (2000) and Alarcon *et al*. (2013). The sites and mode of action of these compounds are being investigated and probably correspond to a combination of antifeedant action, as well as insecticidal and insect growth regulation activities. Although some natural insecticides are found on the market, the search for new compounds with activity against a variety of insects are always necessary either to prevent the emergence of resistance in insects or to guarantee the ready availability of natural insecticides through more widely distributed sources. This study suggests that eudesmanes and plants containing it might be used as new tools for protecting from harmful insects, especially in organic agriculture. Additionally, these compounds could be promising precursors to generate a series of more active derivatives that acted as a chemical defense against predation by certain phytophagous insects.

Conflict of interest

The authors declare that there are no conflicts of interest and they have no actual or potential competing financial interests

ACKNOWLEDGMENTS

Work in Argentina was supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina (CONICET) and Consejo de Investigaciones de la Universidad Nacional de Tucumán (CIUNT).

REFERENCES

[1] ALARCON, J., LAMILLA, C. & CESPEDES, C.L. (2013). Insecticidal activity of sesquiterpenes skeleton synthesized by the conventional Robinson annulations reaction on *Drosophila melanogaster*. *Industrial Crops and Products* **42**, 268-272.

[2] AHMAD, V.U., SULTANA, A. & FIZZA, K.Z. (1990). Two new terpenoids from *Pluchea arguta*. *Zeitschrift für Naturforschung* **45B**, 385-388.

[3] AHMAD, V.U., FIZZA, K.Z., KHAN, M.A. & FAROOQUI, T.A. (1991). Sesquiterpenes from *Pluchea arguta*. *Phytochemistry* **30**, 689-691.

[4] AHMED AA, MELEK FR & MABRY TJ (1987). Sulfated and non-sulfated flavonoids from *Pluchea dioscoridis*. *Journal of Natural products* **50**, 311.

[5] BREMER, K. & ANDERBERG, A. (1994). Asteraceae: cladistics & classification. Portland, Or.: Timber Press.

[6] CÉSPEDES, C.L., ALARCÓN, J., ARANDA, E., BECERRA, J. & SILVA, M. (2001). Insect growth regulator and insecticidal activity of β-dihydroagarofurans from Maytenus spp. (Celastraceae). *Zeitschrift für Naturforschung C* **56c**, 603-613.

[7] CHAKRAVARTY, A.K. & MUKHOPADHYAY, S. (1994). New thiophene derivatives from *Pluchea indica*. *Indian Journal of Chemistry* **33B**, 978-980.

[8] DADANG OHSAWA, K., KATO, S. & YAMAMOTO, I. (1996). Insecticidal compound in tuber of *Cyperus rotundus* L. against the diamondback moth larvae. *Journal of Pest Science* **21**, 444-446.

[9] FAINI, F., LABBE, C., TORRES, R., DELLE MONACHE, G., DELLE MONACHE, F. & COLL, J. (1997). Eudesmane derivatives from Flourensia

thurifera: structure and biological activity. *Natural Products Letters* **11**, 1-4.

[10] FARRAR, R.R., BARBOUR, J.D. & KENEDY, G.G. (1989). Quantifying food consumption and growth in insects. *Annales of the Entomological Society of America* **82**, 593-598.

[11] FIGUEREDO, S.M., PINTO DO NASCIMENTO, F., FREITAS, C.S., HATSUKO BAGGIO, C., SOLDI, C., PIZZOLATTI, M.G., CHELLION DE IBARROLA, M., DEGEN DE ARRUA, R.L. & SOARES SANTOS, A.R. (2011). Antinociceptive and gastroprotective actions of ethanolic extract from *Pluchea sagittalis* (Lam) Cabrera. *Journal of Ethnopharmacology* **135**, 603-609.

[12] GONZALEZ, A., JIMENEZ, I.A., RAVELO, A.G, & BAZZOCHI, I.L. (1993). Minor sesquiterpenes from *Maytenus canariensis* with insecticidal and antifeedant activity. *Tetrahedron* **49**, 6637-6644.

[13] GUAN, Y.K., LI, P., FANG, L.F. & LI, Y.L. (2005). The first total synthesis of 3-oxo-11,12,13-trihydroxyeudesm-4-ene. *Chinese Chemical Letters* 16, 711-712.

[14] GUILHON, G.M.S.P. & MÜLLER, A.H. (1996) Eudesmane derivatives from *Pluchea quitoc*. *Phytochemistry* **43**, 417-421.

[15] GUILHON, G.M.S.P. & MÜLLER, A.H. (1998a). Eudesmane sesquiterpenoids from *Pluchea quitoc*. *Phytochemistry* **47**, 227-229.

[16] GUILHON, G.M.S.P. & MÜLLER, A.H. (1998b). Eudesmanolides and epoxycuauthemones from *Pluchea quitoc*. *Phytochemistry* **49**, 1347-1351.

[17] HERNANDEZ, C. R. & VENDRAMIN, J.D. (1997). Bioactivity evaluation of aqueous extracts of Meliaceae to *Spodoptera frugiperda*. *Revista agrícola* **72**, 305-318.

[18] HUMMELBRUNNER, L.A. & ISMAN, M.B. (2001). Acute, sublethal, antifeedant and synergestic effects of monoterpenoid essential oil compounds on the tobacco cutworm *Spodoptera litura* (Lepidoptera: Noctudiae). *Journal of Agricultural and Food Chemistry* **49**, 715-720.

[19] ISMAN, M.B. (2002). Insect antifeedants. *Pesticide Outlook* **13**,152-157.

[20] KOUL, O. (2005). Insect Antifeedants. CRC Press LLC: Boca Raton, FL, USA.

[21] KOUL, O. & ISMAN, M. B. (1991). Effects of azadirachtin on the dietary utilization and development of the variegated cutworm *Peridroma saucia*. *Journal of Insect Physiology* **37**, 591-598.

[22] LAKSHMANAN, S., KRISHNAPPA, K. & ELUMALAI, K. (2012). Certain plant essential oils against antifeedant activity of *Spodoptera litura* (Fab.), *Helicoverpa armigera* (Hub.) and *Achaea janata* (Linn.) (Lepidoptera: Noctuidae). *International Journal of Current Life Sciences* **2,** 5-11.

[23] MACLEOD, J. K., MOELLER, P. D., MOLINSKI, T. F. & KOUL, O. (1990). Antifeedant activity against *b* larvae and [13C] NMR spectral assignments of the meliatoxins. *Journal of Chemical Ecology* **16**, 2511-2518.

[24] MAHMOUD, A. (1997). 7-*epi*-eudesmanes, eudesmanoic acids, eudesmanolides and other sesquiterpenes from *Pluchea dioscoridis*. *Phytochemistry* **45**, 1633-1688.

[25] MIYAZAWA, M., NAKAMURA, Y. & ISHIKAWA, Y. (2000). Insecticidal sesquiterpene from *Alpinia oxyphylla* against *Drosophila melanogaster*. *Journal of Agricultural and Food Chemistry* **48**, 3639–3641.

[26] PEREZ-GARCÍA, F., MARÍN, E., CAÑIGUERAL, S. & ADZET, T. (1996) Anti-inflammatory action of *Pluchea sagittalis*: involvement of an antioxidant mechanism. *Life Sciences* **59**, 2033-2040.

[27] PEREZ-GARCÍA, F., MARÍN, E., PARELLA, T., ADZET, T. & CAÑIGUERAL, S. (2005). Activity of taraxasteryl acetate on inflammation and heat shock protein synthesis. *Phytomedicine* **12**, 278-284.

[28] SADEK, M.M. (2003). Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep. Noctuidae). *Journal of Applied Entomology* **127**, 396-404.

[29] SALVATORE, A., BORKOSKY, S., WILLINK, E. & BARDÓN, A. (2004). Toxic effects of lemon peel constituents on *Ceratitis capitata*. *Journal of Chemical Ecology* **30**, 323-333.

[30] SCHOLZ, E., HEINRICH, M. & HUNKLER, D. (1994). Caffeoylquinic acids and some biological activities of *Pluchea symphytifolia*. *Planta Medica* **60**, 360-364.

[31] SOCOLSKY, C., SALVATORE, A., ASAKAWA, Y. & BARDÓN, A. (2003). Bioactive new bitter-tasting p-hydroxystyrene glycoside and other constituents from the fern *Elaphoglossum spathulatum*. *Arkivoc* **10**, 347-355.

[32] SOCOLSKY, C., FASCIO, M.L., D'ACCORSO, N.B., SALVATORE, A., WILLINK, E., ASAKAWA, Y. & BARDON, A. (2008). Effects of p-Vinylphenyl Glycosides and other related compounds on the oviposition behavior of *Ceratitis capitata*. *Journal of Chemical Ecology* **34**, 539-548.

[33] SRIVASTAVA, R.P., PROKSCH, P. & WRAY, V. (1990). Toxicity and antifeedant activity of a

sesquiterpene lactone from Encelia against *Spodoptera littoralis*. *Phytochemistry* **29**, 3445-3448.

[34] VERA, N., POPICH, S., LUNA, L., CRAVERO, R., GONZÁLEZ SIERRA, M. & BARDÓN, A. (2006). Toxicity and synergism in the feeding deterrence of some coumarins on *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae). Chemistry & Biodiversity **3**, 21-26.

[35] VERA, N., MISICO, R., GONZALEZ SIERRA, M., ASAKAWA, Y. & BARDON, A. (2008). Eudesmanes from *Pluchea sagittalis*. Their antifeedant activity on *Spodoptera frugiperda*. *Phytochemistry* **69**, 1689-1694.

[36] WANG, M., QIN, H., KONG, M. & LI, Y. (1991). Insecticidal sesquiterpene polyol ester from *Celastrus angulatus*. *Phytochemistry* **30**, 3931-3933.

[37] WALDBAUER, G.P. (1968). The consumption and utilization of food by insects. *Advances Insect Physioloy* **5**, 229-288.

[38] WHEELER, D.A. & ISMAN, M.B. (2001). Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*. *Entomologia Experimentalis et Applicata* **98**, 9-16.

[39] WHEELER, D.A., ISMAN, M.B., SANCHEZ-VINDAS, P.E., ARNASON, J.T. (2001). Screening of Costa Rican Trichilia species for biological activity against the larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). Biochemical Systematic and Ecology **29**, 347-358.

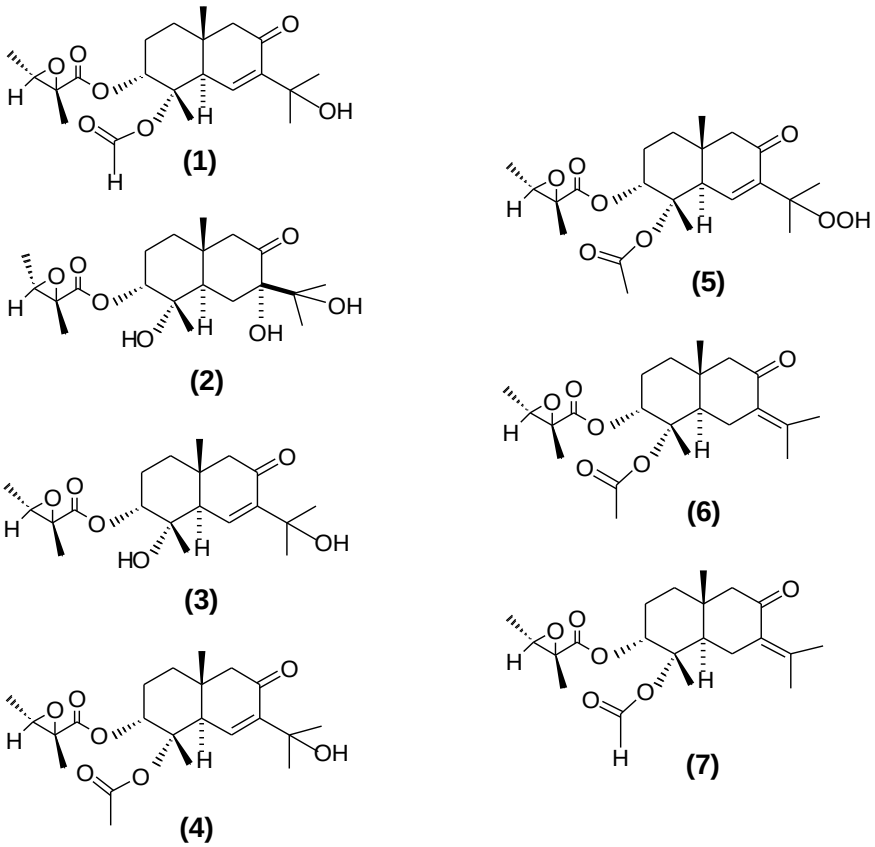


Fig.1: Eudesmanes isolated from Pluchea sagittalis

Table.1: Insect antifeedant activities of eudesmanes (1-7) on S. frugiperda.

	Feeding deterrence index							
	[a] % FEI_{CH} Choice test				[a] % FEI_{NCH} No Choice test			
Doses (µg/g)	25	50	100	200	25	50	100	200
Compounds								
1	10.9 ± 1.0 a	21.0 ± 3.0 b	30.7 ± 5.2 d	50.4 ± 5.0 c	1.0 ± 0.2 a	1.0 ± 0.4 a	4.0 ± 0.4 c	5.4 ± 0.4 d
2	10.1 ± 1.7 a	12.7 ± 0.8 a	13.7 ± 2.0 a	17.9 ± 2.8 b	1.1 ± 0.2 a	2.0 ± 0.8 b	2.0 ± 0.3 b	4.9 ± 0.8 d
3	10.8 ± 2.9 a	13.1 ± 2.1 a	29.8 ± 3.8 b	34.6 ± 4.3 d	0.8 ± 0.1 a	3.1 ± 0.2 bc	3.0 ± 0.4 b	4.6 ± 0.3 d
4	12.4 ± 2.5 a	19.1 ±2.6 b	28.0 ± 3.6 b	37.3 ± 3.4 d	2.4 ± 0.5 b	3.1 ± 0.3 bc	7.0 ± 0.7 e	7.3 ± 0.8 de
5	27.2 ± 4.2 b	51.8 ± 3.6 c	50.1 ± 4.1 c	50.7 ± 5.3 c	2.2 ± 0.2 b	1.8 ± 0.6 ab	7.0 ± 1.0 e	6.7 ± 1.6 de
6	10.9 ± 2.0 a	11.0 ± 3.0 a	19.2 ± 2.2 b	19.4 ± 5.0 b	2.9 ± 0.8 b	3.5 ± 0.5 c	6.0 ± 1.0 de	6.4 ± 1.6 de
7	10.5 ± 2.0 a	31.8 ± 4.2 d	32.3 ± 4.2 d	72.2 ± 6.7 e	2.5 ± 0.2 b	3.8 ± 0.6 c	5.0 ± 0.9 d	6.2 ± 1.5 de

The values followed by the same letter are not significantly different. The significance level $P < 0.05$. * FEI (%) = Feeding election index = [(1 − T/C) × 100].

Table.2: Effect of eudesmanes (1-7) incorporated into larval diet on food consumption and utilization by S. frugiperda larvae.

		Nutritional indices				
Compounds	**Doses (µg/g)**	**[a] RCR = I/(B_a T)**	**[a] RGR=ΔB/(B_aT)**	**[a] ECI= (ΔB/I*100)**	**[a] AD=(I- F/I)*100**	**[a] ECD = (ΔB/I-F)*100**
Control		**0.51 ± 0.06 a**	**0.25 ± 0.03 a**	**21.12 ± 2.9 a**	**73.88±3.5 a**	**23.45±1.5 a**
1	25	0.39 ± 0.06 **b**	0.12 ± 0.03 **b**	23.13 ± 1.8 **a**	73.20±8.0 **a**	22.63±2.2 **a**
	50	0.25 ± 0.05 **c**	0.11 ± 0.03 **b**	18.43 ± 2.2 **b**	68.21±2.6 **a**	18.44±2.8 **b**
	100	0.14 ± 0.03 **d**	0.09 ± 0.03 **b**	18.34 ± 3.8 **b**	69.26±1.5 **a**	17.62±2.5 **b**
	200	0.13 ± 0.02 **d**	0.06 ± 0.06 **c**	14.38 ± 2.6 **c**	67.51±2.4 **a**	14.13±1.8 **c**
2	25	0.45 ± 0.03 **a**	0.09 ± 0.02 **b**	20.09 ± 2.8 **a**	74.22 ± 2.3 **a**	20.09 ± 2.8 **a**
	50	0.44 ± 0.03 **a**	0.08 ± 0.008 **d**	22.32 ± 2.5 **a**	75.64 ± 3.3 a	22.32 ± 2.5 **a**
	100	0.43 ± 0.02 **a**	0.07 ± 0.01 **d**	17.78 ± 2.8 **b**	71.16 ± 3.1 a	17.78 ± 2.8 **b**
	200	0.34 ± 0.04 **b**	0.08 ± 0.02 **d**	17.48 ± 1.5 **b**	76.01 ± 2.2 a	17.48 ± 1.5 **b**
3	25	0.29 ± 0.06 **b**	0.11 ± 0.03 **b**	14.78 ± 2.6 **c**	71.13 ± 6.3 **a**	13.07 ± 7.2 **c**
	50	0.29 ± 0.07 **b**	0.11 ± 0.02 **b**	13.88 ± 1.6 **c**	78.67 ± 8.3 **a**	15.52 ± 1.2 **c**
	100	0.15 ± 0.02 **d**	0.04 ± 0.008 **f**	12.96 ± 2.3 **c**	72.00 ± 3.1 **a**	10.12 ± 3.1 **c**
	200	0.15 ± 0.04 **d**	0.04 ± 0.02 **c**	12.45 ± 1.8 **c**	69.91 ± 4.2 **a**	10.45 ± 4.9 **c**
4	25	0.47 ± 0.05 **a**	0.24 ± 0.04 **a**	20.58 ± 1.2 **a**	69.21 ± 3.0 **a**	27.14 ± 7.2 **a**
	50	0.44 ± 0.06 **a**	0.23 ± 0.04 **a**	22.02 ± 1.8 **a**	73.78 ± 4.2 **a**	26.96 ± 3.2 **a**
	100	0.42 ± 0.06 **a**	0.21 ± 0.05 **a**	21.71 ± 0.7 **a**	74.16 ± 0.8 **a**	24.79 ± 4.8 **a**
	200	0.42 ± 0.06 **a**	0.26 ± 0.05 **a**	21.51 ± 1.3 **a**	73.84 ± 4.0 **a**	23.29 ± 5.9 **a**
5	25	0.36 ± 0.03 **b**	0.06 ± 0.02 **c**	21.32 ± 0.5 **a**	75.49 ± 5.5 **a**	20.12 ± 3.3 **a**
	50	0.34 ± 0.04 **b**	0.05 ± 0.02 **c**	18.45 ± 0.5 **b**	72.79 ± 3.1 **a**	18.53 ± 5.4 **b**
	100	0.13 ± 0.01 **d**	0.02 ± 0.007 **f**	17.67 ± 0.4 **b**	69.21 ± 2.5 **a**	18.28 ± 8.2 **b**
	200	0.13 ± 0.03 **d**	0.05 ± 0.01 **dc**	14.65 ± 0.6 **c**	71.20 ± 2.4 **a**	14.73 ± 7.1 **c**
6	25	0.48 ± 0.05 **a**	0.29 ± 0.04 **a**	20.58 ± 1.2 **a**	71.58 ± 8.0 **a**	20.27 ± 4.2 **a**
	50	0.42 ± 0.04 **ab**	0.24 ± 0.01 **a**	22.02 ± 0.8 **a**	74.16 ± 5.2 **a**	22.61 ± 4.2 **a**
	100	0.43 ± 0.05 **ab**	0.24 ± 0.02 **a**	20.71 ± 0.7 **a**	78.83 ± 7.2 **a**	23.52 ± 5.3 **a**
	200	0.41 ± 2.1 **b**	0.25 ± 0.02 **a**	21.31 ± 1.3 **a**	76.64 ± 2.0 **a**	19.80 ± 2.9 a

7	25	0.38 ± 0.05 **b**	0.14 ± 0.02 **b**	16.17 ± 1.4 **b**	77.01 ± 4.9 **a**	18.12 ± 4.3 **ab**
	50	0.39 ± 0.04 **b**	0.15 ± 0.01 **b**	15.70 ± 0.3 **b**	75.40 ± 6.1 **a**	18.65 ± 7.1 **ab**
	100	0.40 ± 0.03 **b**	0.15 ± 0.02 **b**	12.64 ± 0.8 **c**	75.55 ± 2.5 **a**	13.67 ± 5.4 **b**
	200	0.40 ± 0.04 **b**	0.14 ± 0.03 **bc**	11.93 ± 1.8 **c**	78.20 ± 4.4 **a**	12.78 ± 4.4 **b**

Values are mean ± SE (n = 20).The values followed by the same letter are not significantly different. The significance level P < 0.05.

Table.4: Effect of eudesmanes (1-7) on the oviposition-behavior of C. capitata

Compounds	**Number of Eggs Laid on the Control Fruit**	**Number of Eggs Laid on the Treated Fruit**	**% IO = (1-T/C) *100 30 µg/cm^2**
1*	810 ± 43a	256 ± 40 a	84.18 ± 13.1a
2*	741 ± 25a	254 ± 27b	59.96 ± 7.5b
3*	876 ± 12a	670 ± 14c	34.85 ± 1.1c
4	457 ± 31a	411 ± 34d	11.2 ± 2.0 d
5*	522 ± 51a	91 ± 4^a	87,52 ± 5.5a
6	868 ± 23a	753 ± 14d	13.2 ± 3.0 d
7	894 ± 11a	488 ± 13^a	45.4 ± 4.0 a

Numbers represent mean ± SEM, n=3. Means within a column followed by the same letter are not significantly different (P>0.05, paired t test)

*Table.5: Insecticidal activity of eudesmanes (**6** and **7**) against adults of Ceratitis capitata.*

	Mortality F1 (%)	**Oviposition of F1 Volume of eggs (ml)^a)**	**Hatching eggs of F1 (%)**	**Larval Mortality of F2 (%)**	**Pupal Mortality of F2 (%)**
Control	5 a	1.2 ± 0.1a	94 a	12a	11a
6	3 a	0.4 ± 0.05b	95 a	51b	33b
7	2 a	0.4 ± 0.1b	96 a	54b	39b

F1: Adult insects fed on the treated diet. F2: The first generation larvae of viable eggs oviposited by females consuming the treated diet.
Numbers represent mean ± SEM, n=3. Means within a column followed by the same letter are not significantly different (P>0.05, paired t test)

http://dx.doi.org/10.22161/ijeab/2.1.46 *ISSN: 2456-1878*

Molecular variations due to phylogeographic factors in *Channa punctatus* found in different regions of India

Shobhna[1*], Seema Jain[1], Hridaya Shanker Singh[2], Manu varma[1]

[1]Department of Zoology, R.G.P.G.College,C.C.S University, Meerut - 250004, India
[2]Department of Zoology, Ch. Charan Singh University, Meerut - 250004, India

*Corresponding author: Shobhna, Department of Zoology, R.G.P.G. College, C.C.S. University, Meerut- 250004, India

Abstract*— *Channa punctatus is a freshwater fish belonging to family Ophiocephalidae. This fish is available in almost all over the country. In present communication molecular study of COI region of mitochondrial gene was done to find out intraspecific differences in genome of Channa punctatus habitating in different states of India having variable environmental conditions. Result showed minor variations in mitochondrial genome of Channa punctatus and utility of molecular markers to show intraspecific variations.

Keywords*— *Channa punctatus, mitochondrial COI gene, intraspecific variations.

I. INTRODUCTION

In recent years studies have investigated the effects of climate change on the future of biodiversity. Predicting the response of biodiversity to climate change has become an active field
of research (Dillon *et al*. 2010; Gilman *et al*. 2010; Pereira *et al*. 2010; Salamin *et al*. 2010; Beaumont *et al*. 2011; Dawson *et al*. 2011; McMahon *et al*. 2011). Intraspecific genetic variation is important for maintaining the ability of species to adapt to new environmental conditions (Frankham *et al*. 2002). It is necessary to study the effect of climate changes on intraspecific genetic diversity to trace the evolutionary consequences of climate and its effects on biodiversity.

Aquaculture contributes to over 70% of inland fish production placing India second rank in the world. A number of air breathing fishes are indigenous and many of these are popular as food fish among the Indians. This includes *Channa* Species (Snake heads) belonging to family Ophiocephalidae (Bleeker). According to Jayaram (1981), eight species of *Channa* has been reported so far from India. *Channa punctatus* (Snake head or murrel) is found in almost all parts of the country and can thrive in almost any situation. They assist in keeping water pure by destroying either animal or vegetable substances, which may come in their way (Day, 1878).Snake head or murrels are important food fishes acclaimed all over the country for their flavour, medicinal values, well adapted for Pisciculture recuperative attributes and keeping quality. Giant murrels and stripped murrels are regarded as excellent table fish for attaining large sizes in states like Punjab, West Uttar Pradesh, Madhaya Pradesh. Andhra Pradesh, Karnataka and Kerala.

Phylogeographic studies separates population history to reconstruct the fate of genetic diversity and can provide insight into species reactions to climate change (Paul *et al.* 2013). India is a country of variety of climatic regions, ranging from tropical in South to temperate and alpine in Himalayas in North. Climate of four states of India i.e. Lucknow, Assam, Maharashtra and Tamil Nadu is given below:

Lucknow

Lucknow varies from Temperate to Extreme. Therefore it is extremely difficult to categorize it a particular climatic frame. Summers are very hot and winters are bit chilly. Summer season persists from April to August. The daytime temperature remains very high and usually touches around 45°C. Night are relatively cooler typical of extreme climate and the temperature comes down to as low as 28 °C because of the cool breeze. The winter falls around Mid-November and continue till February end, day is cooler and temperature is pleasant around 24°C. And nights are chilly with temperature getting as low as 2 to 4°C across the state.

In monsoon the rainfall varies considerably. The average annual rainfall varies from 105-110 centimeters. The western disturbance too brings fair amount of rainfall.

http://dx.doi.org/10.22161/ijeab/2.1.46 *ISSN: 2456-1878*

Approximate average annual rainfall in the state is around 65-70 centimeters.

Assam

Assam, generally, observes temperate climate. Its weather is characterized by heavy downpour and humidity. Summer, winter and monsoons are the three seasons that visit the state, rainy season marks the most of the months of a year. Summers prevail for a few months between March and June. However, temperature never goes beyond 35°C - 38°C even in the summer months. Rain showers occur erratically and keep the temperature under control, nevertheless humidity levels shoot up. Heavy precipitation lasts till the month of September. During winter, nights and early mornings are misty. This is only time when Assam observes scanty rainfall.

Maharashtra

The climate of Maharashtra is moderate, with variations in temperature ranging between 16°C and 35°C. July to September are the months when monsoon lashes this state with good rainfall. This does not mean that the whole state gets uniform rainfall; a large part of inner Maharashtra remains dry in comparison to other areas under the rain shadow of the Sahyadri.

Tamil Nadu

Tamil Nadu has a tropical climate with no wild swing between summer and winter temperature. April and May are the hottest months, Coastal regions also get uncomfortably warm and humid during these months but the nights are usually cool. Winter falls between November-February when the climate is pleasantly cool. Minimum temperatures in the plains rarely dip below 20 ºC.

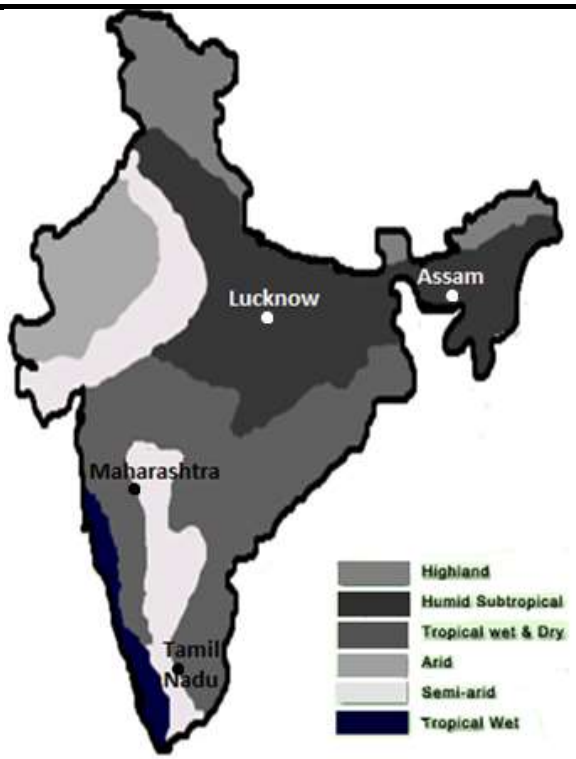


Fig.1: Map of India showing climate of different states.

Genetic variations within a species is important for the long term survival. Intraspecific genetic variation is the fundamental level of biodiversity that provides the basis of any evolutionary change (Frankham *et al.* 2002). Combining genetic data and climate change, represents a significant knowledge in inferring how climate shapes genetic diversity and impact genetic structure.

To check variations in mitochondrial genome due to change in climatic factors of these fish, COI sequences of *Channa punctatus* of four different states of India i.e., Lucknow, Assam, Maharashtra and Tamil Nadu were retrieved from gene bank. These states have different climatic conditions **(Fig.1)** and there is a possibility that these climatic factors can influence some genetic level changes. COI region was chosen because of its rapid evolution rate, this gene also enables the differentiation of closely related species as well as phylogeographic groups within a single species (Cox and Hebert 2001; Wares and Cunningham 2001).

An attempt has been made to evaluate molecular differences in *Channa punctatus* from different states. Same species should have same motifs (conserved regions) in the sequence i.e. Number of motifs, their frequency and position. Therefore, motifs of each sequence have been made by MEME software to compare the conservedness.

http://dx.doi.org/10.22161/ijeab/2.1.46 *ISSN: 2456-1878*

The conserved motifs are found in all species but at variable positions. Gene shifting may be responsible for variable positions of motifs.

II. MATERIALS AND METHOD

Mitochondrial COI sequences of *Channa punctatus* of four states (Lucknow, Assam, Maharashtra and Tamil Nadu) were retrieved from Genbank (available at www.ncbi.nlm.nih.gov) having accession number (Lucknow: FJ459409.1, Assam: JN245992.1, Maharashtra: JX260843.1, Tamil Nadu: EU342202.1). To check the similarity search, COI sequences were uploaded on Basic Local Alignment Search Tool (BLAST). The motifs and their regular expressions were predicted with the help of online available MEME software (Timothy *et al*., 1994). Phylogenetic analysis was performed using Neighbour-Joining (NJ) and Maximum Likelihood (ML) methods with the help of MEGA 6 (Tamura *et al*., 2011) software.

III. RESULTS AND DISCUSSION

All sequences aligned using clustal W online software showed similar conserved regions in their genetic material of same species, *Channa punctatus* but at variable positions. It is noted that same genetic material of all the four species has some variations, incorporated in its position, to adapt to different environmental conditions. The motif prediction confirms conserved regions (motifs) in each sequences and for this, mitochondrial COI sequences of *Channa punctatus* (from Lucknow, Assam, Maharashtra, and Tamil Nadu) retrieved from GenBank have been used for the motif identification with the help of online available software, MEME. The results of MEME showed three different kinds of motifs in mitochondrial COI sequences of *Channa punctatus.* Minimum motif width is found of 29 bases and maximum motif width of 50 bases. Base length of motif one and motif two obtained is 50 bases. However, motif three has 29 bases. Motif one is shown as sky blue, two as deep blue and three as red colour. Motif one (**Fig. 2**), motif two (**Fig. 3**) and motif three (**Fig. 4**) are shown. Motif one was repeated 5 times, motif two 3 times and motif three 2 times respectively in sequences of *Channa punctatus* (FJ459409, JN245992 and JX260843). While in EU342202, motif one was repeated 5 times, motif two 3 times and motif three two times. Positions of motifs in sequences of same species were different from each other which is clearly seen in the combined block diagram (**Fig. 5**). The order of motifs was found same in all sequences. The shifting pattern is clearly seen in combined block diagram of motifs (**Fig. 5**). The p-value of the motifs has been also found to be different for each sequence. The combined P-value of all the three motifs of *Channa punctatus* is 2.46e-37 (in FJ459409), 1.26e-44 (in JN245992), 5.81e-34 (in JX260843) and 1.51e-37 (in EU342202) for all sequences respectively. It indicates that changes in climatic conditions of different regions of India may be responsible for shifting of motifs to adapt themselves.

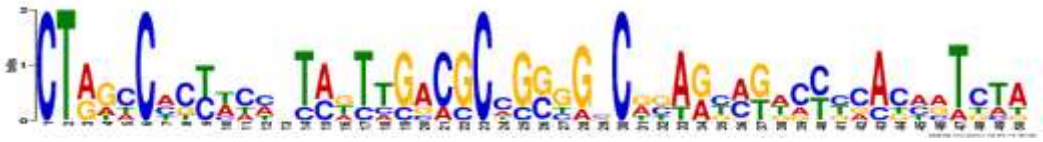


Fig.2: Motif one

Fig. 3: Motif two

Fig. 4: Motif three

http://dx.doi.org/10.22161/ijeab/2.1.46 *ISSN: 2456-1878*

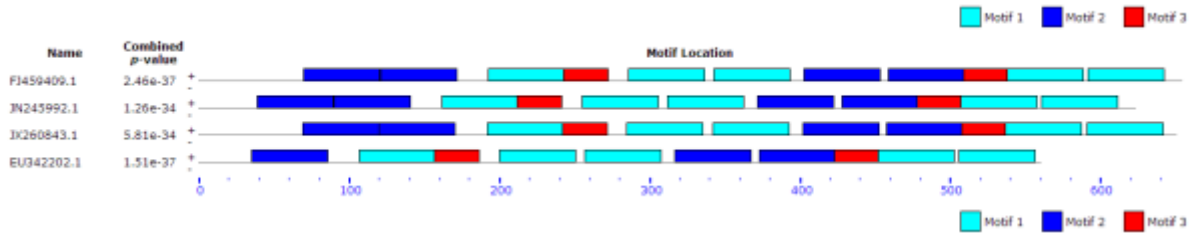


Fig. 5: Combined Block Diagram

Phylogenetic Tree prediction

When sequences of *C. punctatus* were BLAST, they were found 99% similar. To check this one percent variation in the mitochondrial genome, Neighbor Joining (NJ) and Maximum Liklihood (ML) trees were constructed by using MEGA 6 software. In ML tree (**Fig.7**), Lucknow and Assam sequences were found in one clade and Tamil Nadu made an outgroup, which shows that it is slightly different from other groups. For further confirmation, NJ tree (**Fig. 6**) was constructed which shows that Assam and Maharashtra were in one clade and Tamil Nadu again formed an out group.

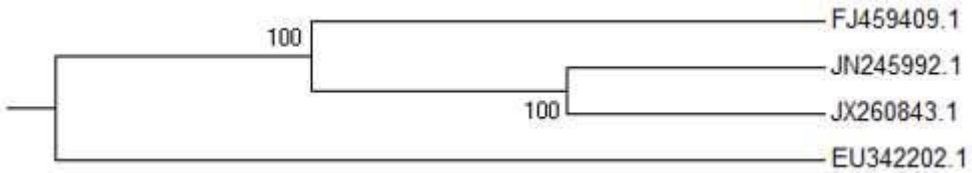


Fig. 6: Neighbor Joining Tree of COI nucleotide sequences

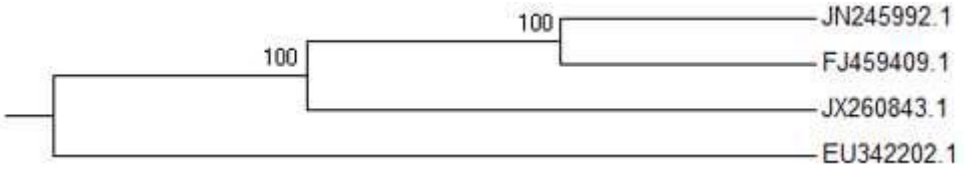


Fig. 7: Maximum likelihood tree of COI nucleotide sequences

IV. CONCLUSION

This molecular analysis reveals the genetic variations between same species of *C. punctatus* from different regions with minor variations. These variations are probably because of gene shifting. Reasons for genetic variations are due to their geographical distribution or adaptation in different regions. At molecular level, this genetic comparison between same species from other parts of different states of India may provide further clues to the understanding of the evolution of the species. Motif prediction and phylogenetic trees prediction could be a valuable tool to see Phylogeographic and intraspecific variations in genome.

REFERENCES

[1] Beaumont, L.J., Pitman, A., Perkins, S., Zimmermann, N.E., Yoccoz, N.G. & Thuiller, W. (2011). Impacts of climate change on the world's most exceptional ecoregions. *Proc. Natl Acad. Sci.* USA, 108, 2306–2311.

[2] Cox A. J., Hebert P. D. N. (2001). Colonization, extinction and phylogeographic patterning in a freshwater crustacean. *Mol. Ecol*. 10, 371-386.

[3] Dawson, T.P., Jackson, S.T., House, J.I., Prentice, I.C. & Mace, G.M. (2011). Beyond predictions: biodiversity conservation in a changing climate. *Science*, 332,53–58.

[4] Day. (1878). The Fishes of India; being a natural history of the fishes know to inhabit the seas and freshwaters of India, Burma and Ceylon. I and II: XX-778; 1-195.

[5] Dillon, M. E., Wang, G. & Huey, R.B. (2010). Global metabolic impacts of recent climate warming. *Nature*, 467, 704–706.

[6] Frankham, R., Ballou, J. D. & Briscoe, D. (2002). An Introduction to Conservation Genetics (Cambridge Univ. Press).

[7] Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G.W. & Holt, R.D. (2010). A framework for

community interactions under climate change. *Trends Ecol. Evol*., 25, 325–331.

[8] Jayaram K. C. (1981). The freshwater Fishes of India, Pakistan, Bangladesh, Burma and Srilanka – A Handbook. Zoological survey of India, Calacutta.

[9] McMahon, S.M., Harrison, S.P., Armbruster, W.S., Bartlein, P.J., Beale, C.M., Edwards, M.E. *et al*. (2011). Improving assessment and modelling of climate change impacts on global terrestrial biodiversity. *Trends Ecol. Evol*., 26, 249–259.

[10] Paul, S. U., Nowak, C., Balint, M. & Pfenninger, M. (2013) The impact of global climate change on genetic diversity within populations and species. *Mol. Ecol*. 22, 925-946.

[11] Pereira, H.M., Leadley, P.W., Proenca, V., Alkemade, R., Scharlemann, J.P.W., Fernandez-Manjarres, J.F. *et al*. (2010). Scenarios for global biodiversity in the 21st century. *Science*, 330, 1496–1501.

[12] Salamin, N., Wu¨est, R.O., Lavergne, S., Thuiller, W. & Pearman, P.B. (2010). Assessing rapid evolution in a changing environment. *Trends Ecol. Evol*., 25, 692–698.

[13] Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution:30 2725-2729.

[14] Timothy L., Bailey and Charles E. (1994). Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proceedings of the Second International Conference on Intelligent Systems for Molecular Biology*, AAAI Press, Menlo Park, California. 28-36.

[15] Wares J. P & Cunningham C.W. (2001). Phylogeography and historical ecology of the North Atlantic intertidal. *Evolution* 12, 2455-2469.

http://dx.doi.org/10.22161/ijeab/2.1.47 *ISSN: 2456-1878*

Stimulatory Effect of the Magnetic Treatment on the Germination of Cereal Seeds

E. Martinez, M. Florez, M.V. Carbonell

Department of Agroforestry Engineering. Physics section. School of Agricultural, Food and Biosystems Engineering. Ciudad Universitaria s/n 28040 Madrid, Spain

Abstract*— *The main objective of this study is to determine the effects of 125 mT magnetic treatment on the germination of different cereals seeds. This objective has a practical application in agriculture science: early growth of seeds. Germination tests were carried out under laboratory conditions by exposing seeds to magnetic field for different times. For each treatment the number of germinated seeds was counted to determine the time necessary to achieve the final maximum percentage of germinated seeds; rate of germination was assessed by determining the mean germination time (MGT) and parameters T1, T10, T25, T50, T75, T90, time required to germinate 1 - 90 percent of seeds. An increase in the percentage and rate of germination of seeds as positive response to magnetic field treatment in rice, wheat, maize and barley seeds have been found for all treatments applied. The mean germination time and parameters were reduced for all the magnetic treatments applied. Most significant differences were obtained for time of exposure of chronically exposure and 24 hours. External magnetic fields enhance seed vigor by influencing the biochemical processes by stimulating activity of proteins and enzymes. Numerous studies suggested that magnetic field increases ions uptake and consequently improves nutrition value.

Keywords*— *cereal seeds, magnetic field, germination, seedling, magnet.

I. INTRODUCTION

The effects on living systems of exposure to a magnetic field, particularly on germination of seeds and growth of plants, have been the object of much research. In general, the enhancement of growth due to magnetic field exposure appears to have been confirmed by many scientists. Some have tried to determine effects related with seed germination, such as changes in biochemical activity, curvature, magnetotropism and germination rate. The main objective of this study is to determine the effects of magnetic treatment, in addition to the geomagnetic field, on the germination of cereal seeds.

Scientists have found an induction of primary root curvature in radish seedlings in a static magnetic field, roots responded tropically to magnetic field with the tropism appearing to be negative; these roots responded significantly to the south pole of the magnet (Yano, et al. 2001). The application of magnetic field doses of 4 mT and 7 mT promoted germination ratios of bean and wheat seeds (Cakmak, et al. 2010). Magnetic treated pea plants grew higher and heavier than control; the greatest differences were observed for seeds treated with doses of 125 mT and 250 mT, for 24 hours or permanent. Germination curves show the earlier germination process and higher germination rate; curves of treated seeds are lightly displaced towards the left side rate. Furthermore, the final percentage of seeds is higher than control seeds (Carbonell et al. 2011). The positive effects on plants characteristics such as seed germination rate, shoot development, length and weight of plants and yield is reported by numerous authors.

II. MATERIAL AND METHODS

Germination tests of cereal seeds were carried out under laboratory conditions with natural light and the minimum and maximum temperature of 18 ºC and 22 ºC, according to guidelines issued by the International Seed Testing Association (ISTA, 2004) with only slight modifications. Cereal seeds (maize, rice, barley and wheat) were supplied by the Spanish Office of Vegetable Varieties, which guarantees high seed viability and homogeneity and thus significant results with smaller samples.

2.1 Magnetic treatment

Magnetic treatment consisted of different doses (D) due to variation in exposure time (t) and magnetic field induction (B). The static magnetic field was generated by permanent ring magnets, with 125 mT strength, external diameter of 7.5 cm, internal diameter of 3 cm, and height of 1 cm. Ring analogous to the ring magnets, of the same material but without magnetic induction, were used as blind (Control). Magnetic doses were obtained by exposing the seeds to each magnetic field for different times: from 1 minute to chronically exposure. The experimental design involves four replicates (n=4) with 25 seeds. Thus, groups of 100 seeds were subjected to each magnetic treatment, and an analogous group was used as control. In each replicate the Petri dishes with seeds was placed on magnet for the corresponding time: 1 min D1, 10 min D2, 20 min

D3, 1 hour D4 24 hours D5 and for all experimental period or chronical exposure D6.

2.2 Germination test

The goal of this test was to determine the possible influence of magnetic treatment on the time required for germination. Germination was tested by placing 25 treated seeds per Petri dish around a circular line, on filter papers soaked with 12 ml of distilled water. Petri dishes with seeds were labeled and randomly located. Experimental groups D1-D6 and control C ran simultaneously. For each treatment the number of germinated seeds was counted to determine the time necessary to achieve the final maximum percentage of germinated seeds (G_{max}). Seeds were considered germinated when their radicle measured at least 1 mm. The rate of germination was assessed by determining the mean germination time (MGT) and time required to germinate 1, 10, 25, 50, 75 and 90 percent of seeds (parameters T_1, T_{10}, T_{25}, T_{50}, T_{75} and T_{90}).

2.3 Statistical analysis

Statistical analysis of variance and mean comparisons ($p \leq 0.001$; $0.001 \leq p \leq 0.01$; $0.01 \leq p \leq 0.05$) was performed using the Seedcalculator software developed for seed germination data analysis by Plant Research International; the software provides the germination curves for each treatment, a comparison of the results of all the treatments and a comparison of those results with the result of the control.

III. RESULTS

Figures 1-4 show the germination curves for each treatment and control: fig. 1 for maize, fig. 2 for rice, fig. 3 for barley and fig. 4 for wheat. Treatment curves are placed to left side that indicates the diminution of germination parameters T_1-T_{90}, consequently the higher rate of germination of cereals seeds. Curves also show the great percentage of germination.

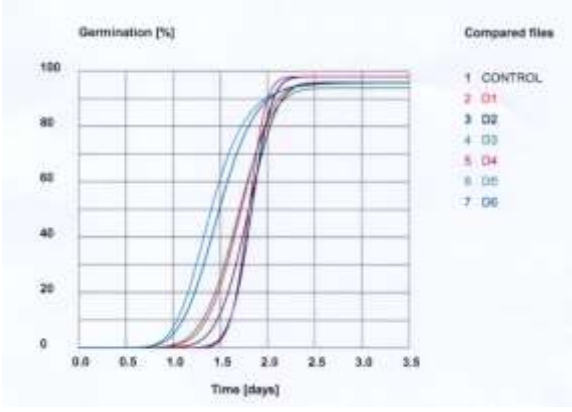


*Fig.1: Germination curves of **maize** seeds subjected to 125 mT magnetic field. Doses D1-D6 versus control C.*

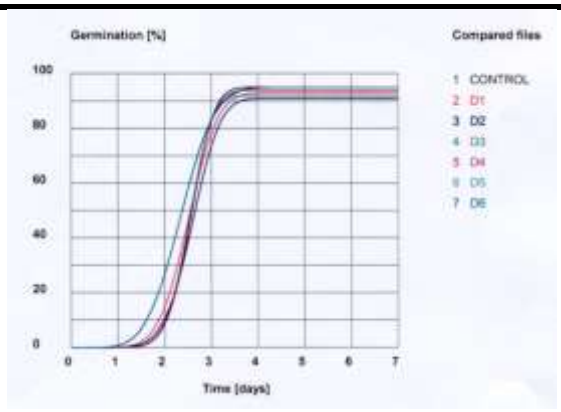


Fig.2: Germination curves of rice seeds subjected to 125 mT magnetic field. Doses D1-D6 versus control C.

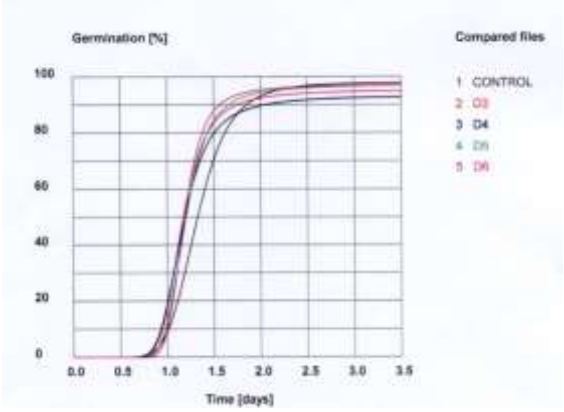


Fig.3: Germination curves of barley seeds subjected to 125 mT magnetic field. Doses D1-D6 versus control C.

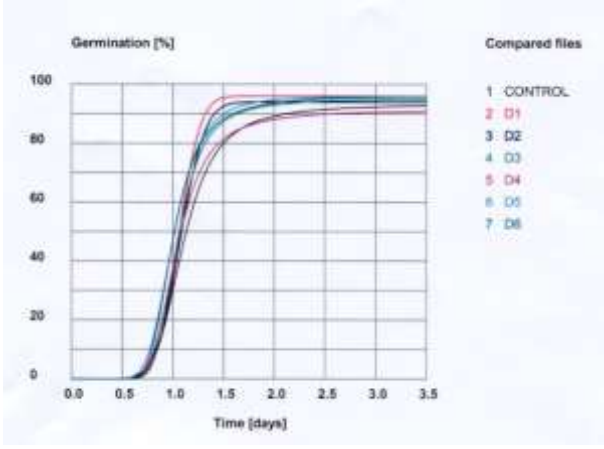


Fig.4: Germination curves of wheat seeds subjected to 125 mT magnetic field. Doses D1-D6 versus control C.

In germination test, percentage of germinated seeds (G_{max}) and the time required for germination (parameters MGT, T_1–T_{90}) were determined for each dose, expressed as the mean of the four replicates and their standard error; the germination parameters obtained from cereals seeds exposed to 125 mT magnetic field (doses D1-D6), are provided in Table 1 for maize, Table 2 for rice, Table 3 for barley and Table 4 for wheat seeds. The number of

germinated seeds (G_{max}), from 80 to 99 %, corroborates the high quality of seeds. Fewer than 90% of seeds exposed to a magnetic field germinated earlier than the control seeds; parameters T_{10} – T_{90} and the mean germination time were reduced for all the applied magnetic doses.

*Table.1: Germination parameters for maize seeds subjected to 125 mT stationary magnetic field for exposure times: 1 minute (D1), 10 minutes (D2), 20 minutes (D3), 60 minutes (D4), 24 hours (D5), chronic exposure (D6) and control (C). G_{max}: number of germinated seeds (%); MGT: Mean germination time; T_1, T_{10}, T_{25}, T_{50}, T_{75}, T_{90}: time needed to obtain 1, 10, 25, 50, 75 and 90 % of seeds to germinate, expressed in hours. Asterisks show significant differences whit control (C): **** extremely significant (p<0.001); *** very significant (0.001<p<0.01); **significant (0.01<p<0.05).*

Parameter D	G_{max} (%)	Time (hours) $\bar{x}$ ± standard error						
		T_1	T_{10}	T_{25}	T_{50}	T_{75}	T_{90}	MGT
C	96.00 ±2.31	28.56 ±2.40	35.28 ±1.92	39.12 ±1.44	43.20 ±0.96	47.52 ±0.72	51.84 ±12.48	**42.72 ±0.96**
D1	96.00 ±0.00	25.20 ±2.16	32.4 ±1.92	36.72 ±1.68	41.28 ±1.44	46.08 ±0.96	50.88 ±0.72	**40.08 ±1.44**
D2	98.00 ±1.15	33.84 ±1.68	38.40 ±1.20 **	41.04 ±0.96 **	43.92 ±0.72	46.80 ±0.48	49.44 ±0.48	**43.68 ±0.72**
D3	94.00 ±1.15	24.00 ±1.20 **	31.44 ±0.96 **	36.00 ±0.72 ***	41.04 ±0.48 ***	46.32 ±0.24 **	52.32 ±0.72	**40.32 ±0.48 *****
D4	98.00 ±1.15	34.56 ±0.48 **	38.64 ±0.48	40.08 ±0.48	43.20 ±0.48	45.84 ±0.24 **	48.24 ±0.24	**43.20 ±0.48**
D5	96.00 ±2.31	19.20 ±1.44 ***	24.96 ±0.24 ****	28.80 ±0.96 ****	33.84 ±1.68 ****	39.60 ±2.40 ****	47.52 ±3.12	**34.32 ±0.96 ******
D6	**96.00 ±2.31**	**19.92 ±1.68 *****	**26.40 ±0.48 ******	**30.72 ±0.48 ******	**35.76 ±1.20 ******	**41.32 ±1.44 ******	**48.48 ±3.36**	**35.76 ±0.24 ******

*Table.2: Germination parameters for **rice** seeds subjected to 125 mT stationary magnetic field for exposure times: 1 minute (D1), 10 minutes (D2), 20 minutes (D3), 60 minutes (D4), 24 hours (D5), chronic exposure (D6) and control (C). G_{max}: number of germinated seeds (%); MGT: Mean germination time; T_1, T_{10}, T_{25}, T_{50}, T_{75}, T_{90}: time needed to obtain 1, 10, 25, 50, 75 and 90 % of seeds to germinate, expressed in hours. Asterisks show significant differences whit control (C): **** extremely significant (p<0.001); *** very significant (0.001<p<0.01); **significant (0.01<p<0.05).*

Parameter Dose		Time (hours) $\bar{x}$ ± standard error						
	G_{max} (%)	T_1	T_{10}	T_{25}	T_{50}	T_{75}	T_{90}	MGT
C	95.00 ±1.91	37.92 ±1.44	48.96 ±0.96	55.20 ±0.72	62.16 ±0.48	69.12 ±0.24	76.56 ±0.96	**61.20 0.72**
D1	93.00 ±1.91	32.40 ±3.60 **	45.12 ±2.64 **	52.80 ±1.44 **	61.44 ±0.96	70.56 ±0.96 **	82.08 ±2.64 ***	**60.00 ±0.72**
D2	91.00 ±4.43	35.04 ±3.84	47.76 2.88	55.20 ±1.92	63.60 ±1.20	72.72 ±0.72 ****	-------	**61.92 ±0.96**
D3	92.00 ±1.00 **	37.92 ±2.40	48.48 ±1.68	54.72 ±1.20	61.68 ±0.48	68.88 ±0.48	78.72 ±1.20 **	**60.24 ±0.72**

http://dx.doi.org/10.22161/ijeab/2.1.47 *ISSN: 2456-1878*

D4	94.00 ±2.00	37.44 ±3.60	48.48 2.64	54.96 ±1.92	61.92 ±0.96	69.12 ±0.48	77.28 ±2.64	**60.96 ±1.20**
D5	95.00 ±1.91	25.68 ±1.44 ****	38.88 ±1.20 ****	47.28 ±0.96 ****	57.12 ±0.48 ****	67.68 ±0.72 **	79.20 ±3.36	**56.40 ±0.48 ******
D6	95.00 ±1.91	24.96 ±1.68 ****	38.64 ±1.68 ****	47.28 ±1.44 ****	57.12 ±0.96 ****	67.20 ±0.96 **	78.48 ±3.12	56.16 ±1.20 ****

*Table.3: Germination parameters for **barley** seeds subjected to 125 mT stationary magnetic field for exposure times: 1 minute (D1), 10 minutes (D2), 20 minutes (D3), 60 minutes (D4), 24 hours (D5), chronic exposure (D6) and control (C). G_{max}: number of germinated seeds (%); MGT: Mean germination time; T_1, T_{10}, T_{25}, T_{50}, T_{75}, T_{90}: time needed to obtain 1, 10, 25, 50, 75 and 90 % of seeds to germinate, expressed in hours. Asterisks show significant differences whit control (C): **** extremely significant (p<0.001); *** very significant (0.001<p<0.01); **significant (0.01<p<0.05).*

Parameter		**Time (hours) $\bar{X}$ ± standard error**						
Dose	**G_{max} (%)**	**T_1**	**T_{10}**	**T_{25}**	**T_{50}**	**T_{75}**	**T_{90}**	**MGT**
C	98 ±1.15	19.44 ±0.96	24.24 ±0.96	27.6 ±0.72	31.92 ±0.72	37.20 ±0.48	44.40 ±0.96	**33.12 ±0.48**
D1	96 ±1.63	19.68 ±0.48	24.24 ±0.48	27.12 ±0.48	31.20 ±0.48	36.24 ±0.24 **	44.16 ±0.72	**31.92 ±0.48 ****
D2	100 ±0.00	19.44 ±0.72	23.52 ±0.24	26.16 ±0.48 **	29.76 ±0.96 **	34.32 ±1.68 **	40.56 ±2.64 **	**31.44 ±1.20 ****
D3	95 ±1.91	19.44 ±0.72	22.80 ±0.48 **	24.96 ±0.72 ***	28.60 ±0.96 ****	32.88 ±1.20 ****	42.00 ±2.16	**29.52 ±0.96 ******
D4	93 ±1.91	18.72 ±0.96	22.32 ±0.72 **	24.96 ±0.72 ***	28.56 ±0.72 ****	34.08 ±1.20 ***	------	**29.76 ±0.48 ******
D5	97 ±1.91	20.16 ±0.72	23.52 ±0.24	25.68 ±0.24 ***	28.80 ±0.48 ****	33.12 ±1.20 ****	39.84 ±3.60 **	**30.00 ±0.24 ******
D6	**97 ±1.00**	**21.12 ±0.72 ****	**24.00 ±0.24**	**25.92 ±0.24 ***	**28.56 ±0.48 ******	**31.92 ±0.24 ******	**37.68 ±1.92 ******	**29.52 ±0.48 ******

*Table.4: Germination parameters for wheat seeds subjected to 125 mT stationary magnetic field for exposure times: 1 minute (D1), 10 minutes (D2), 20 minutes (D3), 60 minutes (D4), 24 hours (D5), chronic exposure (D6) and control (C). G_{max}: number of germinated seeds (%); MGT: Mean germination time; T_1, T_{10}, T_{25}, T_{50}, T_{75}, T_{90}: time needed to obtain 1, 10, 25, 50, 75 and 90 % of seeds to germinate, expressed in hours. Asterisks show significant differences whit control (C): **** extremely significant (p<0.001); *** very significant (0.001<p<0.01); **significant (0.01<p<0.05).*

Parameter		**Time (hours) $\bar{X}$ ± standard error**						
Dose	**G_{max} (%)**	**T_1**	**T_{10}**	**T_{25}**	**T_{50}**	**T_{75}**	**T_{90}**	**MGT**
C	93.00 ±1.91	16.32 ±0.96	20.40 ±0.72	23.28 ±0.48	27.12 ±0.48	33.60 ±0.96	51.12 ±11.28	**28.8 ±0.72**

D1	96.00 ±1.63	16.08 ±0.48	20.16 ±0.48	22.80 ±0.48	25.68 ±0.24 ***	28.56 ±0.24 ****	31.68 ±0.24	**25.44 ±0.48 ******
D2	94.00 ±1.15	15.36 ±0.48	19.92 ±0.48	22.80 ±0.48	26.16 ±0.48 **	29.52 ±0.24 ****	33.84 ±3.60	**25.68 ±0.24 ******
D3	95.00 ±2.52	17.04 ±0.72	20.16 ±0.48	22.56 ±0.24	25.68 ±0.24 ****	30.00 ±1.20 ***	39.12 ±3.36 ***	**26.88 ±0.48 *****
D4	91.00 ±3.42	16.08 ±0.48	19.68 ±0.48	22.32 ±0.24 **	25.92 ±0.48 ***	32.16 ±1.68	-----	**27.12 ±0.96 ** **
D5	97.00 ±1.91 **	15.12 ±0.24 **	18.48 ±0.24 ***	21.12 ±0.24 ****	24.48 ±0.24 ****	29.04 ±0.48 ****	36.48 ±1.68 ****	**25.44 ±0.72 ******
D6	**98.00 ±1.15 *****	**16.08 ±0.48**	**19.20 ±0.24 ** **	**21.36 ±0.24 ******	**24.48 ±0.24 ******	**29.28 ±0.96 ******	**38.64 ±2.40 ******	**25.92 ±0.48 ******

Mean germination time (MGT) of control seeds was significantly reduced for most doses. Time required to germinate 1%, parameter T_1, of seeds exposed to a magnetic field was less than control. As time required for germinate 1% of seeds, T_1, is closely related to the onset of germination, these results indicate that cereals seeds exposed to a magnetic field sprouted earlier. The time required for germination recorded for each treatment was, in general, less than the corresponding control values; thus the rate of germination of treated seeds was higher than that of the untreated seeds (C). Results of maize germination show extremely significant differences for doses D5 and D6 in mean germination time and T_1-T_{75} parameters; significant differences were also founded for doses D2, D3 and D4. Germination curves obtained for all doses are displaced towards left side rate. The onset of rice germination shows significant differences for D1 and extremely for TMG and T_1-T_{50} parameters; the same behavior of germination curves were observed. Table 3 shows the significant differences observed for barley in D2-D6 doses, thus, the germination rate was improved.

Wheat results are similar of other cereal seeds, a higher germination rate was obtained for magnetic doses applied; best results were obtained for D6, D5. The results obtained in this study are in concordance with preliminary researches carried out for authors in grass, legumes, medicinal and others seeds.

IV. DISCUSSION

Although the mechanisms at work in plants and other living systems exposed to a magnetic field are still not well known, several theories have been proposed, including biochemical changes or altered enzyme activities (Phirke, *et al.* 1996). An experimental study on water absorption by lettuce seeds previously exposed to a stationary magnetic field of 1–10mT was carried out; an increase in water uptake due to the applied magnetic field, which could explain of the increase in the germination speed of treated lettuce seeds was reported (Gracía *et al.* 2001). Exposure to magnetic fields improved parameters like water uptake, leaf photosynthetic efficiency and leaf protein content was found in soybean Shiene *et al.* 2011). Our results are in agreement with the germination data of maize seeds, an increase in germination and shoot development in seeds exposed to 150 mT magnetic field for 10, 15, 20 and 30 minutes was found (Aladjadjiyan, 2002). Magnetic treatment of 30 mT and 85 mT on two broad bean cultivars affected positively the germination and emergence (Podlesni *et al.* 2004). The effect of a magnetic field on *Asparagus officinalis* and *Ocimum basilicum* seed germination and seedling growth to be positive (Soltani *et al.* 2006 a, b). The leaf, stem and root relative growth rates of tomato plants grown from magnetically treated seeds were greater than those of control plants (Souza *et al.* 2006). Magnetic field application enhanced chickpea seed germination speed, seedling length and seedling dry weight. Seeds were exposed in batches to static magnetic fields ranging in intervals of 50 mT of from 0 to 250 mT for 1-4 h in steps of 1 hour for all fields. Best results were obtained from exposures of 50 mT for 2 h, 100 mT for 1 h and 150 mT for 2 h (Vashisth *et al.* 2008). Accelerated germination after magnetic stimulation of wheat seeds was observed working with 30, 45 and 60 mT magnetic field strengths (Pietruszewski *et al.* 2010).

In previous studies authors found an increase in the rate of germination of seeds and a stimulation of growth of seedlings. They found a positive growth response to a 125 mT and 250 mT magnetic field in rice, wheat and barley seeds (Flórez *et al.* 2004; Martínez *et al.* 2000, 2007). The greatest stimulation of growth was observed in seeds chronically exposed to a magnetic field or in seeds treated for 24 hours. An increase of the initial growth stages and an early sprouting of maize seeds exposed to a stationary magnetic field was also observed (Flórez *et al.* 2007), treated plants grew higher and heavier than control. Effect of exposure of grass seeds to 125 mT and 250 mT was studied (Carbonell *et al.* 2008), finding that mean germination time was reduced by 10% compared with control seeds; the time required for germination onset was also reduced and the roots of grass seedlings from chronically exposed seeds grew higher and longer than those of untreated ones. Recently they have also obtained an early germination in *Salvia officinalis* L. and *Calendula officinalis* L, (Flórez, 2012).

Different theories have been proposed on the biological changes: external magnetic field effects at biochemical level and activating proteins and enzymes can increase the growth potential of seeds; magnetic field can interact with internal electric field of biological systems though its resonating behavior and cold be effective on the diffusion of biological particles in solutions (Liboff *et al.* 1988). The orientation of ferromagnetic particles and modulation of radical-pair reactions are reported as the mechanism of magnetic field effect (Faten *et al.* 2009). Living cells possess electric charges exerted by ions or free radicals, which act as endogenous magnets. Treatments with magnetic field are assumed to enhance seed vigor by influencing the biochemical processes that involve free radicals, and by stimulating activity of proteins and enzymes. Numerous studies suggested that magnetic field increases ions uptake and consequently improves nutrition value which could be a good alternative for chemical treatments (Stange *et al.* 2002). The influence of pre-sowing magnetic field treatments on tomato seed germination, seedling growth, emergence and plant growth parameters were studied. Seeds were exposed to 60 Hz induced by an electromagnet at 80, 120, 160 and 200 mT(rms) for 1- 20 min. An improved seed performance in terms of reduction of time required for the first seeds to complete germination, time to reach 50% germination, time between 10 and 90% germination with increasing germination rate, and increased germination percentage at 4 and 7 days, seedling shoot and root length compared to the untreated were found Souza *et al.* 2010). Results of the study of the effects of magnetic treatment of irrigation water and snow pea (*Pisum sativum* L) and Kabuli chickpea (*Cicer arietinum* L) suggest that both magnetic treatment of seeds and magnetic treatment of irrigation water and seeds have the potential to improve the early seedling growth and nutrient contents of seedlings (Harsham *et al.* 2011). Revealing the relationships between MF and plant responses is becoming more and more important as new evidence reveals the ability of plants to perceive and respond quickly to varying MF by altering their gene expression and phenotype. The recent implications of MF reversal with plant evolution opens new horizons not only in plant science but also to the whole biosphere, from the simplest organisms to human beings (Maffei, 2014).

V. CONCLUSIONS

Results obtained in this study allow us to conclude that magnetic treatment improves germination rate of cereal seeds. In general, most of the parameters recorded for all the doses applied to maize, rice, barley and wheat seeds were better than control values. Thus, the rate of germination of treated seeds was higher than the untreated seed (C) rate. The greatest reductions in the mean germination time, the most relevant parameter, were for chronically exposure and 24h to 125 mT. The early germination process are plotted in germination curves showing the left side displacement. Consequently, 125 mT magnetic field have a positive effect on cereal germination process.

ACKNOWLEDGEMENTS

The Technical University of Madrid (UPM) has supported this research, carried out by members of the research group "Investigation in Bioelectromagnetism Applied to Agroforestry Engineer", through the Project N.CCG07-UPM/0003348.

REFERENCES

[1] Aladjadjiyan, A. (2002). Study of the influence of magnetic field on some biological characteristics of *Zea mais*. Journal of Central European Agriculture, 2 (3), 89–94.

[2] Cakmak, T. Dumlupinar, R. Erdal, S. (2010). Acceleration of germination and early growth of wheat and bean seedlings grown under various magnetic field and osmotic conditions. Bioelectromagnetics, 2 (31), 120–129.

[3] Carbonell, V. Flórez, M. Martínez, E. Maqueda, R. Amaya, J.M. (2011). Study of stationary magnetic fields on initial growth of pea (*Pisum sativum* L.) Seeds. Seed Science and Technology 39, 673–679.

[4] Carbonell, M.V. Martínez, E. Flórez, M. Maqueda, R. López-pintor, A. Amaya, J.M. (2008). Magnetic field treatments improve germination and seedling growth

in *Festuca arundinacea* Schreb. and *Lolium perenne* L. Seed Science and Technology, 36, 31–37.

[5] Faten, D. Jameel, M. Essam, H. (2009). Static Magnetic Field Influence on Elements Composition in Date Palm *Phoenix dactylifera* L. J. Agr. Biol. Sci 5, 161.

[6] Flórez, M (2004). Efecto de campos magnéticos estacionarios de 125 mT y 250 mT en la germinación de semillas y desarrollo de plántulas. Tesis doctoral. Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Politécnica de Madrid.

[7] Flórez, M. Carbonell, M.V. Martínez, E. (2007). Exposure of maize seeds to stationary magnetic fields: effects on germination and early growth. Environmental and Experimental Botany, 1 (59) 68–75.

[8] Flórez, M. Martínez, E. Carbonell, M.V. (2012). Effect of magnetic field treatment on germination of medicinal plants *Salvia officinalis* L. and *Calendula officinalis* L. Polish Journal of Environmental Studies,1 (21) 57–63.

[9] García, F. Arza, L.I. (2001). Influence of a stationary magnetic field on water relations in lettuce seeds. Part I: Theoretical considerations. Bioelectromagnetics, 8(22) 589–595.

[10] Harsharn S. G. and Basant L. M. (2011). Magnetic treatment of irrigation water and snow pea and chickpea seeds enhances early growth and nutrient contents of seedlings. Bioelectromagnetics 32:58–65, 2011.

[11] ISTA. (2004). International Seed Testing Association. International Rules for Seed Testing. Seed Science and Technology, Zurich.

[12] Liboff, A.R. McLeod, B.R. (1998). Kinetics of channelized membrane ions in magnetic field Bioelectromagnetic*s* 9, 39-51.

[13] Maffei, M.E. (2014). Magnetic field effects on plant growth, development, and evolution. Frontier Plant Science. http://dx.doi.org/10.3389/fpls.2014.00445

[14] Martínez, E. Carbonell, M.V. Amaya, J.M. (2000). Static magnetic field of 125 mT stimulates the initial growth stages of barley (*Hordeum vulgare* L.). Electromagnetobiology, 3 (19) 271–277.

[15] Martínez, E. Carbonell, M.V. Flórez, M. (2002). Magnetic biostimulation of initial growth stages of wheat (*Triticum aestivum*, L.). Electromagnetic Biology and Medicine. 1 (21) 43–53.

[16] Phirke, P.S. Kubde, A.B.. Umbakar, S.P. (1996). The influence of magnetic field on plant growth. Seed Science and Technology, 24, 375–392.

[17] Pietruszewski, S. Kania, K. (2010). Effect of magnetic field on germination and yield of wheat. International Agrophysics, 24, 297–302.

[18] Podlesni, J. Pietruszewski, S. Podlesna, A. (2004). Efficiency of the magnetic treatment of broad bean seeds cultivated under experimental plot conditions. International Agrophysics, 1(18) 65–71.

[19] Shine, M.B. Guruprasad, K.N. Anand, A. (2011). Enhancement of germination, growth and photosynthesis in soybean by pre-treatment of seeds with magnetic field. Bioelectromagnetics, 6 (32) 474–484.

[20] Soltani, F. Kashi, A. Arghavani, M. (2006a). Effect of magnetic field on *Asparagus officinalis* L. seed germination and seedling growth. Seed Science and Technology, 5 (34) 349–353.

[21] Soltani, F. Kashi, A. Arghavani, M. (2006b). Effect of magnetic field on *Ocimum basilicum* seed germination and seedling growth. Acta Horticulturae, 723, 279–282.

[22] Souza, A. García, D. Sueiro, L. Gilart, F Porras, E. Licea, L. (2006). Pre-sowing magnetic treatments of tomato seeds increase the growth and yield of plants. Bioelectromagnetics, 4, 27, 247–257.

[23] Souza, A.; Sueiro, L.; García, D.; Porras, E. (2010). Extremely low frequency non-uniform magnetic fields improve tomato seed germination and early seedling growth. Seed Science and Technology, 38, 1. 61-72.

[24] Stange, B.C. Rowlans, R.E. Rapley, B.I. J.V. Podd, B.I. (2002). ELF magnetic fields increase aminoacid uptake into *Vicia faba* L. roots and alter ion movement across the plasma membrane. Bioelectromagnetics 23, 347-354.

[25] Vashisth, A. Nagarajan, N. (2008). Exposure of seeds to static magnetic field enhances germination and early growth characteristics in chickpea. Bioelectromagnetics, 7 (29) 571–578.

[26] Yano, A. Hidaka, E. Fujiwara, K. Iimoto, M. (2001).Induction of primary root curvature in radish seedlings in a static magnetic field. Bio electromagnetics,

http://dx.doi.org/10.22161/ijeab/2.1.48 ISSN: 2456-1878

Protease activity of extracellular enzyme produced by *B. subtilis* isolated from soil

Md. Gahangir Alam[1], Md. Ekhlas Uddin[2]*, Sezanur Rahman[3], Tasnim Ahmad[5], Mohammad Reazul Karim[4], Dr Mohammad Shakil Mahmood[2], Mohammad Shah Alam[1], Md. Nazmuzzaman[5], Md Jakir Hossain[6], Pulak Maitra[5], Md. Sharifull Islam[1]

* **(Corresponding Author)** Dept. of Biochemistry & Molecular Biology, Gono Bishwabidalay, Savar, Dhaka, Bangladesh

Abstract*— *Background: *Proteases produced by enzymatic method are more environments friendly than chemical process, and they have tremendous potential in the leather industry and in other several industries. In this study extracellular protease producing nonpathogenic Bacillus subtilis was isolated from soil sample and relationship between sporulation and extracellular protease synthesis in large scale cultivation was studied.The enzyme was further characterized, purified, and tested for potential application.*

Result: *The molecular weight of the protease was found to be ~30 KDa. Enzyme activity was checked on the presence of different metal ions and effectors. The enzyme was slightly modulated by MG^{++} ion, and significantly by Hg^{++} ion, while Zn^{++} ion slightly decrease the proteolytic activity. Sulfahydryl reagents, DTT slightly and β-ME significantly inhibit the enzyme. EDTA showed no effect on the enzyme suggesting that the enzyme might not be metallo protease. PMSF, a known serine protease inhibitor was seen to totally inhibit the enzyme which indicates that the enzyme is a serine protease. The optimum enzyme activity was observed after 22 hours of incubation of B. subtilis at 37° C.*

Conclusions: *Crude enzyme contains 285 units of enzyme which have direct dehairing activity. The enzyme was also seen to be able to remove blood and curry stain from clothes; making it a very promising candidate to be used in a leather and detergent industry. Apart from protease the bacterium was also seen to have lipase and collagenase activity. So, the bacteria are potentially good candidate for industrial application.*

Keywords — Bacillus subtilis, Microbial Proteases, Extracellular enzyme,* Enzymatic dehairing, D*etergent activity.

I. INTRODUCTION

The global market for industrial enzymes in 2012 and 2013 was nearly $4.5 and $4.8 billion[1]. A compound annual growth rate (CAGR) of 8.2% from 2013 to 2018 expect that it'll reach around $7.1 billion by 2018[2]. Proteases have a large variety of applications mainly in the detergent at food industries and account for nearly 60% of the industrial market in the world[3]. They find application in a number of biotechnological processes, food processing, Pharmaceuticals, leather industry, silk, bakery, soy processing, meat tendering and brewery industries[4-7]. However, its application in the production of peptide synthesis in organic media is limited by the presence of organic solvents, but microbial proteases are more environments friendly when compared with the chemical process[5, 8]. Microbial proteases are extracellular in nature and directly secreted into the fermentation broth, thus simplifying downstream processing of the enzyme as compared to proteases obtained from plants and animals [9, 19]. Though, optimization of protease could involve several variables such as temperature, pH and incubation period. In this regard, the *Bacillus* species specially (*B. subtilis)* were exploited for their ability to produce extracellular enzymesin submerged fermentation [10-12].

The aim of the study were to culture dehairing protease producing *Bacillus* sp. in large scale for production and purification of extracellular protease from it, than evaluating practical application of the enzyme in leather and detergent industry.

II. MATERIAL AND METHODS

Sample preparation: Bacterial sample isolate by pure culture followed by stick culture from tannery waste rich soil.Gram staining & spore staining showed that the organism is gram positive and forms spore during adverse condition in the growth medium. After various tests it was suggested and the features agreed with the description of *B. subtilis* in Bergey's Manual of Systematic Bacteriology [13]. It was also identified as *B. subtilis* with 99.9% identity by API 50 CHB[14, 22] and was also characterized and identified by using a bioinformatics tool PIB (Probabilistic

Identification of Bacteria)[15] that suggests the organism was *B. subtilis* (ID=0.9760). *B. subtilis* was cultured in nutrient broth and incubated in Psychrotherm Incubator-Shaker at 37°C for 22-24 hours until the absorbance of growing culture reached 1.5-1.8. Spore staining was carried out to check the shape and the position of bacterial spores and culture was centrifuged at 5000 rpm for 25 minutes to use supernatant as crude enzyme.

Protease activity analysis: The growth was measured at every two hours interval at 37˚C by 660 nm light absorbance and proteolytic activity by Kreger and Lockwood [16]method to Determination of co-relation between bacterial growth and extracellular protease synthesis.

Effect of different metal ions (ZnSO4, MgSO4, CuSO4, NaCl, KCl, HgCl2) and inhibitors (EDTA, PMSF, sodium thiosulfate , β-mercaptoethanol, dithiothreitol) on Protease Activity was determined at 1mM to 10mM concentration range by co-incubation of enzyme solution and each ion and inhibitor for 30 minutes at room temperature and then the residual protease activity was determined.

Azocasien assay [17]was carried out to determine extracellular and intracellular protease activity; for extracellular protease crude enzyme was taken but for intracellular, crude enzyme was washed in saline water and disrupted by ultrasonic treatment than centrifuged at 6,000g for 15 minutes before the assay.

To evaluate the detergent activity of the enzyme, crude enzyme was used and compared it with several commercial detergents at room temperature.

Enzymatic dehairing: 5 mL Crude enzyme was added on detergent washed cow hide (2×2 inches) to observe enzymatic dehairing capability of the organism. Sodium azide was used at 1% so other organism cannot grow on subject and incubated for overnight.

Enzyme Purification: For enzyme purification crude enzyme was re-dissolved in buffer and ammonium sulfate was removed by dialysis. After that centricon (ultra-filtration device) is used for collecting enzyme larger than 30 KDa. These enzymes were further purified by DEAE cellulose column ion-exchange chromatography, where 1.0 M Tris-HCl used as buffer and 0.1-3.0 M NaCl solution as gradient. Enzyme purification was confirmed by SDS-PAGE.

III. RESULTS

Microscopic observation (figure 1) along with growth profile and enzyme activity shows that spore formation start at 10 hour incubation time; it also shows that synthesis of enzyme increases with the increase of sporulation (figure 2).At 37ºC temperature we also find higher protease activity for extracellular enzyme (0.28 absorbance at 440 nm) than intracellular enzyme (0.03 absorbance at 440 nm).

Effect of metal ions and other effectors on the protease activity

Caseinolytic result (Figure 3) show that phenyl methyl sulfonyl fluoride (PMSF) can completely inhibit protease activity which suggest that extracellular might be a serine protease and β- mercaptoethanol considerably inhibit the enzyme activity which suggesting that a HS-group may be present at or near the active site. Thiosulfate, potassium chloride, DTT and Zn^{++} ion also inhibit protease activity but not very significantly.

The result also shows that Mg^{++} ion slightly where Hg^{++} ion significantly increased the proteolytic activity. EDTA and other ion have no effect on the protease activity which suggested that the enzyme might not be metallo protease.

Evaluation of detergent activity of the protease enzyme

To evaluate the ability of the enzyme to remove stain (blood, curry) from clothes crude enzyme supernatant was used along with/without commercial detergent powder (Figure 4).

Direct dehairing and Collagenase activity

The crude enzyme was shown to remove the hair completely. As it was shown in Table.1 that the crude enzyme solution contain 285.0 units of enzyme per mL, 356.25 units of enzyme was required to completely remove hair from per square inch of cow hide.So, this enzyme can be used in leather processing and provides an alternative to decrease the environmental contamination load and for a better future.Collagenase activity Test show cow ligment can completely digests in bacterial culture while cell free supernatant could only digest part of it.The fraction collected from ion exchange chromatography showing protease activity (table in supplement file) was subject to SDS-PAGE. After SDS-PAGE a single band was found. Hence we can assume the enzyme has been purified.

IV. DISCUSSION AND CONCLUSION

During sporulation the organism produces both extracellular and intracellular proteases. From our study isolated extracellular protease enzyme functions at alkaline pH,high salt concentration and temperatures higher than 37^0C.It can effectively remove the blood strain from fabrics suggesting that it may used to fortify of the detergents. Intracellular protease might be associated with some generalized cellular development or normal cellular activity. It might not be involved in this sporulaion or extracellular activity. This

http://dx.doi.org/10.22161/ijeab/2.1.48 *ISSN: 2456-1878*

prediction comes from the result that during 30 hours of monitoring of intracellular enzyme activity no increase in the enzyme activity could be established. Intracellular enzyme activity is not an artifact of enzyme extraction from the cell. Since intracellular enzymes activity is not inhibited by PMSF, or B-Mercapto ethanol, it may be safely concluded that this enzyme is different from extracellular protease reported in this work.

Green chemistry, also called sustainable chemistry, is a chemical philosophy encouraging the design of the products and processes that reduce or eliminate the use and generation of hazardous substance[18]. Leather industry contributes to one of the major industrial pollution problems facing the country[19-21].The application of enzymes its fits many of the principles of green chemistry. Because the optimum enzyme activity was observed after 22 hours of incubation of our isolated *B. subtilis* at 37º C where crude enzyme contain 285.0 units of enzyme which have direct dehairing activity.

Competing interests

The authors declare that they have no competing interests.

Author details

[1] Dept. of Microbiology, Gono Bishwabidalay, Dhaka, Bangladesh

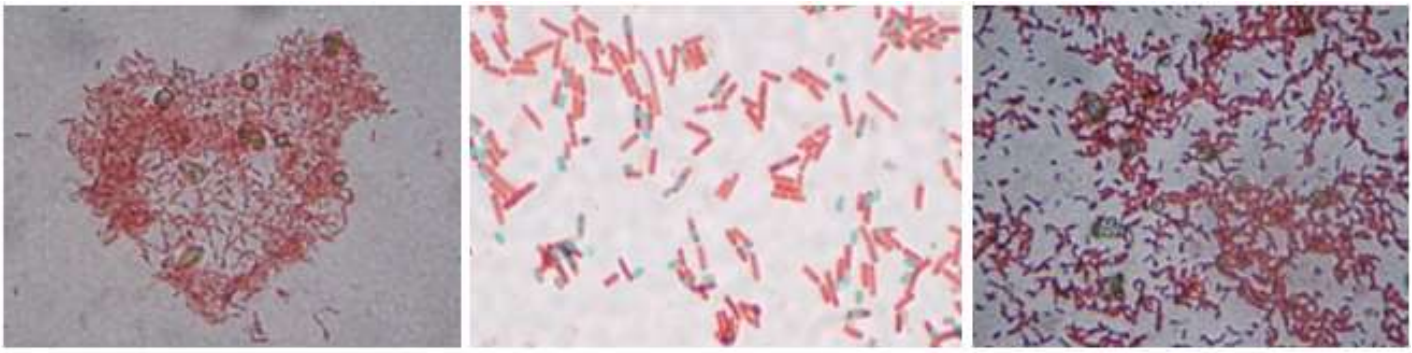
[2] Dept. of Biochemistry & Molecular Biology, Gono Bishwabidalay, Dhaka, Bangladesh

[3] Dept. of Biology & Chemistry, North South University, Bangladesh.

[4]Dept. of Microbiology, Chittagong University, Bangladesh

[5]Dept. of Biotechnology & Genetic Engineering, Islamic University, Kushtia, Bangladesh

[6]Dept. of Biochemistry & Microbiology, North South University, Bangladesh



(a) At 10x *(b) At 100x* *(c) At 50x*

Figure 1: Microscopic observation of sporulation at different time intervel (a) at 10th hour (b) at 18th hour (c) at 22nd hour.

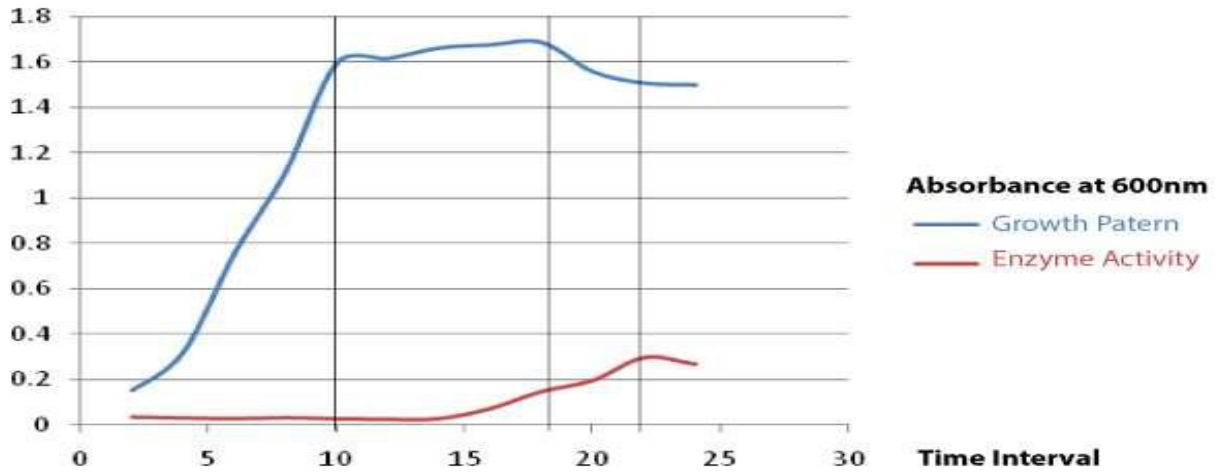


Figure 2: Sporulation start at 10th optimum growth at 18th and highest enzyme activity show at 22nd hour of incubation.

http://dx.doi.org/10.22161/ijeab/2.1.48 ISSN: 2456-1878

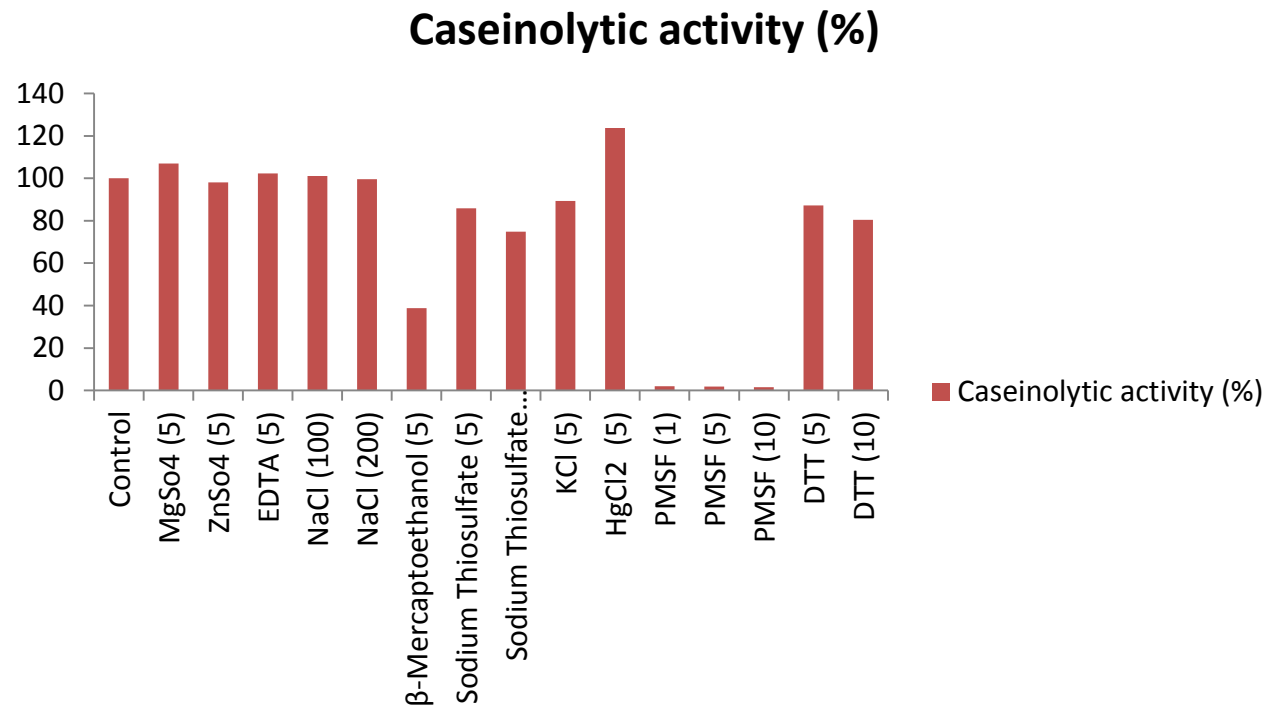


Figure 3: Graphical presentation of the effect of different metal ions and effectors on protease activity.

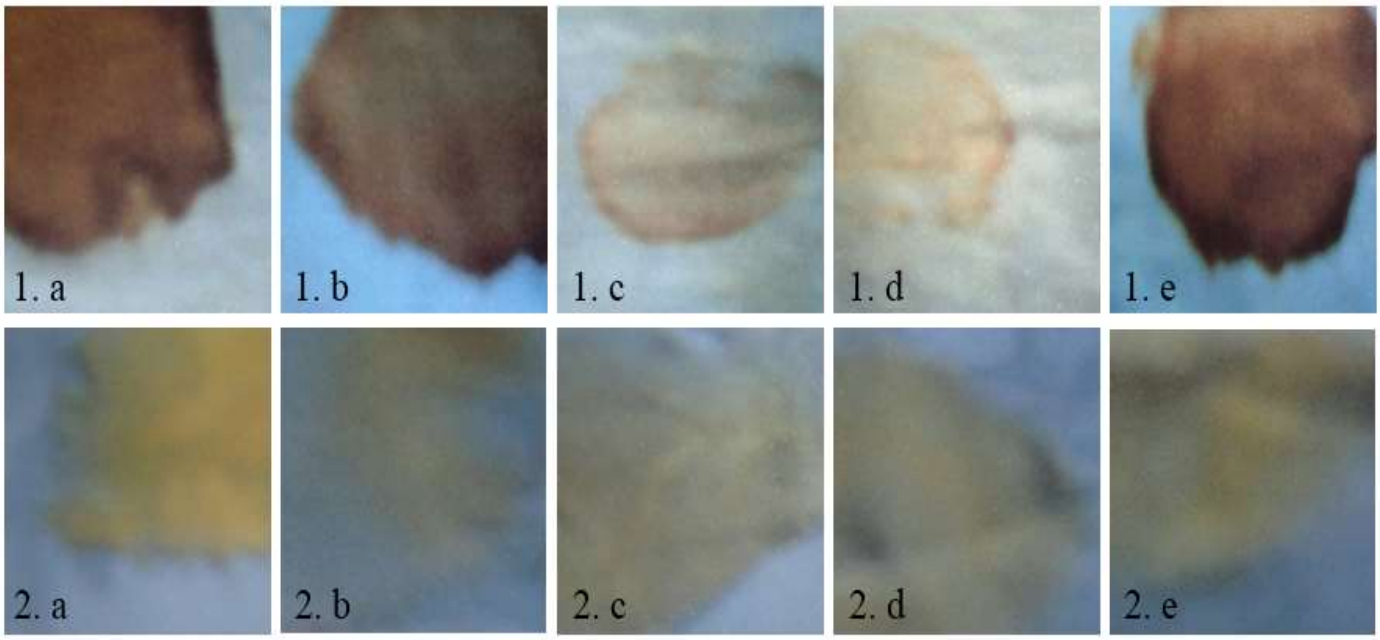


Figure 4: Evaluation of different commercial detergent and the enzyme to remove blood stain from clothe. (1.a) Control, (1.b) Wheel powder slightly removed the blood stain, (1.c) Wheel powder + enzyme nearly remove all blood stain, (1.d) Enzyme alone is sufficient to remove blood stain, (1.e) Surf Excel could not remove blood stain. And for curry (2.a) Control, (2.b) Wheel powder, (2.c) Wheel powder + enzyme (2.d) Enzyme alone, (2.e) Surf Excel; all of them were shown to be very negligibly removing curry stain.

http://dx.doi.org/10.22161/ijeab/2.1.48 *ISSN: 2456-1878*

Table.1: Different enzymatic properties of protease at several stage.

Stage	***Protein concentration (mg/mL)***	***Total protein (mg)***	***Activity***	***Specific activity (U/mg protein/mL)***	***Enzyme Unites/mL***	***Purification fold***
Before purification	*0.75*	*825.0*	*1.25*	*380*	*285*	
75% saturation with ammonium sulfate	*1.35*	*270.8*	*2.872*	*77.4*	*104.5*	*3.04*
After dialysis	*0.49*	*98*	*2.872*	*213.33*	*104.5*	*2.8*
After ultra-filtration by centricon	*0.415*	*2.075*	*1.980*	*130.1*	*54.0*	*3.25*

Supplementary Table. S.1: Growth profile and protease activity of the organism at 37^0C.

Time (hours)	***Absorbance at 600 nm (For Growth)***	***Absorbance at 600 nm (For Protease Activity)***
2	*0.15*	*0.035*
4	*0.319*	*0.031*
6	*0.757*	*0.028*
8	*1.119*	*0.032*
10	*1.599*	*0.027*
12	*1.619*	*0.025*
14	*1.665*	*0.028*
16	*1.679*	*0.073*
18	*1.689*	*0.149*
20	*1.559*	*0.198*
22	*1.509*	*0.299*
24	*1.501*	*0.269*

http://dx.doi.org/10.22161/ijeab/2.1.48 ISSN: 2456-1878

Supplementary Table. S.2: Effect of metal ions and other effectors on the protease activity of the protease.

Compound (concentration in mM)	*Caseinolytic activity (%)*
Control	*100*
$MgSo_4$ (5)	*107*
$ZnSo_4$ (5)	*98*
EDTA (5)	*102.3*
NaCl (100)	*101*
NaCl (200)	*99.5*
β-Mercaptoethanol (5)	*38.8*
Sodium Thiosulfate (5)	*85.9*
Sodium Thiosulfate (10)	*74.82*
KCl (5)	*89.3*
$HgCl_2$ (5)	*123.7*
PMSF (1)	*2.04*
PMSF (5)	*1.9*
PMSF (10)	*1.5*
DTT (5)	*87.2*
DTT (10)	*80.47*

Supplementary Table.S.3: Ion exchange chromatography Result (Absorbance at 280 nm).

Fraction no.	*Eluent Condition*						
	Tris Buffer	*0.1 M NaCl*	*0.2 M NaCl*	*0.5 M NaCl*	*1.0 M NaCl*	*2.0 M NaCl*	*3.0 M NaCl*
1.	*0.005*	*0.038*	*0.103*	*0.025*	*0.049*	*0.005*	*0.001*
2.	*0.004*	*0.029*	*0.127*	*0.030*	*0.044*	*0.005*	*0.001*
3.	*0.004*	*0.027*	*0.111*	*0.041*	*0.070*	*0.005*	*0.001*
4.	*0.209*	*0.03.4*	*0.132*	*0.040*	*0.052*	*0.005*	*0.001*
5.	*0.008*	*0.094*	*0.198*	*0.021*	*0.052*	*0.005*	*0.001*
6.	*0.007*	*0.179*	*0.103*	*0.047*	*0.039*	*0.006*	*0.001*
7.	*0.010*	*0.206*	*0.007*	*0.016*	*0.038*	*0.006*	*0.001*
8.	*0.016*	*0.209*	*0.005*	*0.024*	*0.039*	*0.005*	*0.001*
9.	*0.023*	*0.217*	*0.015*	*0.018*	*0.037*	*0.005*	*0.001*
10.	*0.063*	*0.236*	*0.017*	*0.025*	*0.025*	*0.003*	*0.001*

REFERENCES

[1] Singhal P, Nigam V, Vidyarthi A: **Studies on production, characterization and applications of microbial alkaline proteases**. *International Journal of Advanced Biotechnology and Research* 2012, **3** (3):653-669.

[2] Dewan S: **Global markets for enzymes in industrial applications**. *BCC Research, BIO030G* 2012.

[3] Anwar A, Saleemuddin M: **Alkaline proteases: a review**. *Bioresource Technology* 1998, **64**(3):175-183.

[4] Kumar CG, Takagi H: **Microbial alkaline proteases: from a bioindustrial viewpoint**. *Biotechnology advances* 1999, **17**(7):561-594.

[5] Gupta R, Beg Q, Lorenz P: **Bacterial alkaline proteases: molecular approaches and industrial applications**. *Applied microbiology and biotechnology* 2002, **59**(1):15-32.

[6] Kamath P, Subrahmanyam V, Rao JV, Raj PV: **Optimization of cultural conditions for protease production by a fungal species**. *Indian journal of pharmaceutical sciences* 2010, **72**(2):161.

[7] Fujimaki M, Kato H, Arai S, Yamashita M: **Application of microbial proteases to soybean and other materials to improve acceptability, especially through the formation of plastein**. *Journal of Applied Bacteriology* 1971, **34**(1):119-131.

[8] Ogawa J, Shimizu S: **Microbial enzymes: new industrial applications from traditional screening methods**. *Trends in Biotechnology* 1999, **17**(1):13-20.

[9] FURHAN J, SHARMA S: **INTERNATIONAL JOURNAL OF INVENTIONS IN PHARMACEUTICAL SCIENCES**.

[10] Reddy MN, Kumar C, Swathi K, Nagamani B, Venkateshwar S, Rao L: **Extracellular alkaline protease production from isolated Bacillus subtilis svr-07 by using submerged fermentation**. *International journal of pharma Research and development* 2011, **3**:216-223.

[11] Nigam PS: **Microbial enzymes with special characteristics for biotechnological applications**. *Biomolecules* 2013, **3**(3):597-611.

[12] Rifaat HM, El-Said OH, Hassanein SM, Selim MS: **Protease activity of some mesophilic streptomycetes isolated from Egyptian habitats**. *Journal of Culture Collections* 2007, **5**(1):16-24.

[13] Sneath PH, Mair NS, Sharpe ME, Holt JG: **Bergey's manual of systematic bacteriology. Volume 2**. *Bergey's manual of systematic bacteriology Volume 2* 1986.

[14] Seldin L, Penido EG: **Identification of Bacillus azotofixans using API tests**. *Antonie van Leeuwenhoek* 1986, **52**(5):403-409.

[15] Lapage S, BASCOMB S, Willcox W, Curtis M: **Identification of bacteria by computer: general aspects and perspectives**. *Microbiology* 1973, **77**(2):273-290.

[16] Kreger A, Lockwood D: **Detection of extracellular toxin (s) produced by Vibrio vulnificus**. *Infection and immunity* 1981, **33**(2):583-590.

[17] Iversen SL, Jørgensen MH: **Azocasein assay for alkaline protease in complex fermentation broth**. *Biotechnology techniques* 1995, **9**(8):573-576.

[18] Anastas P, Eghbali N: **Green chemistry: principles and practice**. *Chemical Society Reviews* 2010, **39**(1):301-312.

[19] Md. Ekhlas Uddin, Dr. M. Rahman*, H. M. Faruquee, Md. R. I. Khan, M. Feroz Mortuza, M. H. Rahman & Pulak Maitra: **Isolation, Identification and Partial Characterization of Protease Producing Bacteria that Exhibiting Remarkable Dehairing Capabilities**. *Global Journals Inc. (USA)* 2015, **15**(1): 09-18.

[20] Paul H, Antunes APM, Covington AD, Evans P, Phillips PS: **Bangladeshi leather industry: an overview of recent sustainable developments**. *J Soc Leather Technol Chem* 2013, **97**(1):25-32.

[21] Sharif MI, Mainuddin K: **Country case study on environmental requirements for leather and footwear export from Bangladesh**. *Dhaka: Bangladesh Centre for Advanced Studies* 2003.

[22] Strasser J, Kulke E: **Temporary resource availability and quality constraints in the global leather value chain–The impact of the festival of sacrifice on the leather industry in Bangladesh**. *Applied Geography* 2013, **45**:410-419.

[23] Uddin M. E., Alam M. G., Ahmad T., Mahmud S., Nazmuzzaman M., Alam M. F., Karim M. R., Barman N. C., Arif-uz-Zaman M., Ahammed T. and Faruquee H. M.*: **Screening, Optimization and Exploration of Microbial Enzymes with Special Characteristics for Biotechnological Applications**. *Research J. of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences* 2016, **2**(3): 178-191.

http://dx.doi.org/10.22161/ijeab/2.1.49 *ISSN: 2456-1878*

Formulation, Acceptability and Storage Stability of Appetized Ginger Plum Leather

Manisha Kaushal, Aarti Dhiman, Anil Gupta, Devina Vaidya

Department of Food Science & Technology, Dr Y S Parmar University of Horticulture & Forestry Nauni, Solan, HP, India

Abstract*— *Appetized ginger plum leather was prepared by using different combinations of ginger and plum pulp with varying concentrations of appetizing mixture. The TSS of ginger and plum pulp were raised to 25°B by adding sugar and 1.0 to 2.5% appetizing mixture, followed by drying thin layers in dehydrator (55±2°C) to 12-14% moisture content. The standardization of most palatable recipe was done by evaluating sensory properties and highest score was obtained by ginger: plum (50:50) and 1.5% appetizing mixture. The appetized leather contained comparatively higher amount of ascorbic acid (13.16mg/100g), total phenols (55.89mg/100g) and antioxidant activity (72.94%). The leather was found most stable when packaged in laminated aluminium pouches during storage. The leather did not exhibit appreciable changes in titratable acidity, ascorbic acid, total sugars, phenols and antioxidant activity after 6 months. Thus the appetized ginger plum leather can be stored under ambient storage after packing in aluminium laminated pouches.

***Keywords*— Ginger, plum, leather, appetizing mixture, aluminium laminated pouches, antioxidant activity.**

I. INTRODUCTION

Fruit leather or fruit bar also known as fruit roll means a sheet of dried pureed fruit prepared by blending fruit pulp, fat or milk solids & other ingredients required for the product which can be mould into desired shape or size (FSSAI 2011). Fruit leather is a confectionery product made by dehydration of fruit puree into leathery sheets and can be prepared from pulpy fruits such as mango (Gujral and Brar 2003), pear (Huang and Hsieh 2005), guava (Vijayanand et al. 2000), longan (Jaturonglumlert and Kiatsiriroat 2010), banana, kiwifruit (Vatthanakul et al. 2010), grape (Maskan et al. 2002), chiku, jackfruit (CheMan and Taufik 1995) and papaya Gowda et al. (1995). Further innovations can be made in the preparation of leathers by adding appetizing constituents like mint, salt, black salt, thyme seed etc. The edible portion of fruit (single or in combination) is pureed, mixed with other ingredients to improve its physico-chemical & sensory characteristics (Phimpharian et al. 2011). Fruit leathers can be dried by using sun drying, oven drying, cabinet drying & dehydrator drying. Sun drying has traditionally being the process employed for preparing fruit leather from ripe fruits, the process can be unhygienic, lengthy and discolour the products (Teshome 2010). Fruit leathers are dehydrated fruit made from fresh fruit pulp or a mixture of fruit juice and are generally low in calories. Fruit pulp-based fruit leathers are nutritive & organoleptically acceptable and contains ample quantities of dietary fibers, minerals, vitamins & antioxidants (Gujral ad Brar 2003; Damodaran *et al*. 2010; Sharma et. al. 2013).

Ginger (*Zingiber officinale* Roscoe), generally consumed as a spice and is highly valuable in the international market for its aroma, pungency and high oleoresin content (Onwuka et al, 2002). The major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes (Grzanna et al. 2005) and phenolic compounds like gingerol, paradols, shogaol which cause the characteristic odour and flavour of ginger (Harold 2004), aromatic constituents like zingiberene and bisabolene and the pungent constituents like gingerols and shogaols (Tyler 1994). Besides these; amino acids, raw fiber, ash, protein, phytosterols, vitamins and minerals are also present (Langner et al. 1998; Shukla and Singh 2007). Thus ginger can be utilized for value addition and various value added products prepared from ginger are ginger flakes, ginger oil, oleoresin, candy, preserve, paste, and powder (Arya, 2001, Camacho and Brescia, 2009).

Plum (*Prunus domestica* L.) is one of the important stone fruit crops cultivated in temperate regions of the world, consumed mostly as fresh (Pino and Quijano, 2012). The fruits have attractive colour, flavour and taste and are an excellent source of antioxidants, calcium, magnesium, iron, potassium and fibre besides substantial amounts of vitamin C (Sabarez *et al*. 1997). The plum fruits with high antioxidant contents can be used for development of different value added products and dried fruit leather is a well known traditional healthy food of Bulgaria and Turkey (Momchilova et al. 2016). The preservation of fruit leather depends on their low moisture content (15-20%), the natural acidity of the fruit and high sugar content. Major quality parameters associated with dried fruit products are change

of colour, visual appeal, flavour, shape, texture, microbial load, and retention of nutrient, rehydration properties, water activity and chemical stability (Perera, 2005).

Thus, keeping in mind the therapeutic properties of ginger and the presence of substantial amount of anthocyanins from plum fruits, the present investigations were therefore undertaken to optimize the recipe for the preparation of ginger plum leather by adding palatable concentration of appetizing mixture.

II. MATERIALS AND METHODS

The farm fresh ginger and plum fruits were utilized for the preparation of ginger plum leather. The ginger pulp was extracted by the hot break method after adding 30 percent water followed by heating for 60 minutes (Dhiman 2015) and plum pulp was extracted by adding 10 percent water, heating for 15-20 minutes followed by passing the whole mass through the pulper (M/S B. Sen Berry and Co. New Delhi, India). The pulp was preserved by heat pasteurization method (over-flow method) as advocated by Lal and Sharma (1989) and packed in pre-sterilized glass bottles for its use in product development.

Fruit leather/bar was prepared by mixing ginger and plum pulp in different proportions (100:0, 90:10, 80:20, 70:30, 60:40 and 50:50) followed by homogenization and heating. The total soluble solid was raised to 25°B by adding cane sugar powder and the mixture was spreaded in a thin layer (3-6mm) on the stainless steel trays (30×20cm^2) with a tray load of 440g/tray and dried in a mechanical dehydrator at 55±2°C. The combinations of ginger plum leather were evaluated on the basis of sensory characteristics and the treatment with higher sensory score was further taken for the standardization of suitable concentration of appetizing mixture. The appetizing mixture prepared by mixing thyme seed powder (5g); mint powder (10g); salt (10g) and black salt (10g) was tried in different concentrations of 1.0, 1.5, 2.0 and 2.5 percent. Further, the best treatment combination of ginger plum leather with appetizing mixture was selected on the basis of drying behavior and sensory score. The dried fruit leather was then cut into strips of suitable dimensions (8cm^2) followed by wrapping in a butter paper and packing in aluminium laminated pouches (20 g) and stored at ambient temperature (14.6–26.1°C) for further storage studies.

Analysis

Physico-chemical analysis of ginger rhizome, plum fruits and prepared leather was conducted by using standard analytical procedures (Ranganna 1997; Ting and Rouseff 1986; AOAC 1995). Average weight and dimensions of ginger and plum were determined gravimetrically and the results were expressed as g and per cent (w/w) on whole fruit basis. Total soluble solid (TSS) content of fresh and processed products was determined by hand refractometer and sugars were estimated by Lane and Eyon method as detailed in Ranganna (1997). Acidity was determined by titrating the aliquots against 0.1N NaOH solution to a pink end point using phenolphthalein as an indicator (Ranganna 1997). The ascorbic acid content was determined using 2, 6-dichloro phenol indophenol dye as per the method given by Ranganna (1997). Total phenols were extracted in 80% ethanol and were estimated using Folin-Ciocalteau reagent (AOAC 1995). The rate of dehydration per unit time was calculated by placing a weighed quantity of pulp on a stainless steel tray (30×20 cm^2) followed by drying in mechanical dehydrator (55± 2 °C) to a moisture content of 12–14% (w/w). The loss in weight during drying (% dwb) was calculated by plotting the moisture on dry weight basis against time in hours (Fellows 1988). The sensory evaluation of fruit leather was done by a semi-trained panel of 7–9 judges for various quality attributes viz., colour, texture, flavour, taste and overall acceptability on 9 point hedonic scale. Data pertaining to sensory evaluation of ginger plum leather was statistically analyzed according to Randomized Block Design (RBD) as described by Mahony (1985) while, the data on chemical analysis was analyzed by following Completely Randomized Design (CRD) Cochran and Cox (1967).

III. RESULTS AND DISCUSSION

Physico chemical characteristics of ginger rhizome and plum: The ginger rhizome utilized for product development contained a moisture content of 82.39 ± 0.05 percent with total soluble solids as 2.7 ± 0.10°B (Table 1). The rhizomes were found to be a good source of ascorbic acid (8.48 ± 0.53 mg/100 g), total phenols (10.18 ± 0.03 mg/100 g), antioxidant activity (57.45 ± 0.60 %), protein (2.73 ± 0.06 %), crude fibre (1.41 ± 0.02%) and total ash (1.66 ± 0.02%), while oleoresin and oil was noticed to be 5.01 ± 0.05 and 1.63 ± 0.01 percent, respectively. These values were in conformity with the result reported by Onyenekwe and Hashimoto (1999), Abeyesekera *et al*. (2005), Sultan *et al*. (2005) and Shahid and Hussain (2012).

Further, the plum fruits contribute 13.86°B total soluble solids with 2.94 ± 0.02 percent of malic acid, with an appreciable amount of ascorbic acid (18.30 ± 1.09 mg/100 g), total phenols (96.66 ± 2.89 mg/100g), antioxidant activity (71.6 ± 0.55%), crude fibre (0.07 ± 0.01 %) and total ash (0.42 ± 0.03%) (Table 1). These values were found in conformity with the result found by Erturk *et al.* (2009) and Esehaghbeygi *et al.* (2013). Thus, keeping view the nutritional as well as medicinal properties of ginger and

plum, they were suitably blended to provide leather with an acceptable acidity, colour and flavour without the addition of exogenous colour, flavour and acid.

Standardization of recipe for leather preparation: The results pertaining to the sensory evaluation of plum ginger leather are presented in Table 2, reveals that the significantly higher scores for colour (8.25), texture (8.20), flavour (7.99), taste (8.00) and overall acceptability (8.50) were received by ginger: plum 50:50 proportion (L_6). The leather prepared by using 100 percent ginger was rated as least preferred with colour, texture, flavour, taste and overall acceptability scores of 5.16, 5.07, 5.21, 5.81 and 5.11 respectively and was further could not form into leather. However, on the basis of sensory score, 60: 40 proportion (L_5) was also rated at par with 50:50 (L_6) but on basis of overall quality and higher yield, the 50: 50 proportion was found superior and thus optimized for further studies.

Drying Behaviour: In comparison to total drying period, the rate of dehydration was very fast during initial period of drying. About 50-55 percent (fwb) of the moisture was lost within the initial 2 to 2.5 hours of drying, thereafter the rate of drying slowed down (Fig 1). It took about 8.0 to 9.30 hours to dry the pulp combination to moisture content of about 13.04 to 13.93 percent (Table 3). The dehydration ratio calculated on the basis of the yield of dried plum ginger leather varied between 3.48: 1 to 3.91: 1 and the maximum yield (28.76%) was noticed in 50:50 proportion and time taken for drying was 9.30 hours.

Standardization of concentration of appetizing mixture in leather: The plum ginger leather combination of 50:50 attaining the highest sensory scores were mixed with appetizing mixture in different concentrations (1.0%, 1.5%, 2.0% and 2.5%) and was subjected to sensory evaluation for the selection of best recipe for the preparation of spiced ginger plum leather. The results pertaining to the sensory evaluation are presented in Table 4 and Fig 2 showed that the score for colour (7.40), texture (8.16), flavour (7.90), taste (7.97) and overall acceptability (8.46) were highest for plum: ginger 50:50 with 1.5 percent appetizing mixture (LA_2). Although, at par score were given to all combinations by the panellists, but treatment LA_2 (50:50 + 1.5% AM) was liked very much and thus optimized for the development of appetized ginger plum leather and selected for further storage studies.

Drying characteristics: The total time taken for drying by the different plum ginger leather combinations (50:50) containing appetizing mixture in varying concentrations was about 9.45 to 10.40 hours with dehydration ratio of 3.43: 1 to 3.55: 1 and yield ranging between 25.9 to 26.3 percent. The total solids and moisture contents were found in range of 87.33 to 87.67 per cent and 12.42 to 12.67 percent (Table 5).

Physico-chemical characteristics of ginger plum leather: The data presented in Table 6 shows that the appetized ginger plum leather contains slightly higher amount of ascorbic acid (13.16 mg/100g), phenols (55.89mg/100g) and thus having better antioxidant activity (72.94%) as compared to leather without appetizing mixture. Thus, the appetizing mixture (1.5%) adds more value to ginger plum leather.

Storage Studies

The storage stability of ginger plum leather prepared with or without the addition of appetizing mixture after packing them in aluminium laminated pouches was evaluated at periodic intervals of 0, 3 and 6 months at ambient temperature (14.6–26.1^{o}C) .

The plum ginger leather with or without appetizing mixture did not exhibit appreciable changes in total phenols, antioxidant activity and crude fibre content during the entire storage period. The results were in conformity with those obtained by Kaushal et al (2013) in foam mat dried seabuckthorn leather. However, increase in the total soluble solids of the leather is correlated well with the corresponding decrease in the moisture content. The appetized ginger plum leather exhibited 75 percent retention of ascorbic acid (9.95mg/100g) as against 70 percent retention (8.45 95mg/100g) in the leather without appetizing mixture after six months of storage under ambient conditions. However, at end of the storage period the mean ascorbic acid in leather was found to be 9.2 mg/100 g against its initial value of 12.60 mg/100 g thus representing a reduction of about 22% (Table 8). The degradation of ascorbic acid during storage is attributed partially to its oxidation and partially to its involvement in browning reactions in the presence of high acidic environment (Clegg 1966). Marginal decrease in total sugars during storage has been attributed to its possible participation in maillard browning reactions (Cheftal et al. 1985).

Further, the sensory quality of ginger plum leather for various attributes during storage is presented in Table 8. The leather prepared with or without the addition of appetizing mixture was acceptable in all sensory quality

parameters with a hedonic score more than 7.0 out of 9.0. However, the acceptability score exhibited slight decrease with the increase in period of storage. Slight decrease in flavour scores observed during 6 months might be attributed to the loss of aromatic compounds during storage. The ginger plum leather made with the addition of appetizing mixture had a higher score (8.32) for overall acceptability which was non-significantly different from the leather without appetizing mixture (8.35). Further, the sensory score for all organoleptic parameters in the product during six months storage remained above 7.0, thus exhibiting good storage stability of this product.

IV. CONCLUSION

On basis of sensory evaluation during this study, it can be concluded that among different treatments, ginger: plum 50:50 proportion (L_6) was found best with overall acceptability score (8.50) and further the plum: ginger 50:50 proportion with 1.5 percent appetizing mixture (LA_2) optimized for development of appetized ginger plum leather. Thus the method for the preparation of appetized ginger plum leather 50:50 ratio followed by drying and packing in aluminium laminated pouches was found the most appropriate to increase antioxidant activity The developed technology can be commercially explored at industry level for the production of appetized leather and profitable utilization of ginger and highly perishable plum fruits for ensuring better returns to the growers.

Table.1: Physico-chemical characteristics of fresh ginger rhizome (Zingiber officinale) and plum fruit (Prunus domestica)

Characteristics	**Mean ± SD***	
	Ginger rhizome	**Plum**
Moisture (%)	82.39 ± 0.05	86.93 ± 0.09
TSS (^{0}B)	2.7 ± 0.10	13.86 ± 0.41
Titratable acidity (% citric acid for ginger, % malic acid for plum)	0.15 ± 0.02	2.94 ± 0.02
Total sugars (%)	1.26 ± 0.02	9.26 ± 0.34
Ascorbic acid (mg/100 g)	8.48 ± 0.53	18.30 ± 1.09
Total phenols (mg/ 100g)	10.18 ± 0.03	96.66 ± 2.89
Antioxidant activity (%)	57.45 ± 0.60	71.6 ± 0.55
Crude Protein (%)	2.73 ± 0.06	0.6 ± 0.06
Crude fibre (%)	1.41 ± 0.02	0.07 ± 0.01
Total ash (%)	1.66 ± 0.02	0.42 ± 0.03
Oleoresin (%)	5.01 ± 0.05	-
Oil (%)	1.63 ± 0.01	-

**All values are the mean of 10 observations, SD = Standard deviation*

Table.2: Sensory evaluation of recipes for the preparation of plum ginger leather*

Treatments	**Plum: ginger ratio**	**Colour**	**Texture**	**Flavour**	**Taste**	**Overall Acceptability**
L_1	100:0	7.98	7.80	7.59	7.59	7.78
L_2	90:10	8.10	7.82	7.62	7.62	7.87
L_3	80:20	8.14	7.87	7.68	7.78	7.89
L_4	70:30	8.16	7.98	7.76	7.81	7.92
L_5	60:40	8.20	8.16	7.92	7.91	8.00
L_6	50:50	8.25	8.20	7.99	8.00	8.50
L_7	0:100	5.16	5.07	5.21	5.81	5.11
$CD_{0.05}$		**0.04**	**0.03**	**0.02**	**0.08**	**0.03**

**On 9 point hedonic scale*

http://dx.doi.org/10.22161/ijeab/2.1.49 *ISSN: 2456-1878*

Table.3: Effect of different recipes on drying behaviour of plum ginger leather

Treatments	Plum: ginger	Drying times (hrs)	Yield (%)	Dehydration ratio	Total solids (%)	Moisture (%)
L_1	100: 0	8.00	27.10	3.91: 1	86.07	13.93
L_2	90: 10	8.10	27.23	3.67 :1	86.37	13.63
L_3	80: 20	8.35	27.55	3.63: 1	86.59	13.41
L_4	70: 30	8.55	28.21	3.55: 1	86.72	13.28
L_5	60: 40	9.15	28.70	3.48: 1	86.89	13.15
L_6	50: 50	9.30	28.76	3.48: 1	86.99	13.04

*Table.4: Effect of different concentrations of appetizing mixture on *sensory quality of appetized plum ginger leather*

Treatments	Plum: Ginger + #AM (%)	Colour	Texture	Flavour	Taste	Overall Acceptability
LA_1	50:50 + 1.0	7.36	7.94	7.86	7.88	7.62
LA_2	50:50 + 1.5	7.40	8.16	7.90	7.97	8.46
LA_3	50:50 +2.0	7.18	7.68	7.68	7.58	7.58
LA_4	50:50 + 2.5	7.13	7.62	7.64	7.47	7.42
$CD_{0.05}$		**0.03**	**0.02**	**0.05**	**0.14**	**0.08**

* On 9 point hedonic scale

#AM = Appetizing mixture

Table.5: Effect of different concentrations of appetizing mixture on drying behaviour of spiced ginger leather

Treatments	Plum: Ginger + #AM (%)	Drying times (hrs)	Yield (%)	Dehydration ratio	Total solids (%)	Moisture (%)
LA_1	50:50 + 1.0	9.45	25.90	3.55: 1	87.33	12.67
LA_2	50:50 + 1.5	10.00	26.03	3.48: 1	87.36	12.64
LA_3	50:50 +2.0	10.15	26.10	3.44: 1	87.62	12.50
LA_4	50:50 + 2.5	10.40	26.30	3.43: 1	87.67	12.42

#AM = Appetizing mixture

Table.6: Physico-chemical characteristics of ginger plum leather

	Ginger : Plum	
Parameters	**50:50**	**50:50 + 1.5%AM**
Moisture (%)	13.04	12.64
Total soluble solids (^{0}B)	49.07	49.02
Titratable acidity (%)	2.82	2.86
pH	3.52	3.46
Reducing sugars (%)	13.68	13.71
Total sugars (%)	37.29	37.04
Ascorbic acid (mg/100g)	12.05	13.16
Total ash (%)	3.72	3.82
Total phenols (mg/100g)	53.54	55.89
Antioxidant activity (%)	72.61	72.94
Crude fibre (%)	0.41	0.42
Salt (%)	------	1.89

http://dx.doi.org/10.22161/ijeab/2.1.49 *ISSN: 2456-1878*

Table.7: Changes in quality characteristics of ginger plum leather during storage at ambient temperature (14.6-26.1°C)

Parameter	leather	0 month	3 month	6 month	Mean	CD 0.05%
Moisture (%)	50:50	13.04	12.93	12.80	**12.92**	T= 0.03
	50:50+ 1.5% AM	12.64	12.55	12.35	**12.51**	S=0.03
	Mean	**12.84**	**12.74**	**12.57**		T × S= NS
TSS (°B)	50:50	49.07	50.06	50.56	49.90	T= NS
	50:50+ 1.5% AM	49.02	50.02	50.49	49.84	S=0.19
	Mean	**49.04**	**50.04**	**50.52**		T × S= NS
Titratable acidity (%)	50:50	2.82	2.60	2.38	2.60	T= 0.04
	50:50+1.5% AM	2.86	2.70	2.48	**2.68**	S=0.04
	Mean	**2.84**	**2.65**	**2.43**		T × S= NS
Total sugars (%)	50:50	37.29	36.01	35.49	**36.26**	T= NS
	50:50+1.5% AM	37.04	36.0	35.22	**36.09**	S=0.55
	Mean	**37.16**	**36.00**	**35.35**		T × S= NS
Ascorbic acid (mg/100g)	50:50	12.05	10.14	8.45	**10.21**	T= 0.31
	50:50+1.5% AM	13.16	11.97	9.95	**11.69**	S=0.03
	Mean	**12.60**	**11.05**	**9.2**		T × S= 0.05
Total phenols (mg/100g)	50:50	53.54	53.51	53.42	**53.49**	T= NS
	50:50+1.5% AM	55.89	55.70	55.42	**55.67**	S=NS
	Mean	**54.71**	**54.60**	**54.42**		T × S= NS
Antioxidant activity (%)	50:50	72.61	72.37	72.05	**72.34**	T= NS
	50:50+1.5% AM	72.94	72.65	72.30	**72.63**	S=NS
	Mean	**72.77**	**72.51**	**72.17**		T × S= NS

S=Storage, T= Temperature, AM=appetizing mixture, NS=non significant

Table.8: Changes in sensory quality of ginger plum leather during storage at ambient temperature (14.6-26.1°C)

Sensory Characteristics	leather	0 month	3 month	6 month	Mean	CD 0.05%
	50:50	8.22	8.19	8.11	**8.17**	T= 0.05
Colour	50:50+ 1.5% AM	7.40	7.34	7.28	**7.34**	S=0.04
	Mean	**7.81**	**7.76**	**7.69**		T × S= NS
Texture	50:50	8.17	8.10	8.05	**8.11**	T= 0.05
	50:50+ 1.5% AM	8.16	8.08	8.01	**8.08**	S=0.04
	Mean	**8.16**	**8.09**	**8.03**		T × S= NS
Flavour	50:50	7.91	7.85	7.77	**7.84**	T= NS
	50:50+1.5% AM	7.90	7.82	7.72	**7.81**	S =NS
	Mean	**7.90**	**7.83**	**7.74**		T × S= NS
Taste	50:50	7.99	7.93	7.84	**7.92**	T= NS
	50:50+1.5% AM	7.97	7.88	7.80	**7.88**	S =NS
	Mean	**7.98**	**7.90**	**7.82**		T × S= NS
Overall Acceptability	50:50	8.48	8.43	8.35	**8.42**	T= NS
	50:50+1.5% AM	8.46	8.40	8.32	**8.39**	S =NS
	Mean	**8.47**	**8.41**	**8.33**		T × S= NS

http://dx.doi.org/10.22161/ijeab/2.1.49 *ISSN: 2456-1878*

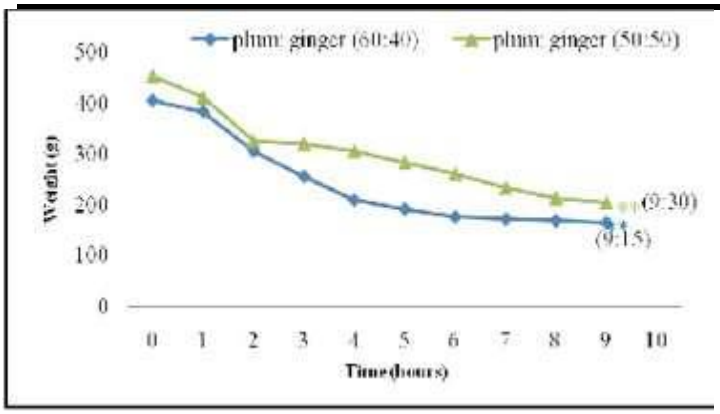


Fig.1: Drying curve of plum ginger leather

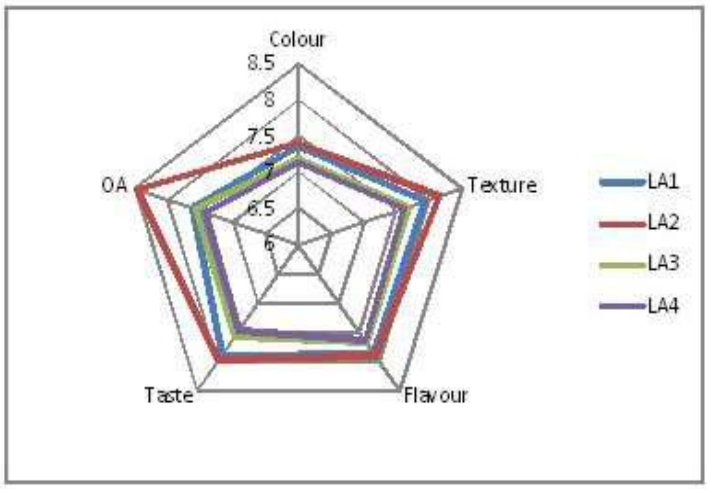


Fig.2: Pictorial representation of sensory scores of appetized ginger leather

REFERENCES

[1] Abeysekera WKSM, Iueperuma CK, Amunugoda PNRJ, Wijeratnam SW. 2005. Comparison of ginger varieties dried at different temperatures for oil and oleoresin contents. *Sri lankan J Agric Sci*. 42: 34-42.

[2] AOAC.1995. Official Methods of Analysis, 16th edn. Association of Official Analytical Chemists, Washington DC.

[3] Arya PS. 2001. Ginger Production Technology. Kalyani Publishers, New Delhi. 169p.

[4] Camacho EH, Brescia A. 2009. The Australian ginger industry: Overview of market trends and opportunities. Ginger: The Genus *Zingiber*. CRC Press, Florida, U.S.A. *http://www.daff.qld.gov.au/data/assets*

[5] Cheftal JC, Cuq JL, Lorient D. 1985. Amino acids, peptides and proteins. In: Fennema O.R. (ed) Food Chemistry. Marcel Dekker, Inc., New York

[6] CheMan YB, Taufik. 1995. Development and stability of jack fruit leather. *Tropical Sci.* 35(3): 245–250.

[7] Clegg KM. 1966. Citric acid and the browning of solutions containing ascorbic acid. *J Sci Food Agric*. 17(12): 546-549

[8] Cochran WG, Cox CM. 1967. Experimental Designs. John Wiley and Sons, Inc., New York.

[9] Damodaran S, Parkin KL, Fennema O R. 2010. AminoAcids, Peptides, and Proteins. In: Fennema O.R.(4th edn.), *Food Chem.*, 900 p.

[10] Dhiman A. 2015. Development and evaluation of noble products from ginger. M.Sc. Thesis. Dr Y S Parmar University of Horticulture and Forestry. Nauni, Solan (HP).

[11] Erturk Y, Ercislib S, Tosunc M. 2009. Physico-chemical characteristics of wild plum fruits (*Prunus spinosa* L.). *Int J Plant Production*. **3**(3): 89-92

[12] Esehaghbeygi A, Pirnazari K, Kamali M and Razavi J. 2013. Physical, and mechanical properties of three plum varieties (*Prunus domestica* L.). *Thailand J Agric Sci*. 46(2): 95-101

[13] Fellows P. 1988. Food Processing Technology-Principles and Practice. Woodhead Publishing Ltd, Cambridge, England, pp 281–313.

[14] FSSAI. 2011. Food Safety and Standards Authority of India. Food Safety and Standards Regulations, 2011, 435-436.

[15] Gowda D, Ambadan IN, Ramanjaneya KH. 1995. Studies on mango fruit bar preparation. *Ind Food Packer*. 49(2):17–24

[16] Grzanna R, Lindmark L, Frondoza CG. 2005. Ginger-an herbal medicinal product with broad anti-inflammatory actions. *J Medicinal Food*. 8(2):125–132.

[17] Gujral HS, Brar SS. 2003. Effect of hydrocolloids on the dehydration kinetics, color, and texture of mango leather. *Int J Food Prop*. 6 (2):269–279.

[18] Harold M. 2004. Food and Cooking: The Science and Lore of the Kitchen, Scribner, Second Edition, New York, NY, USA, p896.

[19] Huang X, Hsieh FH. 2005. Physical properties, sensory attributes, and consumer preference of pear fruit leather. *J Food Sci.* 70: E177–E186.

[20] Jaturonglumlert S, Kiatsiriroat T. 2010. Heat and mass transfer in combined convective and far-infrared drying of fruit leather. *J Food Eng.* 100: 254–260

[21] Kaushal M, Sharma PC, Sharma R. 2013. Formulation and acceptability of foam mat dried seabuckthorn (*Hippophae salicifolia*) leather. *J Food Sci Technol*.50(1):78–85

[22] Lal BB, Sharma TR. 1989. Apricot is a potential fruit crop for processing. *Ind Horti*. 33 p.

[23] Langner E, Greifenberg S, Gruenwald J. 1998. Ginger: history and use. *Adv in Therapy*. 15 (1):25–44.

[24] Mahony MO. 1985. Sensory evaluation of food. **In**: Statistical Methods and Procedures. CRC Press, Marcel Dekker, Inc., New York, p510.

[25] Maskan A, Kaya S, Maskan M. 2002. Hot air and sun drying of grape leather (pestil). *J Food Eng.* 54: 81–88.

[26] Momchilova M, Zsivanovits G, Tomova LM, Buhalova D, Dojkova P. 2016. Sensory and texture characterisation of plum (Prunus Domestica) fruit leather. *Bul Chem Comm.* 48: 428-434.

[27] Onwuka GL, Nwokoha GU, Asumugha GN. 2002. Evaluation of processing methods for the production of ginger drinks. Proceedings of 26th Annual NIFST Conference, Owerri.

[28] Onyenekwe P, Hashimoto S. 1999. The composition of the essential oil of dried Nigerian ginger (*Zingiber officinale* Roscoe). *European Food Res Technol*. **209**: 407-410

[29] Perera CO. 2005. Selected quality attributes of dried foods. *Drying Technol*. 23(4):717-730.

[30] Phimpharian C, Jangchud A, Jangchud K, Therdthai N, Prinyawiwatkul W, No HK. 2011. Physico-chemical characteristics and sensory optimization of a pineapple leather snack as affected by glucose syrup and pectin concentrations. *Int J Food Sci Technol*. **46** (5): 972-981

[31] Pino JA, Quijano CA. 2012. Study of the volatile compounds from plum (*Prunus domestica* L. cv. Horvin) and estimation of their contribution to the fruit aroma. *Food Sci Technol (Campinas)*. **32**(1): 76-83

[32] Ranganna S. 1997. Handbook of analysis and quality control for fruit and vegetable products, 2nd edn. Tata McGraw Hill Pub. Co.Ltd., New Delhi, India

[33] Sabarez H, Price WE, Back P, Woolf LA. 1997. Modeling the kinetics of drying of d' Agen plums (*Prunus domestica*). *Food Chem*. **60**: 371-382

[34] Shahid M, Hussain F. 2012. Chemical composition and mineral content of *Zingiber officinale* and *Alpiniaallughas* (Zingiberaceae) rhizomes. *Int J Chemical Biochem Sci*. **2**: 101-104

[35] Sharma SK, Chaudhary SP, Rao VK, Yadav VK , Bisht TS. 2013. Standardization of technology for preparation and storage of wild apricot fruit bar. *J Food Sci Technol*. 50 (4): 784-90

[36] Shukla Y, Singh M. 2007. Cancer preventive properties of ginger: a brief review. *Food Chem Toxicol*. 45(5):683–690.

[37] Sultan MHN, Bhatti, Iqbal Z. 2005. Chemical analysis of essential oil of ginger *(Zingiber officinale*). *Pak J Biol Sci*. **8**(11): 1576-1578

[38] Teshome B. 2010. Effect of Processing on some quality attributes of mango (*Mangifera indica*) fruit leather. M.Sc. Chemical Engineering (Food Engineering), Deptt Chemical Engineering Institute of Technology, Addis ababa University School of Graduate Studies, p146.

[39] Ting SV, Rouseff RL. 1986. Citrus fruits and their products-analysis and technology. Marcel Dekker, Inc., New Delhi, 293p

[40] Tyler VE. 1994. The therapeutic use of phytomedicinals, pharmaceutical products Press, New York, NY, USA, 1994.

[41] Vatthanakul S, Jangchud A, Jangchud K, Therdthai N, Wilkinson B. 2010. Gold kiwi fruit leather product development using quality function deployment approach. *Food Qual Preference*. 21: 339–345.

[42] Vijayanand P, Yadav AR, Balasubramanyam N, Narasimham P. 2000. Storage stability of guava fruit bar prepared using a new process. *Food Sci Technol.* 33:133–137.

http://dx.doi.org/10.22161/ijeab/2.1.50 *ISSN: 2456-1878*

Postharvest Management and Value Addition of Ginger (*Zingiber Officinale* Roscoe): A Review

Manisha Kaushal[1], Anil Gupta[1*], Devina Vaidya[1], Meenu Gupta[2]

[1]Department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan-173230, India.

[2]Department of Vegetable Science, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan-173230, India.

Abstract*— *Ginger is an important spice crop and India is one of the leading producer and exporter of ginger in the world. Ginger is widely used around the world in food as a spice both in fresh and dried form which adds flavour to the meal by creating spicy pungent taste. The chemical components of the ginger rhizome vary considerably depending on the location of cultivation and postharvest treatments. Ginger contains polyphenol compounds such as gingerol and its derivatives like zingiberone, bisabolene, camphene, geranial, linalool, borneol and oleoresin (combination of volatile oils and resin) that accounts for its characteristic aroma and therapeutic properties. Fresh ginger are perishable in nature and are spoiled due to improper handling, growth of spoilage microorganisms, susceptibility to rhizome rot, wilting and sprouting, action of naturally occurring enzymes, chemical reactions and structural changes during storage. Keeping in mind the low shelf-life of fresh ginger and inadequate facility for their modern storage leading to distress sale, value addition could be a viable alternative which will fetch remunerative price to the growers. The present scenario, nutritional importance, postharvest management, value added products of ginger have been discussed in detail in the review.

Keywords*— *Ginger, novel products, scenario, appetized flakes, bars, salted ginger, crystallized ginger, quality, storage, value added products.

I. INTRODUCTION

Ginger originated from India from where it was introduced to Africa and Caribbean, however no definite information on the primary centre of domestication of ginger is available (Prabhakaran, 2013). It is now cultivated throughout the humid tropics (Meadows, 1998) and is a most widely used spice worldwide. Ginger is a monocotyledonous perennial herb with robust, heavily branched rhizomes called hands or laces. India is the largest producer of ginger and the annual production is around 2, 63,170 tonnes from an area of about 77,610 hectares, contributing approximately 30 to 40% of the world production (Ravindran and Nirmal, 2005).

Ginger is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases (Ali et al. 2008). Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infections (Tan & Vanitha, 2004). Ginger belongs to Zingiberaceae family and an English botanist William Roscoe (1753-1831) gave this plant the name *Zingiber officinale* in the year 1807. The name of the genus *Zingiber* derives from a Sanskrit word denoting "horn shaped" in reference to the protusions on the rhizomes (Ghosh *et al*, 2011). It is an oldest rhizome widely domesticated as a spice where the edible part is the swollen underground stem or rhizome. The Zingiberaceous plants have strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes (Chen *et al*. 2008).

Ginger is valued as a spice because of its aroma and pungency (Paull et al. 1988) and has been used through ages in almost all systems of medicine against many maladies due to its medicinal properties (Kubra and Rao 2012). Despite of its use as flavoring agent, ginger is also appreciated in ayurvedic, tibbe-e-unani (Srivastava and Mustafa 1989), allopathic (Fessenden *et al.* 2001), aromapethy (Shelly *et al.* 2004) and household medicines (Sloand and Vessey 2001). Ginger rhizome can be employed in the form of fresh paste, ginger tea (flavoring), dried powder and preserved slices (El-Ghorab *et al.*, 2010). Ginger can be utilized in different commercial products like cookies, candy, teas, tinctures, sodas, jam, beer, capsule and syrup (Maxwell, 2008). Ginger bread, confectionery, ginger ale, curry powders, certain curried meats, table sauces, in pickling and in the manufacture of certain soft drinks like cordials, ginger cocktail, carbonated drinks, etc are some of the value added products of ginger. Ginger is also used for the extraction of ginger oil, oleoresin, essences, tinctures etc (Pruthi, 1998).

http://dx.doi.org/10.22161/ijeab/2.1.50 *ISSN: 2456-1878*

II. PRODUCTION AND CONSUMPTION

The ginger is an erect perennial growing plant from one to three feet in height, consisting of thick scaly rhizomes (underground stems) which branch with thick thumb like protrusions, known as "hands". The rhizomes are 7-15 cm long and 1-1.5cm broad and laterally compressed depending on cultivars and growing conditions (Awang 1992; Bisset and Wichtl 1994). The outer surface is buff coloured and longitudinally striated or fibrous in nature (Ghosh *et al*, 2011). Ginger is commercially cultivated in India, China, South East Asia, West Indies, Mexico and other parts of the world. Most promising cultivars of ginger are Nadia, Maran, China, Ernad, Suprabha, Suruchi, Surabi, Varad, Himgiri etc. The variety IISR Varada is suited for fresh ginger, dry ginger and making candy while, IISR Rejatha is rich in essential oil.

The world production of ginger is 20,95,056 tonnes from an area of 3,22,157 hectares, while India's share is 7,03,000 tonnes from an area of 1,50,000 hectares (FAO, 2014). It is difficult to compare import data because they usually do not distinguish fresh from dried ginger. For instance, Japan is the number one importer and consumer of ginger with 1, 04,379 t in the year 2002 (ITC, 2002), but Japanese traditionally consume preserve ginger made from a mild fresh rhizome (Govindarajan 1982). Other major importing countries are: US (19,035 t), UK (10,337 t), Saudi Arabia (8,248 t), Singapore (import 7,566 t, re-export 2,989 t), Malaysia (import 7,652 t, re-export 1,334 t), Korea (6,805 t), the Netherlands (import 6,981 t, re-export 2,858 t), Canada (4,680 t), Germany, and France. Within the last decade China has become a major competitor overtaking some traditional exporting countries.

Traditional use

Zingiber officinalis is one of these traditional folk medicinal plants that have been used for over 2000 years by Polynesians for treating diabetes, high blood pressure, cancer, fitness and many other illnesses (Tepe *et al.*, 2006). *Zingiber officinalis* contains a number of antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, and polyphenols such as flavonoids, flavones glycosides, rutin, *etc.* (Aruoma *et al.,* 1997). Ginger has been used as a spice and as natural additives for more than 2000 years (Bartley and Jacobs, 2000). Also, ginger has many medicinal properties. Studies have shown that, the long term dietary intake of ginger has hypoglycaemic and hypolipidaemic effect (Ahmed and Sharma, 1997). In traditional Chinese and Indian medicine, ginger has been used to treat a wide range of ailments including stomach aches, diarrhea, nausea, asthma, respiratory disorders (Grzanna *et al*., 2005).

Medicinal Properties

Ginger has been identified as an herbal medicinal product with pharmacological effect. It is known as Sunthi in ayurveda and description of the plant appears in the old text like Charaka, Sushruta, Vagbhatta and Chakra-dutta. Pharmacologically, the drug in ayurveda medicinal part of the herb is dried and is described as appetizer. Ginger has been used as a traditional medicine to treat stomach disorders, nausea, diarrhea, colic, arthritis, heart conditions, menstrual period, dyspepsia, rheumatism and flu like symptoms, bronchitis and many more (Gosh *et al*. 2011, Latona et al, 2012, Grzanna *et al*, 2005). It is extensively used around the world in foods as a spice and highly value in the international market for its aroma, pungency and high oleoresin content (Onwuka *et al*. 2002). It is used as a flavouring agent in foods and beverages and as a fragrance in soaps and cosmetics (Alam 2013). Ginger is thought to act directly on the gastrointestinal system to reduce nausea. Therefore, it is used to prevent nausea resulting from chemotherapy, motion sickness, and surgery. It is known as a popular remedy for nausea during pregnancy (Langner *et al*. 1998). Ginger is also used to treat morning sickness, colic, upset stomach, gas, bloating, heartburn, flatulence, diarrhea, loss of appetite and dyspepsia (discomfort after eating) and is recommended to enhance the digestion of food (Ali *et al*. 2008). Because of its warming effect, ginger acts as antiviral for treatment of cold and flu (Qidwai *et al*. 2003).

Mechanism of action of ginger

The rhizome of the *Zingiber officinale* plays an important role in prevention of diseases, but the exact mechanism of action in diseases management is not understood fully. It is thought that ginger act as anticancer due to various constituents such as vallinoids viz; 6-gingerol and 6-paradol, shogaols, zingerone, and galanals A and B (Aggarwal and Shishodia 2006; Miyoshi *et al*. 2003; Shukla and Singh 2007) and constituents show a therapeutics role in diseases control via modulation of various biological activities, describe as following:

1. Ginger and its constituents show antioxidant activity and prevent the damage of macro-molecules, caused by the free radicals/oxidative stress.
2. It also shows a vital role as anti-inflammatory processes. Earlier studies on In-*vitro* investigations of ginger preparations and some isolated gingerol-related compounds showed that anti-inflammatory effects of ginger such as inhibition of COX (Tjendraputra *et al*. 2001) and inhibition of nuclear factor κB (Grzanna *et al.* 2005).
3. Ginger also acts as antitumor via modulation of genetic pathways such as activation tumour suppressor

gene, modulation of apoptosis and inhibition of VEGF. Earlier study has shown that terpenoids, constituents of ginger induce apoptosis in endometrial cancer cells through the activation of p53 (Liu *et al*. 2012).

4. Ginger also shows antimicrobial and other biological activities due to gingerol and paradol, shogaols and zingerone. An important finding showed that 10% ethanolic ginger extract was found to possess antimicrobial potential against pathogens (Giriraju and Yunus 2013).
5. Antioxidants are substances that play a role in the neutralization of free radicals and oxidative.

Chemistry and chemical structure of active constituents

Numerous active ingredients are present in ginger including terpenes and oleoresin which called ginger oil. Ginger also constitutes volatile oils approximately 1% to 3% and non-volatile pungent components oleoresin (Zick *et al*. 2008). The major identified components from terpene are sesqui-terpene hydrocarbons and phenolic compounds which are gingerol and shogaol (Hasan et al. 2012) and lipophilic rhizome extracts, yielded potentially active gingerols, which can be converted to shogaols, zingerone, and paradol. The chemical analysis of ginger shows that ginger contains over 400 different compounds and the major constituents in ginger rhizomes are carbohydrates (50–70%), lipids (3–8%), terpenes and phenolic compounds (Grzanna *et al*. 2005). Terpene components of ginger include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, paradols, and shogaol. The characteristic odour and flavour of ginger are due to a mixture of volatile oils like gingerols (23–25%) and shogaol (18–25%) and these constituents are found in higher quantity as compare to others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid, vitamin A) and minerals are also present (Langner *et al*. 1998; Shukla and Singh 2007). The aromatic constituents include zingiberene and bisabolene. Other gingerol- or shogaol-related compounds (1–10%), which have been reported in ginger rhizome, include 6-paradol, 1-dehydrogingerdione, 6- gingerdione and 10-gingerdione, 4- gingerdiol, 6-gingerdiol, 8-gingerdiol, and 10-gingerdiol, and diaryl-heptanoids (Michiein *et al.* 2009, Elaissi *et al*. 2011, Hossain *et al*. 2011). The mode of action and active constituents present in ginger are listed in Table 1.

Antimicrobial property: Ginger is reported to have antibacterial effect especially against the *Staphylococci* species and also exhibits antifungal activity against a wide variety of fungi including *Candida albicans* (Ficker *et al*. 2003), though *Penicillium brevicompactum* actually grows on ginger causing rot during post-harvest storage (Overy and Frisvad 2005). According to Islam et al. (2014), the antimicrobial activity of the ginger was found highest against *Salmonella spp.* while lowest activity was found against *Escherichia coli*. *Staphylococcus aureus* showed lower sensitivity to ginger extract as compare to the most other Gram-negative bacteria.

The chemical structures of some of the constituents present in ginger are given in Figure 1.

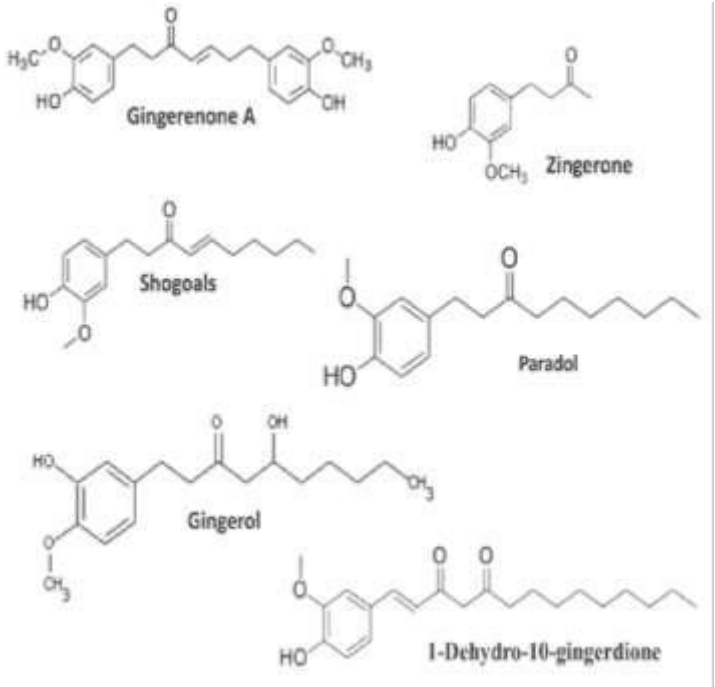


Fig.1: Chemical structure of active ingredients present in ginger.

Quality characteristics of fresh ginger rhizome

Antioxidant activity: Ginger is a source of a large number of antioxidants and also plays an important role in the reduction of the lipid oxidation and inhibits the pathogenesis of diseases. Ginger extract possesses anti-oxidative characteristics and shows a role in scavenge superoxide anion and hydroxyl radicals (Cao *et al*. 1993; Krishnakantha and Lokesh 1993) and gingerol, inhibited ascorbate/ferrous complex induced lipid peroxidation in rat liver microsomes (Reddy and Lokesh 1992). Ginger grown in different parts of the country varies considerably in its intrinsic physical and biochemical properties and its suitability for processing. The important quality parameters of ginger are its fibre, volatile oil and non volatile ether extracts. The research finding reported by different researchers on fresh ginger is detailed in the Table 2.

Ginger oleoresin, a substance having a pungent property is obtained from ground rhizome by extraction with volatile solvents, while the essential oil which lacks pungency is derived from the rhizome by steam distillation. The yield of ginger oleoresin and oil depends upon the cultivar, location, stage of harvesting and method of extraction. Ginger oil is extracted from dried ginger commercially after steam distillation, however fresh ginger can also be used for oil extraction purposes but the yields are quite low (Purseglove *et al*, 1981). The commercial ginger essential oil is characterized by pale yellow colour, warm, spicy and woody flower with slight lemony notes (Koroch *et al*, 2007).

Major Post-harvest Problems

Postharvest Diseases: Postharvest disease in ginger is normally due to rough harvesting and handling practices which result in injury to the skin and flesh of the rhizome (Table 3). Holding ginger at a less than optimal temperature and relative humidity (RH) will accelerate postharvest decay. Postharvest losses from diseases are caused by various microorganisms. Decay can be kept to a minimum by following careful harvesting and handling practices, sanitation of the wash water, curing of the rhizomes after washing to promote wound healing, application of a postharvest fungicide, and holding the rhizomes at 12.5°C (55°F) and 70 to 75% RH.

Post-harvest Disorders

Sprouting: Ginger rhizomes will sprout at temperatures above 15.6°C (60°F). Sprouting may begin after several weeks storage at ambient temperature. The rate of sprouting grows as the temperature increases. There is no effective chemical sprout inhibitor for ginger.

Chilling Injury: Physiological disorder that results in pitting and sunken lesions on the rhizome surface, shriveling, softening, flesh darkening, and postharvest decay. Ginger rhizomes are very sensitive to chilling injury or low temperature breakdown if stored below 12°C (54°F).

Shriveling/desiccation: This is a common postharvest disorder of ginger held under low relative humidity (RH) conditions (i.e. less than 65% RH). Shriveling of the rhizome becomes noticeable after the loss of more than 10% of the initial harvest weight. On the other hand, surface mould will begin to grow at a RH above 90% and sprouting will be stimulated, especially if the temperature is above 16°C. In order to minimize weight loss and avoid surface mould, an optimum relative humidity range of 70 to 75 percent is recommended for storing ginger.

Harvesting and Postharvest management

Maturity Indices: The principal indices used to determine ginger harvest maturity are foliage senescence, age of leaves and rhizome size. Rhizomes do not continue to enlarge and grow without healthy foliage. Delaying harvest until all of the leaves have died is not recommended as it will reduce rhizome quality, increase fiber content, decrease storage life and increase the incidence of sprouting. The drawback of harvesting less mature ginger is that bulbs are susceptible to greater weight loss.

Stage of ginger harvesting for various end uses

End use	**Stage of harvest (months after planting)**
Vegetable purpose and preparation of ginger preserve, candy, soft drinks, pickles and alcoholic beverages.	5-6
Dried ginger and preparation of ginger oil, oleoresin, dehydrated and bleached ginger.	7-8

Harvest methods: Ginger is almost entirely harvested by hand, although mechanical digging devices are available for use on large-scale planting. The initial step in harvesting is to remove a significant portion of the senescent foliage to make the rhizomes more accessible. Ginger is dug by hand using a fork to loosen the soil around the crown of the plant. The process is done carefully in order to avoid damaging the rhizomes. The rhizomes are gently pulled out of the soil using the remaining length of stem as a handle. Ginger harvested early will still have an actively growing green

stem attached to the rhizome which needs to be snapped or cut off slightly above the point of attachment to the rhizome. Ginger is then pre-graded in the field for any unmarketable, damaged or diseased rhizome. Harvesting during very wet or very dry conditions is not desirable as this will increase the amount of skinning and make removal of the rhizomes from the soil much more difficult.

Pre-cooling conditions: Forced-air or room cooling to 12 to 14 °C (54 to 57 °F) should be used.

Preparation for market: Relatively clean bulbs sold in the domestic market may not require any further cleaning. However, ginger intended for export must be thoroughly cleaned before packing. The ginger intended for long term storage should be washed immediately after harvest and then cured. Ginger should be scrubbed by hand or with a soft-bristled brush in clean water sanitized with 150 ppm hypochlorous acid. Care is required during cleaning to prevent bulb breakage, which increases decay and shrinkage. A fungicide treatment benomyl (500 ppm) or thiabendazole (1000 ppm) can also be applied in the wash tank or as a separate overhead spray application after cleaning.

Remove all damaged and injured bulbs. The remaining marketable bulbs should be sorted according to size and overall appearance. The ginger surface should be clean, bright yellow-brown and appear fresh. It should not be wilted or have any evidence of sprouting. Export quality ginger should be smooth and firm, with uniform shape and size, be free from insect damage and decay, and have a uniform peel colour typical of the variety. The internal flesh should be firm and uniformly cream or pale-yellow coloured, without any indication of darkening.

Curing: Ginger intended for storage should be cured by drying the rhizomes in air at ambient temperature (22°C to 26°C or 71°F to 79°F) and 70% to 75% RH for several days to allow the skin to thicken and the cut surfaces to suberize. Curing will help reducing postharvest weight loss and decay. After curing, the ginger should be kept in well-ventilated containers for long-term storage.

Packaging: Ginger of roughly similar size should be packed in each market container. The container should be strong, well ventilated, and capable of being stacked without damaging the bulbs. For the domestic market, wooden crates provide better protection to the ginger than mesh or synthetic sacks. For the export market ginger bulbs should be placed in a clean, strong, well-ventilated fibreboard carton. The surface of the bulbs should be thoroughly dry prior to packing.

Optimum storage conditions: Mature ginger rhizomes can be stored at 12 to 14 °C (54 to 57 °F) with 85 to 90% RH for 60 to 90 days. Storage at 13 °C (55 °F) with 65% RH leads to extensive dehydration and a wilted appearance (Paull and Chen 2015). Holding ginger at ambient temperatures will result in high moisture loss, surface shriveling and sprouting of the rhizome.

Processing and Value addition

Ginger is used in three forms namely, fresh or green ginger, whole dry ginger and split dry ginger. Fresh ginger are sometimes unsuitable for converting to the dry spice, due to its initial high moisture content causing difficulty in drying and thus a heavy wrinkled product is obtained with low volatile oil content (Balakrishnan, 2005). Fresh ginger suffers from weight loss, shrinkage, sprouting and rotting during storage after 3 to 4 weeks of harvesting. This spoilage can be overcome by processing fresh produce to some value added products (Nath *et al*, 2013). The various value added products prepared from ginger are ginger oil, oleoresin, ginger candy, ginger preserve, ginger puree, ginger powder, ginger beer and ginger paste (Arya, 2001, Camacho and Brescia, 2009). Ginger is consumed worldwide as flavouring agent which is used extensively in food, beverage and confectionary industries in the products such as marmalade, pickles, chutney, ginger beer, ginger wine, liquors, and other bakery products (wang et al. 2011).

In South India, ginger is used in the production of a candy called Injimurappa meaning ginger candy in Tamil (Sebioma et al. 2011). Nath *et al,* (2013) optimized the protocol for production of instant ginger candy with a slice thickness of 5.0-25.0 mm and blanching time of 10-30 minutes. Further, Siddiqui *et al.* (2012) standardized the recipe for the preparation of ginger preserve from 70 percent sugar concentration and ginger candy from 75 percent sugar concentration. Similarly, Gupta *et al.* (2012) also optimized osmo-convective process conditions for preparation of honey ginger candy. Bhuyan and Prasad (1990) evaluated the effect of varied drying temperature (70-90^{o}C) on the quality of dried ginger and optimized ginger drying at 60 to 70^{o}C air temperature.

Further, Jadhav *et al,* (2012) studied the drying characteristics of ginger (peeled whole, sliced, treated and control) by employing open air sun drying, solar cabinet drying and mechanical tray drying (65^{0}C) methods and drying time with optimization of mechanical tray drying of peeled untreated sliced ginger. According to Eze and Agbo (2011), solar dried unpeeled ginger contains 7.0% moisture which was within the standard (6-9%) acceptable limits as compare to open air dried ginger attaining 17.0% moisture content.

http://dx.doi.org/10.22161/ijeab/2.1.50 *ISSN: 2456-1878*

Peeling of Ginger: Peeling of ginger is an important step and a prerequisite for preparation of various value added products. After washing the ginger rhizomes are subjected to peeling operation. Indigenously, peeling of ginger is done by scrapping with sharpened bamboo stick. Ginger are irregular in shape and not in a spherical geometry, therefore peeling process is a very tedious, time consuming and labour intensive operation. Commonly used methods of peeling ginger rhizomes include hand peeling, gunny bag peeling, lye peeling and sand blasting (Joshi et al, 1991). Ginger peeling is done manually inspite of machines developed (Agarwal *et al*, 1987; Ali *et al*, 1991). Endrais and Asfaw (2011), Eze and Agbo (2011), Yiljep et al. (2005) have reported the adverse effect of peeling.

A mechanical brush type ginger peeling machine has been developed by Rajasthan Agricultural University. The peeling efficiency of the machine was 85% and the capacity was 200 kg/h (Agarwal *et al.* 1987). Another mechanical ginger peeler was developed with its peeling drum made of diamond cut mesh (Jayashree & Visvanathan 2013) which has a peeling efficiency of 59%. Kaushal *et al*, (2014) evaluated different ginger peeling methods found that maximum recovery of peeled rhizome was obtained by mechanical peeler with peeling losses of 4.51 per cent as compared to other peeling methods like hand peeling, gunny bag peeling, abrasive peeling, lye peeling with a peeling losses of 13.10, 18.0, 11.35 and 28.0 percent respectively.

Ginger Slicing/Splitting: Simonyan *et al*. (2003) developed a motorized reciprocating ginger slicer consisting of the feeding unit, slicing mechanism and housing. The ginger rhizomes fed manually into the hopper falls by gravity into the cylinder at the bottom of the piston. It is pushed horizontally to the stationary knife blade as the piston moves towards the top dead center. The pushing of the rhizomes forces the ginger through the blade, which are collected at the outlet. Guwo (2008) also developed a ginger splitting machine having stationary cutting blade and two revolving impellers. Constant rotation of the impellers created a synchronized flow of ginger rhizomes inside the splitting chamber. The centrifugal force of the impellers due to their rotation against the stationary knife blade accomplishes the splitting process. Chatthong et al. (2011) similarly designed and built a semi-automatic ginger slicing machine. The machine produce sheet ginger and line ginger.

Polishing: Polishing of dried ginger is done to remove the wrinkles developed during drying process. In the indigenous method the dried ginger is rubbed against a hard surface. However, hand or power operated polishers similar to turmeric polishers are also employed for the purpose of polishing dried ginger. In the case of hand operated polishers an output of 5-6 quintals per day of 8 hours is obtained with the help of two persons. The dried ginger rhizomes are manually graded. The machines of various capacities to pulverize dried ginger from 25 kg per batch to continuous powdering of 2-3 t/ day for large scale production are available.

Dried ginger: Drying of ginger basically involves two stages; peeling the rhizomes to remove the outer skin and sun or mechanical drying to a safe moisture level (Balakrishnan, 2005). For converting to dry ginger the crop is harvested at full maturity. In most growing areas, the scraped ginger is dried in the sun but where unfavourable seasonal conditions prevail, improved drying methods using mechanical or solar dryers are also used. In mechanical dryers, 57.2ºC is reported to be the highest temperature at which ginger for the spice market could be dehydrated (Ravindran and Nirmal, 2005). Singh et al, 2008 studied the fluidized bed drying of ginger flakes where the ginger were manually cleaned, steeped in portable water for 2 hours followed by soaking in calcium oxide solution (1%, 2% and 2.5%) for 6 hours.

The quality of dried ginger depends on appearance, pungent principles, fibre, aroma and flavor characteristic of volatile oil (Ebewele and Jimoh 1988). Alakali and Satimehin (2009) studied drying kinetics of ginger slices at varying temperature of 40, 45, 50 and 55^0C and found that the drying proceeded faster at high temperatures; while according to Azain *et al.* (2004) the low temperature drying usually preserves the active components in ginger. Eze and Agbo (2011) also recommended the drying temperature of the inner chamber of solar drier not more than 40ºC, as high temperature denatures the protein and alters the organoleptic properties of ginger. The dried powdered ginger reported to contain 9-12% moisture, 3.6% fatty oil, 9% proteins, 60-70% carbohydrates, 3.8% crude fibre, 8.0% ash as compare to fresh ginger which contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals and 2.4% fibre (Panpatil et al.2013).

Ginger Powder: Ginger powder is made by pulverizing the dry ginger to a mesh size of 50 to 60 (Ravindran and Nirmal 2005). Ginger powder forms an important component in curry powder. It also finds direct application in a variety of food products (Balakrishnan 2005). Pulverization is a physical unit operation whose phenomenon involves size reduction or crushing of the cells and separation of granules

and is generally a limiting unit operation in ginger processing (Earle 2003). Aderemi et al., (2009) designed and built a medium scale ginger pulverizer. There are two major methods of crushing as traditional and modern methods.

Candies and preserves: Chinese ginger has been the standard for ginger preserved in syrup. Australia has also developed a ginger industry and it exports mostly candied rhizomes with superior and consistent quality (ASTA 2002; Weis 1997).

Salted ginger*:* Fresh ginger (with relatively low fibre) harvested at 170 -180 days after planting can be used for preparing salted ginger. Tender rhizomes with portion of the pseudo stem is washed thoroughly and soaked in 30% salt solution containing 1 % citric acid. After 14 days it is ready for use and can be stored under refrigeration.

Ginger oil and oleoresin: The commercial ginger essential oil is characterized by pale yellow colour, warm, spicy and woody flower with slight lemony notes (Koroch *et al.* 2007). The yield of ginger oil depends upon the cultivar, stage of harvesting and method of extraction etc. On an average the ginger oil content of different cultivar of ginger ranged between 0.85 to 2.00 per cent on dry weight basis (Noor *et al.* 2004; Sultan *et al.* 2005; Sasidharan and Menon, 2010). Dry ginger on distillation yields 1.5 to 2.5% volatile oil. The main constituent in the oil is zingiberene and contributes to the aroma of the oil.

*Solvent extraction of ginger oil and oleoresin***:** Oil extraction is the process of recovering oil from oil-bearing agricultural products through manual, mechanical, or chemical extraction. Extraction of oil from oil-bearing products could be done in two major ways i.e; traditional and slicing/splitting and drying on the organoleptic attributes and nutritional value of ginger (Ibrahim and Onwualu, 2005). The traditional method is usually a manual process and involves preliminary processing and hand pressing. The improved method consists of chemical extraction and mechanical expression. Essential oil is obtained by steam distillation, while oleoresins are obtained by solvent extraction. Oils and oleoresins are preferred to dried spices as flavoring by the food industry, because they are more stable, cleaner, free from contaminations, and can be standardized by blending oils from different sources (Peter and Zachariah 2000). Essential oils are also used in the manufacture of soft drinks, ginger beer, and in food preparation.

NOVEL PRODUCTS FROM GINGER

Ginger leather/bars: Commercially known as fruit rolls are prepared by dehydrating fruit puree into leathery sheets. It is made by removing the moisture of the fruit puree, using a large flat tray, until the desired cohesive 'leather' is obtained. Fruit leather generally lasts quite a long time and does not require refrigeration (Naz, 2012). Common drying methods used for drying leather are oven-drying (including convection fan forced), sun-drying and electric cabinet drying where cabinet dried leather were found more acceptable (Che Man and Sin, 1997). Dehydrator drying takes approximately four to twelve hours, but the time ultimately depends on the type of raw material, humidity in the room and thickness of the fruit puree. According to Dhiman (2015), a good quality plum ginger leather can be prepared in proportion of 60:40, and 50:50 (plum: ginger) with a recovery of 27.10 to 28.76 per cent with drying time, dehydration ratio, total solids and moisture content of 8.00 to 9.30 hours, 3.48 to 3.69, 86.07 to 86.96 per cent and 13.04 to 13.93 per cent respectively.

Ginger Aroma and juice: The aroma of ginger is pleasant and spicy and its flavour penetrating and biting due to presence of antiseptic or pungent compounds. The aroma and juice can be utilized for the manufacture of a number of food products like ginger bread, confectionery, ginger ale, curry powders, certain curried meats, table sauces, in pickling and in the manufacture of certain soft drinks like cordials, ginger cocktail, carbonated drinks, bitters etc.

Ginger appetized flakes: Kaushal et al. (2016), prepared osmotically dried appetized ginger flakes by using lime juice blanching, followed by dipping in 70 per cent sugar syrup for 4 hrs and rolling of flakes in 2.0% appetizing mixture. The recovery of osmotically dried ginger flakes ranged between 54.14 to 55.83 per cent with drying hours and drying ratio of 4.20 to 4.25 and 1.79: 1 to 1.84: 1 respectively.

Ginger paste: Ginger paste was prepared from fresh ginger by Ahmed (2004) by addition of 8% common salt and citric acid to adjust the pH from 6.38 to less than 4.6, thermally processing at 80ºC for 15 minutes and packing in polyethylene terephthalate or glass containers followed by storing at 5±1ºC. In India, ginger paste is traditionally made with 50% sliced and macerated ginger, 35% garlic and 15% salt (Pruthi 1992).

Crude fibre*:* In fully matured ginger crude fibre varies from 3-8%. It is estimated by acid and alkali digestion of ginger powder and whatever remains is considered as fibre.

Ginger starch: Pure starch is a white, tasteless and odourless powder containing 20-25% amylase and 75-80% amylopectin which is widely used bio-materials in food, textile, cosmetics, plastic, paper and pharmaceutical industries. Thus, ginger is also considered one of the potential source of industrial starch (Kolawole et al. 2013)

and was found to be white, tasteless with no smell just like maize starch. The percent solubility of ginger starch was calculated as 0.9 percent as against 1.2 percent for maize starch. The starch can be obtained from ginger rhizomes after washing and peeling, the grating is intermittently done to prevent the starch from heating up due to heat from grater. The content is then settled for 2hrs followed by decantation of yellowish supernatant. Series of re-dispersion and decanting helps in removing impurities and the process results in good quality starch.

Other products: Sweet and salty products can be prepared from fresh ginger like ginger candy, ginger paste, salted ginger, crystallized ginger etc.

III. CONCLUSION

Ginger is a spice of commercial and medicinal importance. It is valued because of its aroma and pungency and has been used through ages in almost all systems of medicine against many maladies due to its medicinal properties. Despite of its use as flavoring agent, ginger is also appreciated in ayurvedic, allopathic aromapethy and household medicines. Ginger can be used in the form of fresh paste, ginger tea, dried powder, preserved slices, cookies, candy, teas, tinctures, sodas, jam, beer, capsule and syrup etc. Soft drinks like cordials, ginger cocktail, carbonated drinks, etc are some of the value added products of ginger. Ginger is also used for the extraction of ginger oil, oleoresin, essences, tinctures etc. Ginger has a number of chemical constituents like [6]-Gingerol, [6] -Shagol, Methyl [6] – isogingerol, Paradol which are responsible to provide different pharmacological actions like Cardio protective activity, anti-inflammatory activity, anti-microbial activity, antioxidant property, neuro-protective activity and hepato-protective activities which have been proved. The novel products like ginger bars/rolls, appetized flakes, essential oils & aroma, juice, paste and starch alongwith ginger candy, salted ginger, crystallized ginger etc. discussed during this review would add value to this amazing crop.

REFERENCES

[1] Abeysekera WKSM, Illeperuma CK, Amunugoda PNRJ, Wijeratnam S. (2005). Comparison of ginger varieties dried at different temperatures for oil and oleoresin contents. *Srilankan J Agric Sci*. 42: 34-42

[2] Aderemi BO, Ndirika O, Yerima Y. (2009). Design and fabrication of a medium scale ginger pulverizing machine for rural dwellers. *Aus J Basic Appl Sci*. 3(2): 389-394

[3] Agrawal YC, Hiran A, Galundia AS. (1987). Ginger peeling machine parameters. *Agric Mech Asia Africa Latin Amer*. 18(2): 59-62

[4] Aggarwal BB, Shishodia S. (2006). Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem Pharmacol*. 71: 1397-1421.

[5] Ahmed J. (2004). Rheological behaviour and colour changes of ginger paste during storage. *Int J Food Sci Technol*. 39: 325-330

[6] Ahmed R, Sharma S. (1997). Biochemical studies on combined effect of garlic (*Allium sativum* L.) and ginger (*Zingiber officinalis*) in albino rats. *Ind J Experimental Biol, 3(5)*: 841-843.

[7] Akande EA, Adeyanju JA, Oladipo OA, Oyetunji FF. (2014). Effects of different packaging materials on the shelf stability of ginger juice. *Greener* Int *J Sci, Engineering Technol Res*. 4(2): 38-44

[8] Akhtar A, Dash PK, Mannan A. (2013). Physico-chemical characterization and product development from ginger (*Zingiber officinale*). Germplasm available in South Western Region of Bangladesh. *IOSR J Agric Vet Sci*. **5**: 53-62

[9] Alakali JS, Satimehin AA. (2009). Moisture adsorption characteristics of ginger *(Zingiber officinale)* powders. Agricl Engineering Int. 6: 1286-1291.

[10] Alam P. (2013). Densitometric HPTLC analysis of 8-gingerol in *Zingiber officinale* extract and ginger-containing dietary supplements, teas and commercial creams. *Asian Pacific J Trop Biomedicine*. 3(8):634–638.

[11] Ali Y, Jain GC, Kapdi SS, Agrawal YC, Bhatnagar S. (1991). Development of brush type ginger peeling machine. *Agric Mech Asia Africa Latin Amer*. 22: 71-73

[12] Ali BH, Blunden G, Tanira MO, Nemmar A. (2008). Some phyto-chemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food Chem Toxicology. 46(2):409-420.

[13] Altman RD, Macrcussen KC. (2001). Effects of a ginger extract on knee pain in patients with osteoarthritis. *J Phytomedicine*. 44: 2531-2538

[14] Aruoma OI, Spencer JP, Warren D, Jenner P, Butler J, Halliwell B. (1997). Characterization of food antioxidants, illustrated using commercial garlic and ginger preparations. *Food Chem, 6:* 49–156.

[15] Arya PS. (2001). Ginger Production Technology. Kalyani Publishers New Delhi. 169p.

[16] ASTA. (2002). A concise guide to Spices, Herbs, Seeds, and Extractives. *Amer Spice Trade Assoc*. Magra-Hill Pub. Co. Ltd., New York, p. 51.

[17] Awang DVC. (1992). Ginger. *Canad Pharma J*. 125:309-311.

[18] Azain MN, Kamal AAF, Azlina MN. (2004). Changes of cell structure in ginger during processing. *J Food Engineering*. 62:359-364

[19] Balakrishnan KV. (2005). Postharvest and industrial processing of ginger In: Ravindran PN, NirmalBabu K (eds.) Ginger-The genus *Zingiber*. CRC Press, Florida. pp. 391-434.

[20] Bartley J, Jacobs A. (2000). Effects of drying on flavour compounds in Australian-grown ginger (*Zingiber officinalis*). *J Sci Food Agric. 80(2):* 209-215.

[21] Bhuyan S, Prasad S. (1990). Drying characteristics of ginger and development of a small capacity dryer. In: Y. Zeren, Y. Yildiz, M.T. Ozcan, E. Guzel, A. Isik (eds), Proceedings of the 4th International Congress on Agricultural Mechanization & Energy, Adana. Turkey. pp. 501-509.

[22] Bisset NG, Wichtl M. (1994). Herbal Drugs and Phytopharmaceuticals- A handbook for practice on a scientific basis. Wichtl M, Bisset NG (eds.), Boca Raton, FL: CRC Press.

[23] Bliddal H, Rosetzsky A, Schlichting P, Weidner MS, Andersen LA, Ibfelt HH, Christensen K, Jensen ON, Barslev J. (2000). A randomized, placebo-controlled, cross-over study of ginger extracts and ibuprofen in osteoarthritis. *Osteoarthritis Cartilage*. 8(1): 9-12.

[24] Bolanle AO. (2011). Effect of ginger powder (*Zingiber officinale*) on plasma lipid profile and liver enzyme activities of hypercholesterolemic rats. *J Life Sci*. 5:712-716.

[25] Bryer E. (2005). A literature review of the effectiveness of ginger in alleviating mild-to-moderate nausea and vomiting of pregnancy. *J Midwifery Women's Health*. 50(1): e1-e3.

[26] Camacho EH, Brescia A. (2009). The Australian ginger industry: Overview of Market Trends and Opportunities. Ginger: The Genus *Zingiber*. CRC Press, Florida, U.S.A. *http://www.daff.qld.gov.au/data/assets*.

[27] Cao ZF, Chen ZG, Guo P, Zhang SM, Lian LX, Luo L, Hu WM. (1993). Scavenging effects of ginger on superoxide anion and hydroxyl radical. *Chung-Kuo Chung Yao Tsa Chih*. 18:750–764.

[28] Chatthong B, Sarazin Y, Onjun T. (2011) Analytical and Numerical Modelling of Transport Barrier Formation Using Bifurcation Concept. *38th EPS Conference on Plasma Physics (Strasbourg, France)*, Paper P4.097.

[29] Che Man YB, Sin KK. (1997). Processing and consumer acceptance of fruit leather from the unfertilised floral parts of jackfruit. *J Sci Food Agric*. 75(1):102–108.

[30] Chen IN, Chang CC, Ng CC, Wang CY, Shyu YT, Chang TL. (2008). Antioxidant and antimicrobial activity of zingiberaceous plants in Taiwan. *Plants Foods Hum Nutr*. 63:15-20.

[31] Dhiman A. (2015). Development and evaluation of novel products from ginger *(Zingiber officinale)*. M.Sc. Thesis. p114.

[32] Dhingra S, Kumar A. (2005). Post harvest technology of ginger. *Ind CorxArecanut Spice J*. **14**(1): 13-15

[33] Dohroo NP, Sharma IL. (1986). Evaluation of fungicides for the control of rhizome rot of ginger in storage. *Indian Phytopath*. 36:691-693

[34] Earle RL. (2003). Size reduction. In: Unit Operations in Food Processing. www.nzifst/org.nz/unit operations/size reduction.

[35] Ebewele RO, Jimoh AA. (1988). Feasibility study of kaduna state ginger processing industry. Ahmadu Bello University Chemical Engineering consultant, pp. 63-80

[36] Ehsanullah Raheem MI, Ahmad T, Hayat S. (2013). Effect of potassium metabisulphite and salt solution on ginger puree and its storage. *J Agric Res*. **51**(3): 297-305

[37] El-Baroty GSHH, Farag RS, Saleh MA. (2010). Characterization of antioxidant & antimicrobial compounds of cinnamon and ginger essential oils. *African J Biochem Res*. **4**(6): 167-174

[38] Elaissi A, Salah K, Mabrouk S, Larbi K, Chemli R. (2011). Antibacterial activity and chemical composition of 20 *Eucalyptus* species essential oils. *Food Chem*. 129: 1427-1434

[39] EL-Ghorab AH, Nauman M, Anjum FM, Hussain S, Nadeem MA. (2010). Comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). *J Agric Food Chem*. **58**: 8231-8237

[40] Endrais G, Asfaw K. (2011). Production, processing and marketing of ginger in Southern Ethiopia. *J Horti Forestry*. 3(7):207-213.

[41] Eze JI, Agbo KE. (2011). Comparative studies of sun and solar drying of peeled and unpeeled ginger. *Amer J Scientific Indl Res*. **2**(2): 136-143

[42] FAO (2014). Food and Agriculture Organization, Rome. *www.fao.org.in*. Accession date: (15th Jan, 2014)

[43] Fessenden JM, Wittenborn W, Clarke L. (2001). Gingko biloba: A case report of herbal medicine and bleeding postoperatively from a laparoscopic cholecystec-tomy. *Am Surg*. 67(1): 33-35.

[44] Ficker CE, Arnason JT, Vindas PS, Alvarez LP, Akpagana K, Smith Ml. (2003). Inhibition of human pathogenic fungi by ethno-botanically selected plant extracts. *Mycoses*. 46 (1-2): 29-

[45] Ghayur MN, Gilani AH, Afridi MB, Houghton PJ. (2005). Cardiovascular effects of ginger aqueous extract and its phenolic constituents are mediated through multiple pathways. *Vasc Pharm*. 43(4): 234-241.

[46] Ghosh AK, Banerjee S, Mullick HI, Banerjee J. (2011). *Zingiber Officinale*: a natural gold. *Int*

[47] *J Pharma Bio Sci*. 2(1): 283-294.

[48] Giriraju A, Yunus GY. (2013). Assessment of antimicrobial potential of 10% ginger extract against *Streptococcus mutans*, *Candida albicans* and *Enterococcus faecalis*: an in-vitro study. *Indian J Dent Res*. 24: 397-400.

[49] Gopalan C, Ramasastri BV, Balasubramanium SC. (2004) Nutritive value of Indian foods. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad. p 48–50

[50] Govindarajan VS. (1982). Ginger: Chemistry, technology and quality evaluation. *Crit Rev Food Sci Nutr*. **7**: 1-96

[51] Grant KL, Lutz RB. (2000). Alternative therapies: ginger. *Amer J Health-System Pharm.* **57**: 945–947

[52] Grzanna R, Lindmark L, Frondoza C. (2005). Ginger-a herbal medicinal product with broad anti-inflammatory actions. *J Medicine Food*. *8(2):* 125-132.

[53] Guwo AN. (2008). Development of a ginger splitting machine. M.Sc. Thesis. Department of Agric. Engineering, ABU, Nigeria.

[54] Gupta R, Singh B, Shivhare S. (2012). Optimization of osmo-convective dehydration process for the development of honey-ginger candy using response surface methodology. *Drying Technol*. **30**: 750-759

[55] Hasan HA, Rasheed Raauf AM, Abd Razik BM, Rasool Hassan BA. (2012). Chemical composition and antimicrobial activity of the crude extracts isolated from *Zingiber officinale* by different solvents. *Pharmaceut Anal Acta*. 3: 184

[56] Hasnat M, Rajib B, Hossain MA, Anam MM, Kabir H. (2014). Effect of chemicals, bio-agent, plant extract and soil amendments in controlling rhizome rot of ginger. *Int J Nat Soc Sci.* **1**: 1–11.

[57] Hossain MA, Shah MD, Sang SV, Sakari M. (2011). Chemical composition and antibacterial properties of the essential oils and crude extracts of Merremia borneesis. *J King Saud Univ-Sci*. 24: 243-249

[58] Ibrahim I, Onwualu AP. (2005). Technologies for extraction from oil-bearing agricultural products: A review. *J Agricl Engineering Technol*. 13: 58-70.

[59] Islam K, Rowsni AA, Khan M, Kabir MS. (2014). Antimicrobial activity of ginger (*Zingiber officinale*) extracts against food-borne pathogenic bacteria. *Int J Sci, Environment Technol*. 3(3): 867–871.

[60] ITC. (2002). International Trade Center UNCTAD/WTO. Global Spice Market: Imports-1996-2000. Geneva: ITC, 2002. xxii, 80 p.

[61] Jadhav MS, Nimbalkar CA, Kanawade VL. (2012). Drying behaviour of ginger (cv Mahim). *Bioinfolet.* **9**: 778-783

[62] Jayashree E, Visvanathan. (2011). Physical and biochemical parameters of fresh and dry ginger (*Zingiber officinale* Roscoe). *J Spices Arom Crops*. **20**(1): 14-21

[63] Jayashree E, Kandiannan K, Prasath D, Rashid P, Sasikumar B, Kumar SCM, Srinivasan V, Susheela BR, Thankamani CK. (2014). Ginger (Extension Pamphlet). ICAR- Indian Institute of spice Research, Kozhikode, Kerala. pp. 1-10

[64] Jayashree E, Visvanathan R. (2013). Development of hand operated diamond cut mesh drum abrasive ginger peeler. J Spices Arom Crops. 22(2): 174-180.

[65] Joshi AK, Rathore JS, Ali Y. (1991). Economoic analysis of ginger processing technology for rural areas. *Bev Food World*. **18**(2): 27-29

[66] Kaushal M, Kumar N, Vaidya D. (2014). Processing quality of ginger (*Zingiber officinale*) land races in Himachal Pradesh. *Int J Farm Sci*. 4(3): 110-116

[67] Kaushal M, Dhiman A, Gupta A, Vaidya D. (2016). Effect of blanching on quality characteristics of osmotically dried and appetized ginger flakes. *Ind J Natural Prod Res* (In press) MS No-2349-1296.

[68] Kirtiraj K, Gaikwad, Singh S, Shakya BR. (2013). Studies on the development and shelf life of low calorie herbal aonla-ginger RTS beverage by using artificial sweeteners. *J Food Proc Technol*. 4(1):2

[69] Kizhakkayil J, Sasikumar B. 2009.Variabilty for quality traits in a global germplasm collection of

ginger (*Zingiber officinale* R.). *Curr Trends Biotechnol Phar*. **3**(3): 254 -259

[70] Koroch A, Ranarivelo L, Behra O, Juliani HR, Simon JE. (2007). Quality attributes of ginger and cinnamon essential oils from Madagascar. *Botanicals Med*. **5**: 25-38

[71] Kolawole SA, Igwemmar NC, Bello HA. (2013). Comparison of the physicochemical properties of starch from ginger (*Zingiber officinale*) and Maize (*Zea mays*). *Int J Sci Res*. 71-76.

[72] Krishnakantha TP, Lokesh BR. (1993). Scavenging of superoxide anions by spice principles. *Ind J Biochem Biophys*. 30:133–134.

[73] Kubra IR, Rao LJM. (2012). An impression on current developments in the technology, chemistry, and biological activities of ginger (*Zingiber officinale* Roscoe). *Crit Rev Food Sci Nutr*. 52(8):651–688.

[74] Langner E, Greifenberg S, Gruenwald J. (1998). Ginger: history and use. *Adv Therapy*. 15(1):25–44.

[75] Latona DF, Oyeleke GO, Olayiwada OA. (2012). Chemical analysis of ginger root. IOSR *J Applied Chem*. 1(1): 47-49.

[76] Ling H, Yang H, Tan SH, Chui WK, Chew EH. (2010). 6-shogaol, an active constituent of ginger, inhibits breast cancer cell invasion by reducing matrix metalloproteinase-9 expression via blockade of nuclear factor-κb activation. *British J Pharma*, 161(8), 1763-1777.

[77] Liu Y, Whelan RJ, Pattnaik BR, Ludwig K, Subudhi E, Rowland H, Claussen N, Zucker N, Uppal S, Kushner DM, Felder M, Patankar MS, Kapur A. (2012). Terpenoids from *Zingiber officinale* (Ginger) induce apoptosis in endometrial cancer cells through the activation of p53. PLoS One. 7(12): e53178 (1-10).

[78] Mallorea R, Leon De SY, Lim-sylianco CY. (1992). Free glutamic acid in some Philippine fruits and vegetables. *Asean Food J*. **7**(2): 100-110

[79] Maizura M, Aminath A, Wan AWM. (2011). Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) extract. *Int Food Res J*. **18**: 529-534

[80] Meadows AB. (1998). Ginger processing for food and industry. In: Proceedings of first National ginger workshop. Umudike, Nigeria, pp. 34-42.

[81] Maxwell I. (2008). Let's make ginger beer. Dave's Garden.

[82] Minaiyan M, Ghannadi A, Karimzadeh A. (2006). Anti-ulcerogenic effect of ginger (rhizome of *Zingiber officinale* roscoe) on cystemine induced duodenal ulcer in rats. *DARU J Pharma Sci*. 14(2): 97-101.

[83] Miyoshi N, Nakamura Y, Ueda Y, Abe M, Ozawa Y, Uchida K and Osawa T. (2003). Dietary ginger constituents, galanals A and B are potent apoptosis inducers in Human T lymphoma Jurkat cells. *Cancer Lett*. 199: 113-119.

[84] Michiein EM, Salvador AA, Reihi CA, Smania AJr, Smania EF. (2009). Chemical composition and antibacterial activity of *Cordia veribenacea* extracts obtained by different methods. *Bioresource Tech*. 100: 6615-6623.

[85] Nath A, Bidyut C, Deka AK, Paul JD, Misra LK. (2013). Effect of slice thickness and blanching time on different quality attributes of instant ginger candy. *J Food Sci Technol*. **50**(1): 197-202

[86] Naz R. (2012). Physical properties, sensory attributes and consumer preference of fruit leather. *Pak J Food Sci*. 22(4): 188-190.

[87] Nicoll R, Henein MY. (2009). Ginger (*Zingiber officinale* roscoe): A hot remedy for cardiovascular disease? *Int J Cardiol*. 131(3):408-409.

[88] Noor AM, Mustafa K, Azlina MN. (2004). Changes of cell structure in ginger during processing. *Food Engineering*. **62**(4): 359-364

[89] Nwaoha M, Elizabeth I, Okafor, Gabriel I, Apochi, Veronica O. (2013). Production of oleoresin from ginger (*Zingiber officinale*) peels and evaluation of its antimicrobial and antioxidative properties. *Afr J Microbiol Res*. **7**(42): 4981-4989

[90] Nwinuka N, Ibeh G, Ekeke G. (2005). Proximate composition and levels of some toxicants in four commonly consumed spices. *J Appl Sci Environ Mgt*. 9(1): 150-155.

[91] Nurtjahja-Tjendraputra E, Ammit AJ, Roufogalis BD, Tran VH, Duke CC. (2003). Effective anti-platelet and cox-1 enzyme inhibitors from pungent constituents of ginger. *Thromb Res*. 111(4): 259-265.

[92] Offei-Okyne R, Patterson J, Walker LT, Verghese M. (2015). Processing effects on phyto-chemical content and antioxidative potential of ginger. *Food Nutr Sci*. **6**: 445-451.

[93] Okolo SC, Olajide Olutayo O, Idowu DI, Adebiyi AB, Ikokoh PP, Orishadipe AT. (2012). Comparative proximate studies on some Nigerian food supplements. *Ann Biological Res*. **3**(2):773-779

[94] Onu LT, Okafor G. (2002). Effect of physical and chemical factor variations on the efficiency of

mechanical slicing of Nigerian ginger (*Zingiber officinale* Rosc.). *J Food Engineering*. **63**: 299-302

[95] Onwuka GI, Nwokoha GU, Asumugha GN (2002). Evaluation of processing methods for the production of ginger drinks. Proceedings of 26th Annual NIFST Conference, Owerri.

[96] Overy DP, Frisvad JC. (2005). Mycotoxin Production and Postharvest Storage Rot of Ginger (*Zingiber officinale*) by *Penicillum brevibacterium*. *J Food Prot*. 68 (3): 607-609

[97] Panhwar F. (2005). Ginger (*Zingiber officinale*) cultivation in Sindh Pakistan. Digitalverlag Gmbh Publishing, Germany, p1-5.

[98] Panpatil VV, Tattari S, Kota N, Nimgulkar C, Polasa K. (2013). *In vitro* evaluation on antioxidant and antimicrobial activity of spice extracts of ginger, turmeric and garlic. *J Pharmacognosy Phytochem*. 2 (3): 143-148

[99] Paull RE, Chen NJ, Goo TTC. (1988). Compositional changes in ginger rhizomes during storage. *J Amer Soc Hortic Sci*. 113:584-588.

[100] Paull RE, Chen CC. (2015). Ginger postharvest quality-maintenance guidelines. *Vegetable Root Crops J*. UH–CTAHR VC-2:1-3.

[101] Peter KV, Zachariah TJ. (2000). Spice oils and oleoresins: Challenges and opportunities. *J Medicinal Arom Plant Sci*. 22 (1):247-252.

[102] Phoungchandang S, Sertwasana A. (2010). Spray-drying of ginger-juice and physico-chemical properties of ginger powders. *Sci Asia*. **36**: 40-45

[103] Prabhakaran KP. (2013).The agronomy and economy of turmeric and ginger [electronic resource] : the invaluable medicinal spice crops . Burlington : Elsevier Science, p 537.

[104] Pruthi JS. (1992). Postharvest technology of spices: pre-treatments, curing, cleaning, grading and packing. *J Spices Arom Crops*. 1 (1):1-29.

[105] Pruthi, J.S. 1998. Spices and condiments. National Book Trust (5th Ed.) New Delhi, India. p 1-325.

[106] Puengphian C, Sirichote A. (2008). [6]-gingerol content and bioactive properties of ginger (*Zingiber officinale* Roscoe) extracts from supercritical CO_2 extraction. *Asian J Food Agro Industry*. **1**(10): 29-36

[107] Purnomo H, Jaya F, Widjanarko SB. (2010). The effects of type and time of thermal processing on ginger (*Zingiber officinale* Roscoe) rhizome antioxidant compounds and its quality. *Int Food Res J*. **17**: 335- 347

[108] Purseglove JW, Brown EG, Green CL, Robbins SRJ. (1981). Ginger. **In**: Spices. Vol. II. Longman, New York. pp. 447-531

[109] Qidwai W, Alim SR, Dhanani RH, Jehangir S, Nasrullah A, Raza A. (2003). Use of folk remedies among patients in Karachi Pakistan. J Ayub Medical College Abbottabad. 15(2):31-33.

[110] Rahman MJ, Talukder MAI, Rani L, Saha KC, Nahid MSI. (2013). The effect of processing techniques on the shelf life, nutritional and sensory quality of ginger (*Zingiber officinale*) powder and paste. *J Innov Develop Strat*. **7**(3): 60-66

[111] Ravindran PN, Nirmal-Babu K. (2005). Introduction. In: *Ginger:* the genus *Zingiber*. (Eds.): P.N. Ravinderan and B.K. Nirmal. CRC Press, New York, pp 1-14.

[112] Reddy AA, Lokesh BR. (1992). Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol Cell Biochem*. 111:117–124.

[113] Safaa YQ, Ahmed N, Abo-Khatwa, Mona ABL. (2010). Screening of antioxidant activity and phenolic content of selected food items cited in the Holy Quran. *J Biol Sci*. 2(1):40-51.

[114] Sagar SD. (2006). Investigations on the etiology, epidemiology and integrated management of rhizome rot complex of ginger and turmeric. Ph.D. Thesis, Department of Plant Pathology, University of Agricultural Sciences, Dharwad- 580005.

[115] San Chang J, Wang KC, Yeh CF, Shieh DE, Chiang LC. (2013). Fresh ginger (*Zingiber officinale*) has anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines. *J Ethnopharmacology*. 145(1):146-151.

[116] Sasidharan I, Menon AN. (2010). Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (*Zingiber officinale Roscoe*). *Int J Curr Pharma Res*. 2(4):40–43.

[117] Sebioma A, Awofodu AD, Awosanya AO, Awotona FE, Ajayi AJ. (2011). Comparative studies of antibacterial effect of some antibiotics and ginger (*Zingiber officinale*) on two pathogenic bacteria. *J Microbiol Antimicrobials*. 3:18-22.

[118] Shahid M, Hussain F. (2012). Chemical composition and mineral content of *Zingiber officinale* and *Alpinia allughas* (Zingiberaceae) rhizomes. *Int J Chem Biochem Sci*. **2**: 101-104

[119] Sharma SK, Dohroo NP. (1991). Postharvest management of rhizome rot (*Fusarium oxysporum* f.sp. *zingiberi* trujillo) of ginger through chemical

and antagonist. *Ind Cocoa, Areca Spices J*. 14: 150-152.

[120] Shelly TE, Mcinnis DO, Pahio E, Edu J. (2004). Aromatherapy in the Mediterranean fruit fly (diptera: Tephritidae): Sterile males exposed to ginger root oil in prerelease storage boxes display increased mating competitiveness in field-cage trials. *J Economic Entomology*. 97(3): 846-853.

[121] Shanmugam KR, Mallikarjuna K, Kesireddy N, Reddy KS. (2011). Neuroprotective effect of ginger on anti-oxidant enzymes in streptozotocin-induced diabetic rats. *Food Chem Toxicology*. 49(4): 893-897.

[122] Shirin APR, Prakash. (2010). Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). *J Medicinal Plants Res*. **4**(24): 2674-267

[123] Shirshir Md RI, Hossain M, Hossain Md. M. (2012). Processing of ginger powder. *Bangladesh Res Pub J*. 7(3): 277-282.

[124] Shukla Y, Singh M. (2007). Cancer preventive properties of ginger: a brief review. *Food Chemical Toxicol*. 45(5): 683-690.

[125] Simonyan KJ, Jegede KM, Lyocks SW. (2003). Development of a motorized Ginger Slicer. *Agric Mech Asia, Africa Latin Amer*. 34 (1): 37-41.

[126] Singh K, Bawa AS, Ahmed J. (2001). Dehydration of ginger slices. *J Scientific Industrial Res.* 60:510-512

[127] Singh G, Kapoor IP, Singh SP, Heluani CS, Lampasona MP, Catalan CA. (2008). Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinalis*. *Food Chem Toxicol. 4(6)*: 3295-302.

[128] Siddiqui AA, Bhuiyan MHR, Easdani M. (2012). Ginger (*Zingiber officinale*) preserve and candy development. *Bangladesh Res Publ J*. **7**(3): 283-290

[129] Sloand ED, Vessey JA. (2001). Self-medication with common household medicines by young adolescents. *Compr Pediatr Nurs*. 24(1): 57-67.

[130] Srivastava KC, Mustafa T. (1989). Ginger (*Zingiber officinale*) and rheumatic disorders. *Med Hypotheses*. 29(1): 25-28.

[131] Sultan MHN, Bhatti, Iqbal Z. (2005). Chemical analysis of essential oil of ginger (*Zingiber officinale*). *Pak J Biol Sci*. **8**(11): 1576-1578

[132] Tan BKH, Vanitha J. (2004). Immunomodulatory and antibacterial effects of some traditional Chinese medicinal herbs: a review. *Curr Med Chem*. 11(11):1423-1430.

[133] Tanveer S, Shahzad A, Ahmed W. (2014).Compositional and mineral profiling of *Zingiber officinale*. *Pak J Food Sci*. **24**(1): 21-26

[134] Tepe B, Sokmen M, Akpulat HA, Sokmen A. (2006). Screening of the antioxidant potentials of six Salvia species from Turkey. *Food Chem. 9(5):* 200–204.

[135] Thakore BBL, Mathur S, Singh RB. (1988). Effect of rhizome treatment with fungicides for economic control of rot. *J Phytol Res.*1: 83-84.

[136] Tjendraputra E, Tran VH, Biu-Brennan D, Roufogalis BD, Duke CC. (2001). Effect of ginger constituents and synthetic analogues on cyclooxygenase-2 enzyme in intact cells. *Bioorg Chem*. 29: 156-163.

[137] Vats V, Grover JK, Rathi SS. (2002). Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* linn, *Ocimum sanctum* linn and *Pterocarpus marsupium* linn in normal and alloxanized diabetic rats. *J Ethnopharmacol*. 79(1): 95-100.

[138] Virendra V, Tattari S, Kota N, Nimgulkar C, Polasa K. (2013). In vitro evaluation on antioxidant and antimicrobial activity of spice extracts of ginger, turmeric and garlic. *J Pharmacognosy Phytochem*. **2**(3): 143-148

[139] Wang X, Zheng ZJ, Guo XF, Yuan JP, Zheng CC. (2011). Preparative separation of gingerols from *Zingiber officinale* by high-speed counter-current chromatography using stepwise elution. *Food Chem*. **125**: 1476–1480

[140] Weiss EA. (1997). Essential Oil Crops. CAB International publishing, Oxon, UK.

[141] Yiljep Y, Fumen G, Ajisegiri E. (2005). The Effects of peeling, splitting and drying on ginger quality. Agricultural Engineering International: *CIGR E J*. Manuscript FP 05 009, 7.

[142] Zick SM, Djuric Z, Ruffin MT, Litzinger AJ, Normolle DP, Alrawi S, Feng MR, Brenner DE. (2008). Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev*. 17:1930-1936.

Table.1: Mode of action of active constituents present in ginger.

Active constituents	**Biological action**	**Mode of action**	**References**
Gingerols, shogaols, Sesquit-erpenes and monoterpenes)	For the treatment of nausea and vomiting	By anti-cholinergic and anti-serotonin action	Bryer, 2005
Ethanolic extract of ginger	Hypolipidimic agent	By reducing triglycerides and LDL cholesterol and to increase HDL	Bolanle, 2011
Anticancer activities (e.g. breast cancer)	inhibition of cell invasion	reduction of matrix metalloproteinase-9 expression	Ling *et al.*, 2010
Zingiberone and ethanolic extract of ginger	Anti-hyperglycemic effect	Lowering of blood glucose level by inhibition of oxidative stress and anti-inflammatory process, increase insulin sensitivity.	Vats *et al.*, 2002; Shanmugam *et al.*, 2011
Phenolic and favonoids compounds	Neuroprotector effect	by accelerating brain anti-oxidant defense mechanisms	Shanmugam *et al.*, 2011
Ginger extract (highly purified and standardized)	Treatment of osteoarthritis of the knee joints	By reduction of inflammatory mediators.	Bliddal *et al.*, 2000; Altman and Marcussen, 2001
[6]-gingerol and [6]-shogaol	anti-ulcerative effects Hypotensive effects	By Suppressing the gastric contrac-tion, increasing mucin secretion lowering blood pressure by inhibition of voltage-dependent calcium channels as well as by stimulating muscarinic receptors	Minaiyan *et al.*, 2006 Ghayur *et al.*, 2005; Nicoll and Henein, 2009
Sesquiterpenes (B-Sesquiphellandrene	Anti-viral effect		San Chang *et al.*, 2013
Gingerol and shogaol	Antiplatelet activity	lower platelet thromboxane X2 and prostaglandin E2 production	Nurtjahja-Tjendraputra *et al.*, 2003

Table.2: Quality characteristics of fresh ginger rhizome

S.No.	**Parameters**	**Range**	**References**
1.	Length (cm)	4.80-14.99	Kaushal *et al.* 2014; Kirtiraj *et al.* 2013; Akhtar *et al.* 2013; Jayashree & Vishvanathan 2011
2.	Width (cm)	1.82-8.17	Kaushal *et al.* 2014; Akhtar *et al.* 2013; Kirtiraj *et al.* 2013; Jayashree & Vishvanathan 2011
3.	Thickness (cm)	3.25-4.49	Kaushal *et al.* 2014; Jayashree & Vishvanathan 2011
4.	Weight (g)	8.0-161.6	Kirtiraj *et al.* 2013; Akhtar *et al.* 2013; Jayashree & Visvanathan 2011; Onu and Okafor 2002
5.	Volume (cm^3)	64.0-85.0	Jayashree & Visvanathan 2011; Onu and Okafor 2002

6.	Density	0.88-1.02	Ghosh *et al.* 2011; Phoungchandang and Sertwasana 2010
7.	Moisture (%)	75.20-94.17	Tanveer *et al.* 2014; Kaushal *et al.* 2014; Ghosh *et al.* 2011; Eze and Agbo 2011; Phoungchandang and Sertwasena 2010; Puengphian and Sirichote 2008; Singh *et al.* 2001;
8.	Total soluble solids (^oB)	3.33-6.67	Kaushal *et al.* 2014; Eze and Agbo 2011
9.	Carbohydrates (%)	12.3	Ehsanullah *et al.* 2013
10.	Ascorbic acid (mg/100g)	1.04-9.33	Latona *et al.* 2012; Shahid and Hussain 2012; Shirin and Prakash 2010
11.	pH	5.23-6.72	Kaushal *et al.* 2014; Akhtar *et al.* 2013; Rahman *et al.* 2013; Shirshir *et al.* 2012.
12.	Phenols (mg GAE/100g)	24.63-514.02	Oknye *et al.* 2015; Saffa *et al.* 2010; EL-Ghorab *et al.* 2010; Puengphian and Sirichote 2008
13.	Antioxidant activity (%)	51.01-79.19	Kaushal *et al.* 2014;, Nwaoha *et al.* 2013; Maizura *et al.* 2011; Purnomo *et al.* 2010; Grant and Lutz 2000
14.	Starch (%)	40.4-59.0	Akande *et al.* 2014; Jadhav *et al.* 2012
15.	Proteins (%)	1.2-15.0	Ehsanullah *et al.* 2013; Shahid and Hussain 2012; Okolo *et al.* 2012; Ghosh *et al.* 2011; EL-Ghorab *et al.* 2010; Dhingra and Kumar 2005; Nwinuka *et al.* 2005; Panhwar 2005; Mallorea *et al.* 1992
16.	Fat (%)	0.9-1.0	Ehsanullah *et al*. 2013; Ghosh *et al*. 2011
17.	Fibre (%)	0.50-10.8	Kaushal *et al.* 2014; Ehsanullah *et al.* 2013; Virendra *et al.* 2013; Jadhav *et al.* 2012; Kizhakkayil and Sasikumar 2009; Singh *et al.* 2001
18.	Ash (%)	0.69-2.3	Kaushal *et al.* 2014; Rahman *et al.* 2013; Shahid and Hussain 2012; Ghosh *et al.* 2011; El-Ghorab *et al.* 2010; Singh et al. 2001; Gopalan *et al.* 2004
19.	Oleoresin (%)	3.30 – 13.65	Jayashree *et al.* 2014; Abeysekera *et al.* 2005
20.	Volatile oil (%)	0.85 -2.70	Sasidharan and Menon 2010; EI-Baroty *et al.* 2010; Sultan *et al.* 2005; Noor *et al.* 2004; Singh *et al*. 2001

Table.3: Postharvest diseases of ginger

Disease	**Causative agent**	**Symptoms**	**Control**
Soft Rot	*Pythium* common soil-borne fungus	Small brownish spots on the skin, which may rapidly enlarge into sizeable lesions. As the decay progresses, the tissue breaks down into a soft and watery mass.	Chemicals such as mancozeb, ziram, guazatine, propineb and copper oxychloride (30 minutes dip) treatments for rhizomes. Ridomil MZ @ 1.25 g/L increased survival of rhizomes by about 30%.*(Dohroo and Sharma 1986; Thakore et al. 1988)*
Dry Rot/ Yellows	*Fusarium* Soil-borne fungus	Off-coloured dry sunken lesions typically border by a brown margin on the rhizome surface. Internal symptoms include a pale brown discolouration of the vascular tissue.	Systemic and contact fungicides like Bavistin 50WP, Ridomil Gold MZ-72, Captan, Dithane M-¬45, copper oxychloride and Bordeaux mixture etc. were reported effective against the disease (Sagar 2006; Hasnat *et al.* 2014)
Blue Mould	*Penicillium* fungus	Develops on cut ends and injured areas and result in internal tissue decay.	
Watery Rot	*Rhizopus*	Soft, watery rot progresses	The post-harvest fungicide 2,6 dichloro-4

http://dx.doi.org/10.22161/ijeab/2.1.50 *ISSN: 2456-1878*

	fungus	rapidly and rot an entire rhizome in a week. Infected tissue is mottled brown and soft and in a humid atmosphere the infected area is soon covered with white mould which eventually turns black.	nitroaniline (Botran) is applied just prior to packing may reduce watery rot during transport to export market destinations.
Bacterial Soft Rot	*Erwinia carotovora*	Principal postharvest bacterial disease of ginger. A soft wet rot of the tissue, which has a strong foul odour, the severity of disease is rapid under warm humid conditions.	Control includes harvesting at full maturity, careful handling and efficient curing. Ginger should not be stored in used bags (potato, onion etc) and the storage environment should be cool, dry and well ventilated.
Armillaria Rot	*Armillaria mellea*	Development of tough, dark, string-like growth which adhere to the rhizomes.	Uprooting of the infested plants
Sclerotium Rot	*Sclerotium rolfsii*- soil borne fungus	Small spherical fungal resting bodies about 1-2 mm (0.04 inch to 0.08 inch) in diameter develop on the mould. They are initially white but later turn brown.	Benomyl (750 ppm) or gibberellic acid (150 ppm) or 0.3% Ridomil MZ. Steeping of rhizomes in carbendazim (0.1%) for 60 minutes (Sharma and Dohroo 1991)

http://dx.doi.org/10.22161/ijeab/2.1.51 *ISSN: 2456-1878*

First Record of *Emys orbicularis* (Boulenger, 1882), (Reptilia, Testudinati) in the "Castel di Guido" Natural Park (Northern *Latium*, Italy): a Case of Interest for Species Conservation

Antonio Pizzuti Piccoli[1], Ignazio Scalas[2], Luigi Di Stano[2]

[1]Associazione Natura per Tutti Onlus – Via Monteroni n°1265, 00055 Ladispoli (RM) Italy.
[2]Nisus Film – Via Angelo Dubini n°73, 00189 Roma Italy.

Abstract *— The finding of five specimens of Emys orbicularis (Linnaeus, 1758), (Reptilia, Testudinati) in the "Castel di Guido" Natural Park (Northern Latium, Italy), is reported. This is the first record for the species in the area, regularly monitored since 2006. It is assumed that the species has recently colonized the site through highly populated and degraded territory; the data is of conservation interest, proving the capacity of the species to cross polluted environments.*

Keywords —Castel di Guido, Central Italy, colonization, Emys orbicularis, first record.

I. INTRODUCTION

The European Pond Terrapin *Emys orbicularis* (Linnaeus, 1758) is one of the two representatives of the family Emydidae; it has a very wide range, from northern Europe to some places in North Africa (Vamberger et al., 2015). *Emys orbicularis* is a Reptile distributed in the Italian peninsula, with the exclusion of the Sicily (Mazzotti & Zuffi, 2005). The Italian populations are located principally in two main types of wetland habitats, the first represented by the ponds, puddles, swamps and marshes, mainly with rich aquatic vegetation . The second type is represented by little river and canals for drainage of water, usually in open areas or riparian vegetation.(Mazzotti & Zuffi, 2005). In general we assist to a decline of the species in all its areal and in particular in Latium territory.

The vitality of the populations, in Central Italy, is closely linked to the conservation of small wetland and rivers, often temporary. (Utzeri, 2000)

In the Latium region is a species that mainly frequents the coastal strip. (Utzeri, 2000; Bologna et al., 2007).

The "Castel di Guido" Natural Park is a protected area managed by the LIPU ONG organization included in the Litorale Romano National Reserve (Figure 1).

The area is part of the Mediterranean region, the phytoclimatical unit is characterized by a mesomediterranean type and a higher sub-humid ombrotype (Blasi, 1994; Mangianti & Perini, 2001).

The study area is characterized by an evident complexity of vegetation and a great floristic richness, divided in different habitats. The area is characterized by a mosaic of natural and human elements mixed together. We have crops to wheat, corn, barley, olive groves, pasture lands, natural woods mainly composed by *Quercus ilex* L. and *Quercus pubescens* Willd.; We have areas covered by pine forests and reforestation trees, while the rest of the territory is occupied by country roads, cowsheds, farms and irrigation canals.(Chirici et al., 2001; Filesi, 2001)

In the area there are small rivers, springs and ponds both permanent and temporary.

Scientific research in the wetlands of Castel di Guido" Natural Park had begun in 1994. Since 2006 is ongoing continuous monitoring for populations of amphibians and reptiles. *Emys orbicularis* was discovered for the first time in May 2015.

II. RESULTS AND CONCLUSION

On may, 2015, during monitoring activities, five specimens of *Emys orbicularis* were found in a little ditch in the "Castel di Guido" Natural Park. On 12 of May were observed and captured three specimen; on 25 of May two more specimen were caught. They are three adults (two male and one female), one juvenile specimen and one newborn specimen. Before being captured, they were observed together in basking activities.

All the specimens observed (Figure 2) present the typical coloration with the carapace brown with a hint of green, and a spotted yellow skin. In Table 1 are reported the morphometric measures of the captured individuals. The study was conducted in accordance with applicable laws and authorizations provided for this kind of studies.

The discovery of individuals of *Emys orbicularis* in "Castel di Guido" Natural Park is a noteworthy event. This is the first record for the species in the study area,

http://dx.doi.org/10.22161/ijeab/2.1.51 *ISSN: 2456-1878*

where scientific research had begun in 1994 and populations of amphibians and reptiles are monitored continuously from 2006.

The presence in a peripheral area of the Natural Park might suppose that this is a colonization of the site. By comparing data collected with bibliography references (Lebboroni & Chelazzi, 1991; Gariboldi & Zuffi, 1994), the presence of individuals of different size, including a newborn, probably means that a reproductive population has settled in the area, this may signify that the terrapins have colonized the ditch many years ago. This case of expansion of areal and have importance in conservation of species.

The area surrounding the park is highly urbanized (Figure 3); the nearest population of *Emys* is five kilometers away from the study area. This is a positive aspect for the conservation of the species, suggesting the capacity of the terrapins to cover long distances through degraded and polluted areas.

Finally the present note contribute to the knowledge of the status of the population of *Emys orbicularis* and its conservation in "Castel di Guido" Natural Park and Roman area. The Authors illustrate the hypothesis of recent migration and colonization of the area; the fact needs more studies for the future in order to check the quantity of individuals and persistence in the area.

AKNOWLEDGEMENTS

The Authors are grateful to Alessia De Lorenzis for the contribution given to the present work.

REFERENCES

[1] Blasi C., 1994 – Fitoclimatologia del Lazio. *Regione Lazio*, Roma.

[2] Bologna M. A., Salvi D. & Pitzalis M., 2007 – Atlante degli Anfibi e dei Rettili della Provincia di Roma. *Provincia di Roma, Gangemi Editore*, Roma.

[3] Chirici G., Corona P., Filesi L., Vannuccini M., 2001 – Lineamenti ambientali della Tenuta di Castel di Guido. In: Corona P., 2001. I rimboschimenti della Tenuta di Castel di Guido: Materiali di studio. *Supplemento al n° 4/2000 di Innovazione e Agricoltura, ARSIAL*, Roma: 23-30.

[4] Filesi L., 2001 – Vegetazione attuale e potenziale della Tenuta di Castel di Guido. In: Corona P. I rimboschimenti della Tenuta di Castel di Guido: Materiali di studio, 2001. *Supplemento al n° 4/2000 di "Innovazione e Agricoltura", ARSIAL*, Roma: 31-42.

[5] Mangianti F. & Perini L., 2001 – Osservazioni Meteorologiche dell'anno 1998 - 1999 - 2000 - 2001. *Ufficio Centrale di Ecologia Agraria e difesa delle piante coltivate dalle avversità meteoriche. Osservatorio Meteorologico Torre Calandrelli*. Roma, Collegio Romano.

[6] Gariboldi A. & Zuffi M.A.L., 1994 – Notes on the population reinforcement project for *Emys orbicularis* (Linnaeus, 1758) in a natural park of northwestern Italy (Testudines: Emydidae). *Herpetozoa*, 7: 83-89.

[7] Lebboroni M. & Chelazzi G., 1991 – Activity patterns of *Emys orbicularis* L. (Chelonia Emydidae) in Central Italy. *Ethol. Ecol. Evol.*, 3: 257-268.

[8] Mazzotti S. & Zuffi M., 2005 – *Emys orbicularis*, pp. 377- 381. In: F. Barbieri, G. Doria & R. Sindaco (eds), Atlante degli Anfibi e Rettili d'Italia. *Societas Herpetologica Italica. Polistampa*, Firenze.

[9] Utzeri C., 2000 – *Emys orbicularis*, pp. 70-71. In: Bologna M. A., Capula M. & Carpaneto G. (eds.), Anfibi e Rettili del Lazio. *Fratelli Palombi Editori*, Roma.

[10] Vamberger M., Stuckas H., Sacco F., D'Angelo S., Arculeo M., Cheylan M., Corti C., Lo Valvo M., Marrone F., Wink M., Fritz U., 2015 – Differences in gene flow in a twofold secondary contact zone of pond turtles in southern Italy (*Testudines*: *Emydidae*: *Emys orbicularis galloitalica*, *E. o. hellenica*, *E. trinacris*). *Zool. Scr*. 44(3): 233-249.

http://dx.doi.org/10.22161/ijeab/2.1.51 *ISSN: 2456-1878*

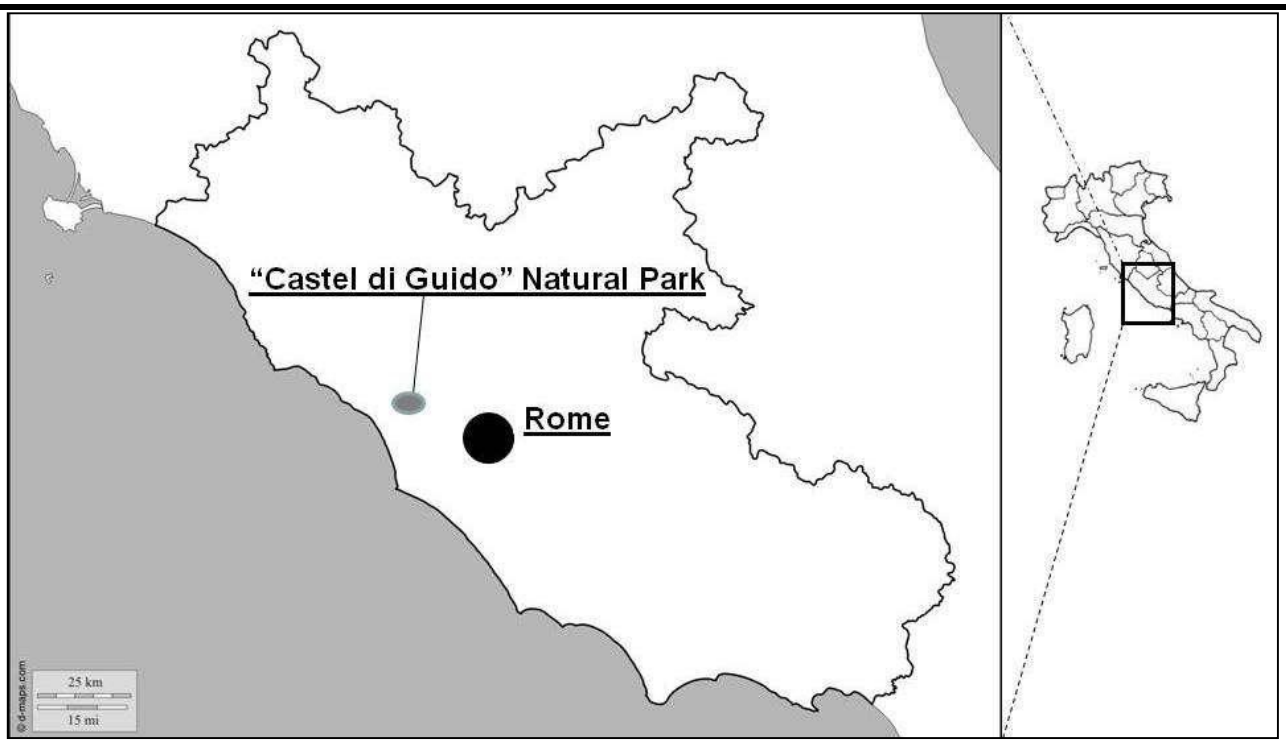


Fig. 1: The "Castel di Guido" Natural Park.

Fig. 2: Three specimens of Emys Orbicularis of "Castel di Guido" Natural Park.

http://dx.doi.org/10.22161/ijeab/2.1.51 *ISSN: 2456-1878*

Table.1: Sizes (in grams and in millimetres) of the specimens of Emys Orbicularis of "Castel di Guido" Natural Park.

	Sex	**Age**	**Weight**	**Length of carapace**	**Width of carapace**
1	Male	Adult	369	136	**110**
2	Male	Adult	311	133	**103**
3	Female	Adult	553	147	**112**
4	-	Juvenile	122	90	**80**
5	-	**Newborn**	**10,4**	**36**	**33**

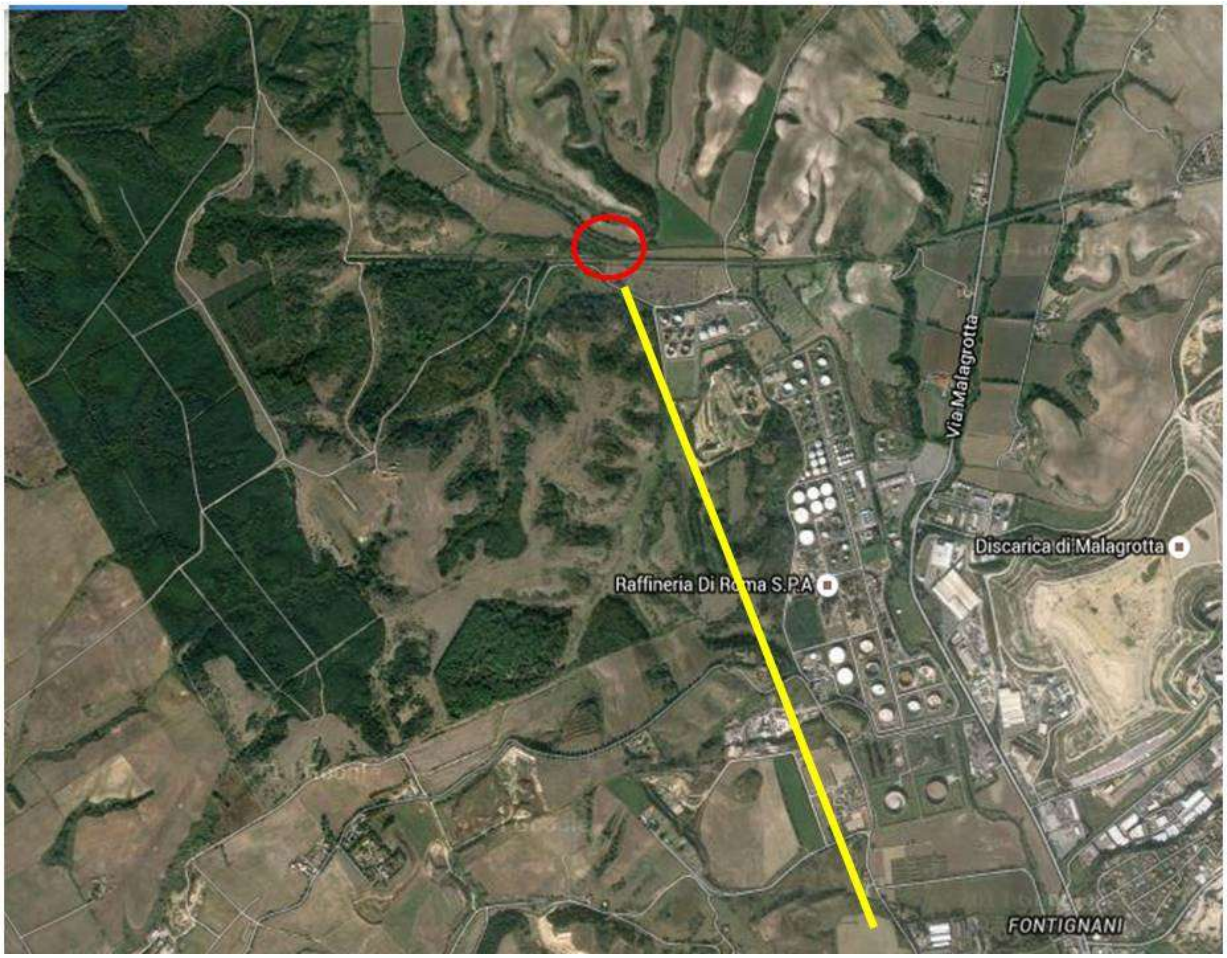


Fig. 3: The location of Emys Orbicularis in "Castel di Guido" Natural Park (red circle). In yellow the direction of provenance of the species.

http://dx.doi.org/10.22161/ijeab/2.1.52 *ISSN: 2456-1878*

Susceptibility of *Eucalyptus* Species and Clones to Red Gum Lerp Psyllid, *Glycaspis brimblecombei*, (Hemiptera: Psyllidae) in Mbizi Forest Plantation, Tanzania

Revocatus Petro, Aloyce Mpiri, Ally Mkude

Mufindi Pulpwood Research Centre, Tanzania Forestry Research Institute, P.O. Box 45, Mafinga – Iringa, Tanzania.

Abstract**—** *Glycaspis brimblecombei is a sap-sucking insect that feeds on Eucalypts. The pest is native to Australia. The nymph feeds on eucalypt leaves and secretes honeydew with which they construct a waxy cover (called a lerp) around themselves. This cover is whitish and conical in shape and shelters the insects until the adult stage. The insect is considered a serious pest that causes leaf discoloration, severe leaf drop, twig dieback and some tree mortality on some Eucalyptus species. In October 2016, the red gum lerp psyllid was recorded for the first time in Mbizi forest plantation in Tanzania infesting Eucalyptus camaldulensis and different Eucalyptus clones. A study was conducted to determine the susceptibility of Eucalypt germplasm to the insect pest. Results showed that E. camaldulensis was more infested followed by GC 514, GC 167, GC 584, GC 15, GC 785 clones while GC 940 was the least infested. Eucalyptus grandis was not infested. Stakeholders can be able to use the susceptibility grouping of the Eucalyptus germplasm to determine what to plant in areas of red gum lerp psyllid infestation. Similar research work should be carried in all major host tree growing areas to determine susceptibility groups for the areas.*

Keywords**— Glycaspis brimblecombei, the red gum lerp psyllid, Tanzania, new record, Eucalyptus germplasm.**

I. INTRODUCTION

Glycaspis brimblecombei (Homoptera: Psylloidea) is commonly referred to as the red gum lerp psyllid. It is native to Australia. The species was first detected outside Australia in *Eucalyptus* plantations causing infestations in California in 1998 (Brennan & Gill, 1999). In a few years, from 2001 to 2008, red gum lerp psyllid was recorded in Florida and Mexico, Chile, Brazil, Argentina, Ecuador, Peru, Hawaii, Uruguay, Venezuela (Burckhardt *et al*., 2008; FAO, 2012). In Europe, the first record of the red gum lerp psyllid dates to 2007 in Portugal and Spain followed by Italy and France in 2010 and 2011 respectively (Valente & Hodkinson, 2009; FAO, 2012; CABI, 2012). In Africa, red gum lerp psyllid was first reported in 2001 in Mauritius, in 2004 in Madagascar and in 2012 in South Africa (EPPO, 2012). In Tanzania, the pest was first recorded in October of 2016 causing infestation on *E. camaldulensis* and different *Eucalyptus* clones on Tanzania Forestry Research Institute (TAFORI) experimental plots planted in Mbizi forest plantation. The origin of the importation has not yet been elucidated.

Lerp insects usually live in colonies of mixed stages. Females of the red gum lerp psyllid lay between 45 and 700 eggs. The eggs hatch in 10 to 35 days depending on temperature and other environmental conditions (FAO, 2012). After hatching, young nymphs or "crawlers" move about the host plant searching for a place to settle, usually settling within 48 hours of hatching. Once settled, they insert their stylets (mouthparts) onto the leaf and begin feeding on the xylem. As the nymphs feed, they secrete honeydew which they use to construct a waxy cover (called a lerp) around themselves. This cover is whitish and conical in shape and shelters the nymphs until they attain the adult stage (Dahlsten & Rowney, 2000; FAO, 2012, CABI, 2012; EPPO, 2012). The red gum lerp psyllid is considered a serious pest that causes leaf discoloration, severe leaf drop, twig dieback and some tree mortality on some *Eucalyptus* species. Red gum lerp psyllid feeds on several *Eucalyptus* species, mainly *E. camaldulensis* Dehnh., but also on other species including *E. globulus* Labill., *E. diversicolor* F. Muell., *E. lehmannii* (Schauer) Benth., *E. blakelyi* Maiden, *E. nitens* H. Deane et Maiden, *E. tereticornis* Sm., *E. bridgesiana* R. T. Baker, *E. brassiana* S. T. Blake and *E. mannifera* Mudie (Brennan & Gill, 1999; 2001; Nagamine and Heu, 2001; Laudonia and Garonna, 2010).

Eucalypts occupy about 10% of the total area of forest plantations in the country of about 550,000 hectares (ha). These eucalypts provide goods and services which contribute to individual livelihoods and national economies in the tropics as well as to reduce pressure on natural forests. There is clear information on a number of

insect pests attacking eucalypts such as stem borers, defoliators, sap suckers and gall forming insects (Kumari, 2009; Petro, 2015). This information includes major hosts, nature and extent of the damage, impact and control. However, little information is available on red gum lerp psyllid which has just recorded in Tanzania for the first time in October of 2016. Therefore, this study was undertaken to determine the susceptibility of Eucalypt germplasm to the insect pest in Mbizi forest plantation, Tanzania. The study was initiated to generate valuable information to forest managers, policy makers, plant protectionists, research and training institutions and individuals' small eucalypt growers in country to find ways of managing the pest situation accordingly.

II. METHODS

Study Area

The study was conducted in a newly established trial plot in Mbizi Forest Plantation. The trial plot was established by TAFORI in March 2015 with the aim of testing growth performance of different *Eucalyptus* germplasm. Mbizi forest plantation is one of 19th forest plantations owned by the Government. The plantation was established in 2013. It has a total area of about 4000 ha. Out of the total plantation area, only 1207 ha are planted. About 99.5% of the planted area is planted with *Pinus patula*. The rest is planted with *Eucalyptus grandis* (5 ha) and *Grevillea robusta* (2 ha) (Petro *et al*., 2016a). The plantation is located in Sumbawanga district, Rukwa region, southern Tanzania and lies between 7°49' and 7°57' S and 31°37' and 31°45'E at altitudes ranging from 1903 to 2373 m a.s.l (Petro *et al*., 2016b). Mbizi is bordered by Lake Rukwa in the North-East and various villages of Sumbawanga Municipality in the South and South East. The area has unimodal rainfall pattern between November to April. The mean annual rainfall ranges between 800 and 900 mm. Drier months are May to October. The area is cool from March to August and warm in the rest of the months.

Sampling and Data Collection

Three plots measuring 12m by 12m were randomly established in each of *E. camaldulensis*, *E. grandis*, *E. camaldulensi* × *E. grandis* (GC) 15, GC 167, GC 514, GC 584, GC 785, GC 940 clones in the trial plot. Assessment of red gum lerp psyllid infestation was done by visual scoring of all trees falling in every plot for incidence (proportion of infested trees) and severity of red gum lerp psyllid. The incidence of red gum lerp psyllid infestation on trees was based on the absence or presence of waxy cover (lerp) on a tree (**Plate 1**). The red gum lerp psyllid severity was assessed visually on the whole crown foliage whereby the following subjective scales were used: (1) none (trees with no visible lerps); (2) minor (trees with lerps in <25% of total shoots); (3) moderate (trees with lerps in 25–50% of total shoots); and (4) severe (trees with lerps in >50% of total shoots).

Plate.1: Red gum lerp psyllid on a Eucalyptus leaf, showing white conical lerps of the nymphs in TAFORI trial plot in Mbizi Forest Plantation

Data Analysis

Descriptive statistics were used to determine the total number of infested trees (incidence) and the number of trees in each severity class of red gum lerp psyllid infestation per plot. The average severity (AS) per plot was calculated as described by Sharma & Sankaran (1988):

$$AS = \frac{(1\times a) + (2\times b) + (3\times c) + (4\times d)}{N \text{ (Total number of trees assessed per plot)}}$$

where 1, 2, 3 and 4 are severity categories and a, b, c and d are the numbers of trees examined in each severity category. Red gum lerp psyllid damage index in each plot was calculated as the product of AS and incidence (proportion of infested trees) per plot. Analysis of variance (ANOVA) in the SAS package was used to test the significance of variation of incidence, AS and damage index between germplasm. In all analyses, significance was determined at $p < 0.05$.

http://dx.doi.org/10.22161/ijeab/2.1.52 *ISSN: 2456-1878*

III. RESULTS AND DISCUSSION

Of the total 536 trees examined, results showed that GC 514 formed the major part (13.8%), followed by *E. camaldulensis* (13.4%), GC 584 (13.2%), GC 167 (13.1%), GC 940 (12.3%), GC 15 & *E. grandis* (11.8%) and GC 785 (10.6%) (Table 1). Distinct differences were observed in incidence, average severity and damage index of red gum lerp psyllid between *Eucalyptus* germplam. The incidence was significantly higher on *E. camaldulensis*, GC 514 and GC 167 than other *Eucalyptus* germplasm ($F_{7,16}$ = 68.02; p < .0001). The average severity and damage index were significantly higher on *E. camaldulensis* and GC 514 than other *Eucalyptus* germplasm ($F_{7,16}$ = 85.17; p < .0001 and $F_{7,16}$ = 94.71; p < .0001 respectively). *Eucalyptus camaldulensi* and GC 514 had higher number of infested trees (21% & 14% of total trees sampled) having lerps on more than 50% of total shoots (severity class 4, Table 1). Results further showed that *E. grandis* was not infested by red gum lerp psyllid. In a study on determining infestation intensity of *Leptocybe invasa* of different *Eucalyptus* species, Nyeko *et al*. (2010) reported that *Eucalyptus* species showing a damage index (DI) = 0 were considered to be resistant, 0 < DI < 0.1 (tolerant), 0.1 ≤ DI < 0.5 (moderately susceptible), and DI ≥ 0.5 (highly susceptible). Therefore, basing on such classification, *E. grandis* (with DI = 0.0) can be classified as resistant, GC 940 (0.1) as tolerant, GC 785 and GC 15 (0.4) as moderately susceptible while GC 584 (0.6), GC 167 (1.1), GC 514 (1.6) and *E. camaldulensis* (1.8) are highly susceptible. This variation in infestation, to a large extent is genetically controlled (Nadel & Slippers, 2011). These findings are in line with Hurley & Greyling (2013) who reported that *Eucalyptus* species differ in their susceptibility to attack by the red gum lerp psyllid with *E. camaldulensis* being highly susceptible and *E. grandis* being more tolerant. Similarly, Paine (2000) reported that although the psyllid feeds on plant fluids from a broad range of *Eucalyptus* species, it prefers to colonize members of the red gum species group, particularly river gum (*E. camaldulensis*). Dahlsten & Rowney (2000) reported that the psyllid was recorded on 27 *Eucalyptus* species in California, including *E. camaldulensis*, *E. rudis*, *E. globulus*, *E. diversicolor*, and *E. sideroxylon* although damage occurs in only a few species, with *E. camaldulensis* being the worst damaged. In 2010, red gum lerp psyllid was observed in different localities of the Campania Region in Italy and in the whole Mediterranean region and it was noticed to be more susceptible to red gum, *E. camaldulensis* than other *Eucalyptus* species (Laudonia & Garonna, 2010).

Table.1: Variation in incidence, severity and damage index of red gum lerp psyllid infestation in different Eucalyptus germplasm in TAFORI trial plot in Mbizi Forest Plantation

Eucalyptus germplasm	Total sample	Incidence (%)	Average severity	Damage index	Severity class (% of total samples)			
					1	2	3	4
E. camaldulensis	72	73.6[a]	2.4[a]	1.8[a]	26	28	25	21
GC 514	74	71.7[a]	2.2[a]	1.6[a]	28	34	24	14
GC 167	70	61.2[a]	1.9[b]	1.1[b]	39	44	9	9
GC 584	71	36.3[b]	1.5[c]	0.6[c]	63	25	7	4
GC 15	63	28.5[b]	1.5[c]	0.4[c]	71	27	2	0
GC 785	57	26.6[b]	1.3[cd]	0.4[c]	74	12	7	7
GC 940	66	4.6[c]	1.1[d]	0.1[d]	95	3	2	0
E. grandis	63	0.0[c]	1.0[e]	0.0[e]	100	0	0	0
F(7,16)		68.02	85.17	94.72				
p-value		<0.0001	<0.0001	<0.0001				

For each incidence, average severity and damage index, values followed by the same letter within a column are not significantly different at 5% probability level.

IV. CONCLUSIONS AND RECOMMENDATIONS

Glycaspis. brimblecombei, commonly called red gum lerp psyllid was recorded for the first time in October, 2016 in Mbizi forest plantation in Tanzania. The study has shown that there is high variability of red gum lerp psyllid infestation on the tested *Eucalyptus* germplasm. Results showed that *E. camaldulensis* was more infested followed by GC 514, GC 167, GC584, GC 15, GC 785 and GC 940 was the least while *E. grandis* was not infested. This is an indication that host plant resistance strategy is a viable management option of this pest. Stakeholders can be able to use the susceptibility grouping of the *Eucalyptus* germplasm to determine what to plant in areas of red gum lerp psyllid infestation. In order to assist management decisions, a survey is recommended in all areas where

eucalypts are planted in Tanzania to obtain accurate information on the distribution and host associations.

ACKNOWLEDGEMENTS

We gratefully acknowledge the Tanzania Forestry Research Institute (TAFORI) for financing this study. We are also thankful to Professor S. Iddi for his useful comments and suggestions.

REFERENCES

[1] Brennan, E. B. & Gill, R., (1999) First record of *Glycaspis brimblecombei* Moore (Homoptera: Psyllidae) in North America: Initial observations and predator associations of a potentially serious new pest of Eucalyptus in California. *Pan-Pacific Entomologist*, 75, 55–57.

[2] Burckhardt, D., Lozada, P. W. & Diaz, B. W. (2008) First record of the red gum lerp psyllid *Glycaspis brimblecombei* (Hemiptera: Psylloidea) from Peru.- *Mitteilungen der Schweizerischen Entomologische Gesellschaft*, 81, 83-85.

[3] CABI. (2012) Invasive species Compendium report - *Glycaspis. brimblecombei* (red gum lerp psyllid). Available at www.cabo.org/isc/?compid5&dsid=25242&loadmodule=datasheet&page=4818&=144. Accessed on 09/11/2016

[4] Dahlsten, D.L. & Rowney, D.L. (2000) The Red Gum Lerp Psyllid, a new pest of Eucalyptus species in California. Available at: http://www.cnr.berkeley.edu/biocon/dahlsten/rglp/index.htm.

[5] EPPO. (2012) *Glycaspis. brimblecombei* EPPO Global Database. Available at: http:// gd3.eppo.int/organism.php/GLYSBR. Accessed on 03/11/2016.

[6] FAO. (2012) *Glycaspis. Brimblecombei*, red gum lerp psyllid (Homoptera: Psylloidea). Major sap-sacking pest of eucalypt species. Forest Pest Species Profile.2 pp.

[7] Hurley, B. & Greyling, I. (2013) PEST ELERT: The red gum lerp psyllid, *Glycaspis brimblecombei*.3 pp.

[8] Kumari, K. N. (2009) Bioecology and management of eucalyptus gall wasp, *Leptocybe invasa* Fisher & Salle (Hymenoptera: Eulophidae). Dissertation for Award of MSc Degree at University of Agricultural Sciences, Bangalore, India. 72pp.

[9] Laudonia, S. & Garonna, A.P. (2010) The red gum lerp psyllid, *Glycaspis brimblecombei*, a new exotic pest of *Eucalyptus camaldulensis* in Italy. *Bulletin of Insectology*. 63(2), 233-236.

[10] Nadel, R., & Slippers, B. (2011) *Leptocybe invasa, the blue gum chalcid wasp* (Information Sheet 01/2011). Retrieved from http://www.forestry.co.za/uploads/File/home/notices/2011/ICFR%20IS01-2011gallwasp.pdf

[11] Nagamine, W.T. & Heu, R.A. (2001) The red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Homoptera: Psyllidae). Available at: http://www.hawaiiag.org/hdoa/npa/npa01-02_rpsyllid.pdf. Accessed on 29/10/2016.

[12] Nyeko, P., Mutitu, K. E., Otieno, B. O., Ngae, G. N. & Day, R. K. (2010) Variations in *Leptocybe invasa* (Hymenoptera: Eulophidae) population intensity and infestation on *Eucalyptus* germplasms in Uganda and Kenya. *International Journal of st Management.* 56(2), 137 - 144.

[13] Paine, T.D. (2000) UC scientists apply IPM techniques to new eucalyptus pests. *California Agriculture.* 54(6), 8-13.

[14] Petro, R. (2015) Effects of Eucalyptus gall wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae) on growth and wood basic density of some *Eucalyptus* species, Tanzania. Thesis for Award of PhD Degree at Sokoine University of Agriculture, Morogoro, Tanzania. 104pp.

[15] Petro, R., Ndomba, O.A., & Mpiri, A. (2016a) The cause of altered growth of *Pinus patula* trees at Mbizi forest plantation, Sumbawanga. Technica report. 13pp.

[16] Petro, R., Ndomba, O.A. & Mpiri, A. (2016b). Soil survey for land suitability mapping for Mbizi Forest Plantation, Sumbawanga. Technical Report Submitted to the Tanzania Forest Services Agency. Tanzania Forestry Research Institute, Mafinga. 36pp.

[17] Sharma, J.K. & Sankaran, K.V. (1988) Incidence and severity of Botryodiplodia dieback in plantations of *Albizia falcataria* in Kerera, India. *For Ecol Manag*. 24,43-58.

[18] URT (2015). National Forest Resources Monitoring and Assessment of Tanzania Mainland (NAFORMA). Ministry of Natural Resources & Tourism, Tanzania Forest Services (TFS) Agency, Dar es Salaam. 106pp.

[19] Valente, C. & Hodkinson, I. (2009). First record of the red gum lerp psyllid, *Glycaspis brimblecombei* Moore (Hem.: Psyllidae), in Europe.- *Journal of Applied Entomology*. 133(4), 315-317.

http://dx.doi.org/10.22161/ijeab/2.1.53 *ISSN: 2456-1878*

Preparation and Foliar Application of Oligochitosan - Nanosilica on the Enhancement of Soybean Seed Yield

Dang Van Phu[1,2], Bui Duy Du[3], Le Nghiem Anh Tuan[3], Hoang Van Tam[4], Nguyen Quoc Hien[1*]

[1]Research and Development Center for Radiation Technology, Vietnam Atomic Energy Institute
[2]Graduate University of Science and Technology, Vietnam Academy of Science and Technology
[3]Institute of Applied Materials Science, Vietnam Academy of Science and Technology
[4]Institute of Agricultural Sciences for Southern Vietnam
[*]Corresponding author: Tel.: +84 8 62829159; Fax: +84 8 38975921

Abstract**—** *Oligochitosan with weight average molecular weight (Mw) of 5000 g/mol was prepared by gamma Co-60 radiation degradation of 4% chitosan solution containing 0.5% H_2O_2 at 21 kGy. Nanosilica with size of 10 – 30 nm was synthesized by calcination of acid treated rice husk at 700° C for 2 h. The mixture of 2% oligochitosan–2% nanosilica was prepared by dispersion of nanosilica in oligochitosan solution. Oligochitosan, nanosilica and their mixture were characterized by gel permeation chromatography (GPC), transmission electron microscopy (TEM), X-ray diffraction (XRD), energy dispersive x-ray spectroscopy (EDX), Ultraviolet-visible spectroscopy (UV-Vis), and Furrier transform infrared spectroscopy (FT-IR). Effect of foliar application of oligochitosan and oligochitosan–nanosilica on soybean seed yield was conducted in experimental field. Results indi-cated that soybean seed yield increased 10.5 and 17.0% for oligochitosan and oligochitosan–nanosilica, respect-tively for the control. Radiation degraded oligochitosan and its mixture with nanosilica can be potentially used for cultivation of soybean with enhanced seed yield.*

Keywords**— Oligochitosan, nanosilica, foliar, soybean, seed yield.***

I. INTRODUCTION

The excessive use of chemical fertilizer and pesticide in agriculture may lead to negative effects of toxic residues in food products causing toxin risk for consumers and in environment causing ecotoxicity and health hazard concerns. Recent trend in agriculture has been focused on organic and vertical farming not only addressing the rising concern for environmental issues but also accommodating the demands of food of increasing world population [1]. Organic farming is considered as a viable alternative in comparison to chemical based farming [2].

Chitosan and oligochitosan have attracted considerable interest due to their many unique biological activities such as antioxidant activity [3], antimicrobial activity [4], and antitumor activity [5]. These features, together with their biocompatibility, biodegradability, and nontoxicity make them as interesting biopolymers for application in medicine, cosmetic, biotechnology, food and agriculture, etc. Oligochitosan is effective at eliciting plant innate immunity against disease in plant such as tomato [6], grapevine [7], etc. Therefore, Yin *et al.* (2010) supposed oligochitosan as a plant vaccine that is similar with general animal vaccine [8]. Beside elicitation effect, oligochitosan also exhibits growth promotion effect for plant such as rice [9], soybean [10,11], etc. It is interesting to note that application of oligochitosan either by seed treatment [10] or through hyponex solution [11] increased seed yield of soybean from 15 to about 36%. In general, oligochitosan has better plant growth promotion and elicitation effect than chitosan [7–9 ,11].

Nanotechnology opens up a wide applicability in various fields like medicine, pharmaceutics, electronics and agriculture. Nanomaterials hold great promise of improved plant disease resistance, controlled release of agro-chemicals, enhanced plant growth, etc [12]. According to Taha (2016), nanomaterials can be used as a magical tool for enhancing growth and improvement of agricultural production [13]. Typically, treatment of tomato seed with nanosilica (SiO_2) of 8 g L^{-1} not only enhanced the characteristics of seed germination but also promoted seedling growth [14]. In addition, Suriyaprabha *et al.* (2012) reported that soil amendment with nanosilica of 15 kg ha^{-1} enhanced growth characteristics of maize particularly stem height, root length, leaf area and chlorophyll content [15]. They concluded that the application of nanoscale fertilizers was found to be superior to bulk silica as soil amendment. Recently,

Kiirika *et al.* (2013) reported for the first time the synergistic effect of mixture of chitosan–silica induced resistance in tomato against bacterial wilt caused by *Ralstonia solanacearum* [16]. To the best of our knowledge, no research on the effect of mixture of oligochitosan–nanosilica for plants has been reported yet. With the aim of contribution to promoting organic farming, in the present study, oligochitosan was prepared by gamma Co-60 irradiation degradation of chitosan in solution and nanosilica was prepared from rice husk. The effect of foliar application of oligochitosan and mixture of oligochitosan–nanosilica on the enhancement of soybean seed yield was investigated.

II. MATERIALS AND METHODS

2.1. Preparation of oligochitosan

Chitosan from shrimp shell with a degree of deacetylation (DDA%) of ~91.4%; the weight average molecular weight (Mw) of 44.5 $\times 10^3$ g/mol and the number average molecular weight (Mn) of 13.5 $\times 10^3$ g/mol was supplied by a factory in Vung Tau province, Vietnam. Oligochitosan was prepared by gamma Co-60 ray irradiation degradation method as described in our previous paper [17] with some modifications. Briefly, chitosan (4 g) was dissolved in 80 ml of 2% (w/v) lactic acid solution, then 1.5 ml of hydrogen peroxide (30% H_2O_2) and 18.5 ml water were added to prepare 4% chitosan (w/v) solution containing 0.5% H_2O_2 (w/v). The 4% chitosan (w/v) solution without H_2O_2 was also prepared by adding 20 ml water. Then, the prepared solutions were irradiated at room temperature and under atmospheric pressure on gamma SVST Co-60/B irradiator at the VINAGAMMA Center up to the dose of 21 kGy, with dose rate of 1.12 kGy/h measured by a dichromate dosimetry system [18]. The Mw and Mn of irradiated chitosan were measured by an Agilent 1100 gel permeation chromatography (GPC; Agilent Technologies, USA) with detector RI G1362A and the column ultrahydrogel models 250 and 500 from Waters (USA). The standards for calibration of the columns were pullulan. The eluent was aqueous solution 0.25 M CH_3COOH/0.25 M CH_3COONa with the flow rate of 1 ml min^{-1} and temperature at 30° C [17]. IR spectra were taken on an FT-IR 8400S spectrometer (Shimadzu, Japan) using KBr pellets. The degree of deacetylation (DDA%) was calculated based on FT-IR spectra according to the following equation [19]:

$$A_{1320}/A_{1420} = 0.3822 + 0.0313 \times (100 - DDA\%)$$

where A_{1320} and A_{1420} are absorbance of chitosan at 1320 and 1420 cm^{-1}, respectively.

2.2. Preparation of nanosilica

Raw rice husk was supplied by rice mills in the south of Vietnam. The nanosilica with particles size of 10 – 30 nm was prepared from rice husk according to the procedure described by Wang *et al.* (2011) with some modifications [20]. Briefly, raw rice husk was first rinsed with water to remove dusts, soluble substances, and other contaminants. It was then dried at 60° C in forced air oven (Yamato, DNF 410, Japan). 50 g of the dried rice husk was then treated with 500 ml of 0.5 N HCl at ambient temperature for 2 h by magnetic stirring. It was kept intact overnight. Then it was decanted and thoroughly washed with distilled water until the rinse became free from acid. The treated-rice husk was subsequently dried in forced air oven until to dry and ground into fine powder. Finally, the rice husk powder was incinerated at 700° C for 2 h inside a programmable furnace (Nabertherm GmbH, Germany) to obtain nanosilica. The silica content and the amount of metallic impurities in the sample were estimated by energy dispersive x-ray spectrometer (EDX), Horiba 7593-H. The X-ray diffraction (XRD) pattern of nanosilica was recorded on an X-ray diffractometer, D8 Advance A25, Brucker, Germany. The particle size of nanosilica was performed using transmission electron microscopy (TEM), model JEM1010, JEOL, Japan.

2.3. Preparation of oligochitosan–nanosilica

Oligochitosan with Mw ~5000 g/mol obtained from 4% chitosan/0.5% H_2O_2 solution irradiated at dose of 21 kGy was used for preparation of oligochitosan–nanosilica mixture. 10 g nanosilica was homogenized in 100 ml NaOH 1N for 1 h. Then, 250 ml of the prepared oligochitosan solution was mixed with homogenized nanosilica and water was added to the mixture to obtain final volume of 500 ml and mixture concentration of 2% oligochitosan–2% nanosilica. In order to increase the adsorption ability of oligochitosan on nanosilica, pH of the mixture was adjusted to ~7.5 [21]. The optical absorbance of samples was performed with an UV-Vis spectrophotometer model UV-2401PC, Shimadzu, Japan in the wavelength range 200 – 800 nm using the quartz cuvettes with a path length of 1 cm and using water as the blank sample. The particle size of nanosilica in the oligochitosan–nanosilica mixture was measured using TEM image, and FTIR spectrum of oligochitosan–nano-silica mixture was also recorded.

2.4. Experimental design, foliar spraying and crop management

The experiment was conducted in the experimental field of the Institute of Agricultural Sciences for Southern Vietnam in Dong Nai Province, Vietnam and was designed as a randomized complete block with three treatments. Each treatment consisted of three replications. The area of each replication was of 30 m^2 (5 m × 6 m). Three treatments included foliar spraying with water

http://dx.doi.org/10.22161/ijeab/2.1.53 *ISSN: 2456-1878*

(control), oligochitosan and oligochitosan–nanosilica mixture. The concentration of oligochitosan and oligochitosan–nanosilica used for foliar spraying was of 50 mg/L and 50 mg/L – 50 mg/L, respectively. Foliar spaying was applied three times after seed sowing of 15, 22 and 30 days. Five plants were randomly selected to determine growth indexes particularly plant dry weight and plant height at flowering stage. In all three treatments, seeds were harvested when plants reached maturity. All data were statistically analyzed by analysis of variance (ANOVA) according to the experimental design and Least Significant Difference (LSD) at 5% probability level was utilized to compare the different means.

III. RESULTS AND DISCUSSION

3.1. Characteristics of radiation degraded chitosan

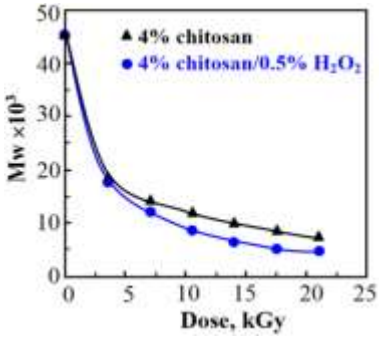


Fig.1: The weight average molecular weight (Mw) of chitosan versus dose.

The results in Fig. 1 indicated that the Mw of irradiated 4% chitosan/0.5% H_2O_2 solution were lower than that of irradiated 4% chitosan solution without containing H_2O_2, particularly Mw was of 5000 g/mol and 7800 g/mol at 21 kGy, respectively. It can be also observed in Fig. 1 that radiation degradation of chitosan in solution containing H_2O_2 is more effective in comparison with that of non-containing H_2O_2 due to synergistic effect, and the same results were also reported by the other studies [17,22]. This process has been put into large-scale production of oligochitosan (OC) with capacity of 500 L day^{-1} for field application as biotic plant elicitor and plant growth promoter for rice, sugarcane, ect. According to Das *et al.* (2015), the greatest challenge in the application of OC for plant protection lies in the development of efficient methods for large-scale production of OC [23]. Thus, the degradation method by gamma Co-60 ray irradiation can be applied on large-scale production of oligosaccharides including OC. The capacity of production could be easily raised by increasing intensity of Co-60 source.

For radiation degradation of chitosan, degradation extent was reported to depend on DDA [24] and initial molecular weight [25] as well as chitosan concentration in solution [17]. It was reported a general tendency that molecular weight distribution of OC obtained by radiation degradation of chitosan in solution is narrower in comparison with that of initial chitosan [17,26]. DDA of OC (Mw ~7800, Mn ~3400 g mol^{-1}) obtained from 4% chitosan solution irradiated at 21 kGy was of 88.3% that was slightly lower compared with initial chitosan (91.4%). The reason of deamination of chitosan by radiation degradation is still unclear. However, according to Mahmud *et al.* (2014) the oxidation reactions, which caused the cleavage of glycosidic linkages to reduce the molecular weight and also act to remove the amino groups of chitosan slightly under irradiation [27].

Table 1. Value of Mn, PI and DDA of irradiated chitosan from 4% chitosan solution and 4% chitosan/0.5% H_2O_2 solution with dose.

Dose, kGy	4% chitosan			4% chitosan – 0.5% H_2O_2		
	Mn ×10^3	PI*	DDA %	Mn ×10^3	PI*	DDA %
0	13.5	3.33	91.4	13.5	3.33	91.4
3.5	7.2	2.63	90.2	6.4	2.78	89.9
7.0	5.7	2.60	89.4	5.0	2.52	89.1
10.5	4.8	2.56	89.0	3.6	2.48	88.6
14.0	4.2	2.49	88.7	3.0	2.18	88.0
17.5	3.8	2.43	88.5	2.8	1.97	87.6
21.0	3.4	2.30	88.3	2.7	1.88	87.2

**PI (polydispersion index) = Mw/Mn*

The results in Table 1 indicated that the PI values were decreased with the increase of dose for both solutions. In addition, the obtained PI values also indicated that the lower the molecular weight of irradiated chitosan the narrower the molecular weight distribution. In other words, molecular weight distribution of OC is more homogenous than chitosan. The DDA% values (87 – 90%) for irradiated chitosan solution were slightly reduced in comparison with that of initial chitosan (~91%). Thus, it can be deduced that gamma Co-60 irradiation of 4% chitosan solution containing 0.5% H_2O_2 did not cause further decrease in DDA% compare to that of 4% chitosan solution without H_2O_2.

3.2. Characteristics of nanosilica and mixture of OC–nanosilica

http://dx.doi.org/10.22161/ijeab/2.1.53 *ISSN: 2456-1878*

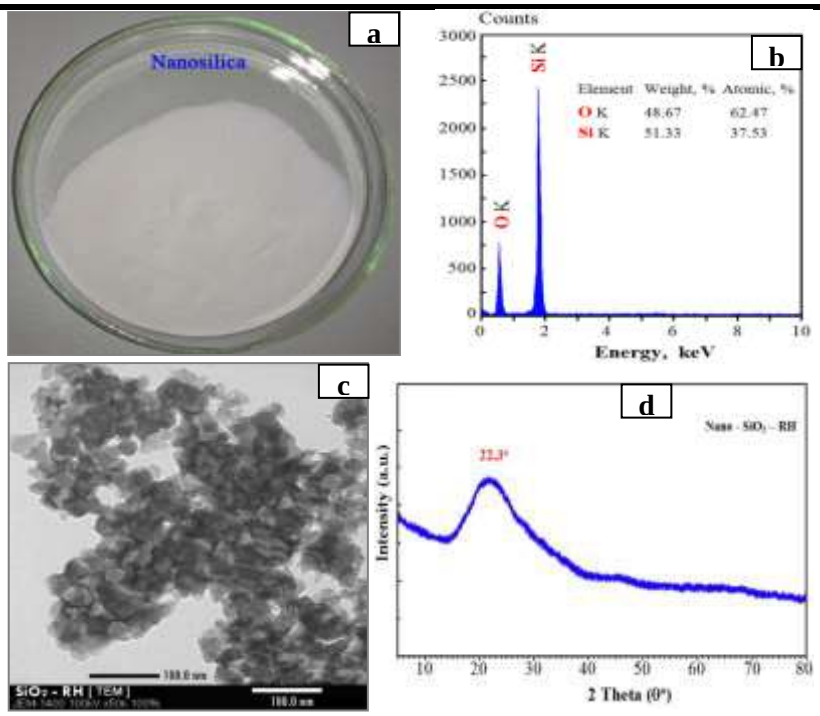


Fig.2: Photograph (a), EDX spectrum (b) TEM image (c) and XRD pattern (d) of nanosilica prepared from rice husk.

The size of as-prepared nanosilica in Fig. 2a was estimated from TEM image to be of 10 – 30 nm (Fig. 2c). The EDX spectrum (Fig. 2b) detected only two peaks for oxigen (O) at 0.525 keV and for silicon (Si) at 1.739 keV. The XRD pattern (Fig. 2d) appreared only one preak at 2θ ≈ 22.3º, which characterized the amorphous structure of nanosilica. Based on the EDX spectrum (Fig. 2b) and XRD pattern (Fig. 2d), nanosilica generated from acid treated rice husk was of high purity and amorphous structure [20].

Fig.3: Photograph of 2% OC solution (left), mixture of 2% OC–2% nanosilica (right), and TEM image of OC–nanosilica.

Photographs of OC solution and mixture of OC–nanosilica were shown in Fig. 3 (left). It was observed that the suspension of OC–nanosilica mixture was homogenous and stable fairly. The TEM image in Fig. 3 (right) indicated that the nanosilica morphology was almost maintained as the origin (Fig. 2c), however some small parts were aggregated that may be presumed due to interaction of nanosilica with OC. Nanosilica (SiO_2) may be changed to $Si(OH)_4$ due to slightly basic medium pH ~7.5 [21]. According to our observation, OC in solution, unlike chitosan, is not precipitated in basic medium at pH 7.5 – 8.5.

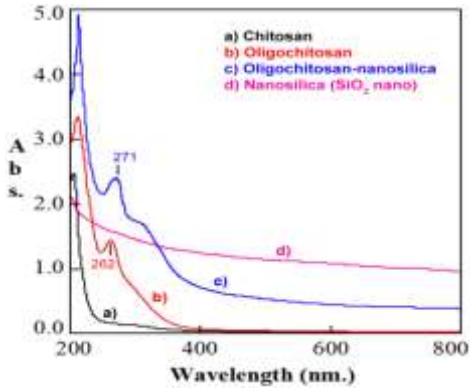


Fig.4: UV-Vis spectra of chitosan (a), OC (b), OC–nanosilica (c) and nanosilica (d).

http://dx.doi.org/10.22161/ijeab/2.1.53 *ISSN: 2456-1878*

The UV-Vis spectra in Fig. 4 showed that the appearance of new peak at 262 nm for OC (Mw ~5000), which was not observed for initial chitosan. The UV-Vis spectrum of nanosilica had no absorption in the range of 200 – 800 nm. The UV-Vis spectrum of OC–nanosilica solution had a peak at 271 nm and a small shoulder around 320 nm. The absorbance band at 262 nm was assigned to C=O in carbonyl groups, which formed in OC molecules during irradiation [25]. In the range of 200 – 800 nm, the nanosilica had no absorption (Fig. 4d), the same result was also obtained by Lu *et al.* (2009) [28]. While the UV-Vis spectrum of OC–nanosilica exhibited a shift of the 262 nm peak of oligochitosan to 271 nm and a shoulder in the range between 300 and 350 nm. This phenomenon may be due to an interaction of OC with nanosilica in the dispersion solution.

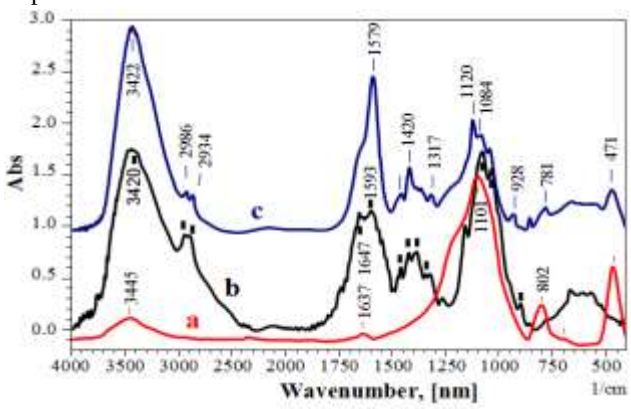


Fig.5: IR spectra of nanosilica (a), OC (b), and their mixture (c)

The FTIR spectroscopy was used to evaluate the interaction between OC and nanosilica. According to results reported our previous articles [17,22], the FTIR spectrum of resultant OC was almost not changed in comparison with that of initial chitosan, suggesting that the main chemical structures of chitosan still remained. The FTIR spectrum of OC (Fig. 5b), the characteristic peaks at 3462; 2850 – 3000; 1647; 1593; 1421; 1319; and 1031 – 1074 cm^{-1} assigned to the vibrations of –OH; C–H; amide I (C=O); amide II (N–H); –OH and C–H in –(CH_2OH); amide III (C–N) of –($NHCOCH_3$); and C–O–C bonds respectively were recorded [29]. The characteristic peaks of silica in Fig. 5a, particularly at 3444; 1637; 1103; 794; 491 cm^{-1} were attributed to stretching vibration of silanol groups (Si–OH); the H–O–H bending vibration of trapped water molecules in silica matrix; the asymmetric stretching; symmetric stretching; and bending vibration of O–Si–O linkages, respectively [30]. In the FTIR spectrum of the OC–nanosilca (Fig. 5c) presented the specific peaks of both OC and silica. Moreover, in this spectrum appeared some of new peaks at 927 cm^{-1} (vibration of silanol group) and the peaks at 1083, 781 cm^{-1} (assumed Si–O–C linkage), but concurrently the peak at 1647 cm^{-1} of OC was disappeared. In addition, the band 1593 cm^{-1} of $–NH_2$ bending vibrations in the spectrum of OC had a shift to 1579 cm^{-1} in the spectrum of OC–nanosilica. All changes in the spectrum of the mixture sample indicating that the interaction of OC and nanosilica in solution actually occurred.

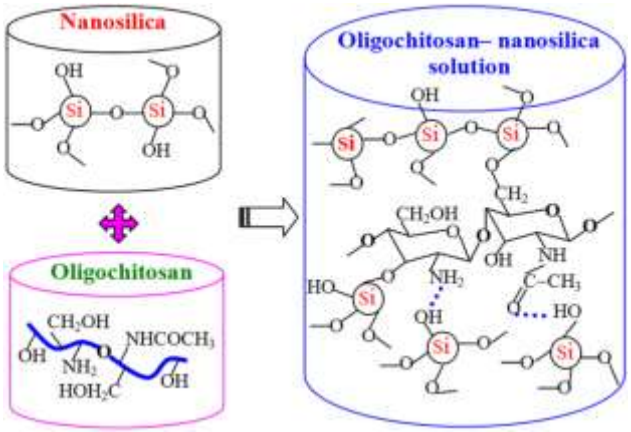


Fig.6: Schematic delineation for the interaction of OC with nanosilica in solution.

According to Al-Sagheer and Muslim (2010) argued that chitosan interacted with tetraethyl orthosilicate by formation of hydrogen bonds between amide groups of chitosan and silanol groups, covalent bonds of chitosan on silanol groups, and ionic bonds between chitosan amino groups and silanol groups of silica network [31]. In this work, however, the pH of OC–nanosilica solution was adjusted to ~7.5, so the ionic bonds between them were unlikely due to the $–NH_2$ groups non-protonated with pH > pKa ~6.3 and the silanol groups negative charge at pH higher than pI ~2 [32]. Consequently, we suggest interaction of OC with nanosilica in OC–nanosilica solution as in Fig. 6.

Table.2: Effect of OC and OC–nanosilica on plant height, dry weight and weight of 1000 soybean seeds.

Treatment	Plant height cm	Dry weight g/5 plants	1000 seed weight, g
Control (water)	45.1	100.1[a]	151.3
OC	48.5	129.7[b]	148.7
OC–nanosilica	48.6	137.3[b]	153.2
$LSD_{0.05}$	NS	28.7	NS

Mean values in each column with the same letter are not different at $P \leq 0.05$.

The results in Table 2 indicated that the treatment of OC and/or OC–nanosilica did not affect the height soybean plant and the weight of 1000 soybean seeds compared with the control. However, the dry weight of soybean plant was increased to 129.7 and 137.3 g/5 plants for OC and OC–nanosilica, respectively compared with the control (100.1 g/5 plants). These results clearly indi-cated

that OC and OC–nanosilica promoted the growth of soybean in term of dry weight of soybean plants.

Table.3: Effect of OC and OC–nanosilica on increase of seed yield and net profit for cultivation of soybean.

Treatment	Seed yield, ton/ha	Increase over control, %	Net profit over control, USD/ha
Control (water)	2.18[a]	-	-
OC	2.41[b]	10.5	120
OC–nanosilica	2.55[c]	17.0	220
$LSD_{0.05}$	0.12	-	-

Mean values in each column with the same letter are not different at $P \leq 0.05$.

The results in Table 3 presented the increase of seed yield of soybean of 10.5 and 17% for OC and OC–nanosilica, respectively compared with the control. Net profit was preliminarily calculated to be of 120 and 220 USD/ha for using OC and OC–nanosilica as plant growth promoter and seed yield enhancer for soybean cultivation based on the price of OC and OC–nanosilica and local labor expense. The results in Table 3 also indicated that nanosilica contributed significantly to increasing the seed yield of soybean together with OC. Although the weight of 1000 seeds was not significantly different among three treatments, but the seed yield even increased when treated with OC and OC–nanosilica. Moreover, further study of the effect of different concentration as well as synergistic effect of combined treatment of OC and nanosilica should be carried out. Khan *et al.* (2003) reported that application of chitin and chitosan oligomers to soybean leaf tissues caused increased activity of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase enzymes [33]. Results of the study by Luan *et al.* (2006) revealed that OC showed not only plant growth promotion effect but also enhancement of the activity of phytoalexin enzymes namely PAL and chitinase which help plants to prevent the infection of microbial diseases [11].

It is noteworthy that combined seed treatment and foliar application of chitosan increased total isoflavone content of mature soybean seeds by 16 to 93% compared to untreated plants [34]. They concluded that elicitors hold great promise as a way for increasing isoflavone content of mature soybean seeds. In addition, in certain conditions, the impacts of elicitors have on plants physiology and defense response may translate into yield increases, as observed by Luan *et al.* (2006) with 16% seed yield increase of soybean treated with OC [11]. Treatment of soybean seed with chitosan made also increase of seed yield but the concentration was of ten times higher in comparison with OC [10,35]. El-Sawy *et al.* (2010) also reported that OC with Mw of 5000 – 10.000 g/mol exhibited better effect on growth promotion and increase of seed yield of faba bean compared to that of chitosan with higher Mw [36]. Recently, Costales *et al.* (2016) reported that under field conditions, foliar application of both chitosan and OC enhances growth and nodulation of soybean plant [37]. However, it was surprising in their remarks that chitosan is more effective than OC. This hold contradictory result as above mentioned. More study works should be carried out to clarify the difference of the effect of chitosan and OC on plants.

IV. CONCLUSION

This study demonstrated that the method of gamma Co-60 ray irradiation degradation of chitosan in solution to prepare oligochitosan can be favorably applied on large-scale. Foliar application of oligochitosan or oligochitosan–nanosilica enhanced the plant growth and seed yield (10 – 17%) of soybean. Therefore, application of oligochitosan and/or oligochitosan–nanosilica may be recommended for soybean cultivation. However, more experiments on the effect of concentration as well as synergistic effect of combined treatment of oligochitosan and nanosilica should be carried out to draw a valid conclusion of foliar application of oligochitosan and oligochitosan–nanosilica for optimal improvement of soybean seed yield.

ACKNOWLEDGMENTS

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number “106-NN.03-2015.84”.

REFERENCES

[1] Garg A. and Balodi R. 2014. “Recent Trends in agriculture: Vertical farming and organic farming”. *Advances in Plants and Agriculture Research*, 1(4):00023.

[2] Stockdale E. A., Lampkin N. H., Hovi M., Keatinge R., et al. 2001. “Agronomic and environmental implications of organic farming systems”. *Advances in Agronomy*, 70(IN1):261–327.

[3] Kim K. W. and Thomas R. L. 2007. “Antioxidative activity of chitosans with varying molecular weights”. *Food Chemistry*, 101(1):308–313.

[4] Zheng L. Y. and Zhu J. F. 2003. “Study on antimicrobial activity of chitosan with different molecular weights”. *Carbohydrate Polymers*, 54(4):527–530.

[5] Qin C. D., Du Y. M., Xiao L., Li Z. and Gao X. 2002. “Enzymatic preparation of water soluble chitosan and their antitumor activity”. *International Journal of Biological Macromolecules*, 31(1-3):111–117.

[6] Benhamou N. 1996. "Elicitor-induced plant pathways". *Trends in Plant Science*, 1(7):233–240.

[7] Aziz A., Trotel-Aziz P., Dhuicq L., Jeandet P., Couderchet M. and Vernet, G. 2006. "Chitosan oligomers and copper sulfate induce grapevine defense reactions and resistance to gray mold and downy mildew". *Phytopathology*, 96(11):1188–1194.

[8] Yin H., Zhao X. and Du Y. 2010. "Oligochitosan: A plant disease vaccine-A review". *Carbohydrate Polymers*, 82(1):1–8.

[9] Chamnanmanoontham N., Pongprayoon W., Pichayangkura R., Roytrakul S. and Chadchawan, S. 2015. "Chitosan enhances rice seedling growth via gene expression network between nucleus and chloroplast". *Plant Growth Regulation*, 75(1):101–114.

[10] Dzung N. A. and Thang N. T. 2002. "Effect of oligoglucosamine prepared by enzyme degradation on the growth of soybean". *Advances in Chitin Science*, 5:463–467.

[11] Luan L. Q., Nagasawa N., Tamada M. and Nakanishi T. M. 2006. "Enhancement of plant growth activity of irradiated chitosan by molecular weight fractionation". *Radioisotopes*, 55(1):21–27.

[12] Huang S., Wang L., Liu L., Hou Y. and Li L. 2015. "Nanotechnology in agriculture, livestock, and aquaculture in China. A review". *Agronomy for Sustainable Development*, 35(2):369–400.

[13] Taha R. A. 2016. "Nanotechnology and its application in agriculture". *Advances in Plants and Agriculture Research*, 3(2):00089.

[14] Siddiqui M. H. and Al-Whaibi M. H. 2014. "Role of nano-SiO_2 in germination of tomato (*Lycopersicum esculentum* seeds Mill.)". *Saudi Journal of Biological Sciences*, 21(1):13–17.

[15] Suriyaprabha R., Karunakaran G., Yuvakkumar R., Prabu P., Rajendran V. and Kannan N. 2012. "Growth and physiological responses of maize (*Zea mays* L.) to porous silica nanoparticles in soil". *Journal of Nanoparticle Research*, 14:1294.

[16] Kiirika L. M., Stahl F. and Wydra K. 2013. "Phenotypic and molecular characterization of resistance induction by single and combined application of chitosan and silicon in tomato against *Ralstonia solanacearum*". *Physiological and Molecular Plant Pathology*, 81:1–12.

[17] Duy N. N., Phu D. V., Anh N. T. and Hien N. Q. 2011. "Synergistic degradation to prepare oligochitosan by γ-irradiation of chitosan solution in the presence of hydrogen peroxide". *Radiation Physics and Chemistry*, 80(7):848–853.

[18] ASTM International 2004. "Standard practice for use of a dichromate dosimetry system, ISO/ASTM 51401:2003(E)". Standards on dosimetry for radiation processing, 69–74.

[19] Brugnerotto J., Lizardi J., Goycoolea F. M., Argüelles-Monal W., Desbrières J. and Rinaudo, M. 2001. "An infrared investigation in relation with chitin and chitosan characterization". *Polymer*, 42(8):3569–3580.

[20] Wang W., Martin J. C., Zhang N., Ma C., Han A. and Sun L. 2011. "Harvesting silica nanoparticles from rice husks". *Journal of Nanoparticle Research*, 13(13):6981–6990.

[21] Tiraferri A., Maroni P., Rodríguez D. C. and Borkovec M. 2014. "Mechanism of chitosan adsorption on silica from aqueous solutions". *Langmuir*, 30(17):4980–4988.

[22] Hien N. Q., Phu D. V., Duy N. N. and Lan N. T. K. 2012. "Degradation of chitosan in solution by gamma irradiation in the presence of hydrogen peroxide". *Carbohydrate Polymers*, 87(1):935–938.

[23] Das S. N., Madhuprakash J., Sarma P. V., Purushothan P., et al. 2015. "Biotechnological approaches for field applications of chitooligosaccharides (COS) to induce innate immunity in plants". *Critical Reviews in Biotechnology*, 35(1):29–43.

[24] Taşkın P., Canisağ H. and Şen M. 2014. "The effect of degree of deacetylation on the radiation induced degradation of chitosan". *Radiation Physics and Chemistry*, 94:236–239.

[25] Tahtat D., Mahlous M., Benamer S., Khodja A. N. and Youcef S. L. 2012. "Effect of molecular weight on radiation chemical degradation yield of chain scission of γ-irradiated chitosan in solid state and in aqueous solution". *Radiation Physics and Chemistry*, 81(6):659–665.

[26] Feng T., Du Y., Li J., Hu Y. and Kennedy J. F. 2008. "Enhancement of antioxidant activity of chitosan by irradiation". *Carbohydrate Polymers*, 73(1):126–132.

[27] Mahmud M., Naziri M. I., Yacob N., Talip N. and Abdullah Z. 2014. "Degradation of chitosan by gamma ray with presence of hydrogen peroxide". *Advancing Nuclear Research and Energy Development, AIP conference Proceeding*, 1584(1):136-140.

[28] Lu J., Kosuda K. M., Van Duyne R. P. and Stair P. C. 2009. "Surface acidity and properties of TiO_2/SiO_2 catalysts prepared by atomic layer deposition: UV-visible diffuse reflectance, DRIFTS, and visible raman spectroscopy studies". *The*

Journal of Physical Chemistry C, 113(28):12412–12418.

[29] Mourya V. K., Inamdar N. N. and Choudhari Y. M. 2011. "Chitooligosaccharides: Synthesis, characterization and applications". *Polymer Science Series A*, 53(7):583–612.

[30] Rafiee E., Shahebrahimi S., Feyzi M. and Shaterzadeh M. 2012. "Optimization of synthesis and characterization of nanosilica produced from rice husk (a common waste material)". *International Nano Letters*, 2:29.

[31] Al-Sagheer F. and Muslim S. 2010. "Thermal and mechanical properties of chitosan/SiO_2 hybrid composites". *Journal of Nanomaterials*, 2010: Article ID 490679.

[32] Podust T. V., Kulik T. V., Palyanytsya B. B., Gun'ko V. M., Tóth A., Mikhalovska L,, Menyhárd A. and László K. 2014. "Chitosan-nanosilica hybrid materials: Preparation and properties". *Applied Surface Science*, 320:563–569.

[33] Khan W., Prithiviraj B. and Smith D. 2003. "Chitosan and chitin oligomers increase phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities in soybean leaves". *Journal of Plant Physiology*, 160(8):859–863.

[34] Al-Tawaha A. M., Seguin P., Smith D. L. and Beaulieu C. 2005. "Biotic elicitors as a means of increasing isoflavone concentration of soybean seeds". *Annals of Applied Biology*, 146(3):303–310.

[35] Zeng D., Luo X. and Tu R. 2012. "Application of bioactive coatings based on chitosan for soybean seed protection". *International Journal of Carbohydrate Chemistry*, 2012:Article ID 104565.

[36] El-Sawy N. M., El-Rehim H. A. A., Elbarbary A. M. and Hegazy E. A. 2010. "Radiation-induced degradation of chitosan for possible use as growth promoter in agricultural purposes". *Carbohydrate Polymers*, 79(3):555–562.

[37] Costales D., Falcón A. B., Nápoles M. C., de Winter J., et al. 2016. "Effect of chitosaccharides in nodulation and growth *in vitro* of inoculated soybean". *American Journal of Plant Sciences*, 7(9):1380–1391.

http://dx.doi.org/10.22161/ijeab/2.1.54 *ISSN: 2456-1878*

Haematological and Serum Biochemical Parameters of Mature Harco Cocks Treated with Human Menopausal Gonadotrophin (Diclair®) For Spermatogenesis

Egu U.N.

Department of Animal Science and Fisheries, Abia State University, PMB 7010, Umuahia, Nigeria

***Abstract*—** *Twenty sexually matured (24 weeks old) healthy Harco cocks were used to determine the effect of Gonadotrophin (Diclair®) on haematology and serum biochemistry. The cocks were divided into 4 treatment groups of 5 cocks per group identified as T_1 (control) administered with 1ml physiological saline, T_2, administered with 6.75i.u Diclair® and T_4, administered with 20.25i.u Diclair®, with one cock per replicate in a completely Randomized Design (CRD). The injections were dividedinto three doses each and administered intramuscularly in the thigh for three consecutive days. One week after Diclair® treatments, five birds from each group were bled from the wing veins for haematology and serum biochemistry. Results of this study showed significant differences ($P<0.05$) among the treatment groups in all the haematological parameters except mean corpuscular haemoglobin concentration and monocytes which were similar ($P>0.05$) among the treatment groups. Basophils were not detected among the treatment groups. The results further showed significant differences ($P<0.05$) among the treatment groups in the serum biochemical parameters except total serum protein which was similar ($P>0.05$) among the treatment groups. However, the values were within the normal ranges, indicating that Diclair® had no deleterious effect on these parameters.*

***Keywords*— Harco cocks, haematology, serum biochemistry, Diclair®.**

I. INTRODUCTION

For several decades, natural or synthetic hormones have been used to improve the productive and reproductive potentials of animals. In reproductive management of farm animals, human menopausal gonadotrophin is reputed to be effective in improving semen quality of local cocks (Abu *et al.,* 2006). Diclair® is a human menopausal gonadotrophin lyophilized in vials containing a mixture of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in a ratio of 1.1 (Dixon and Hopkins, 1996). Follicle stimulating hormone and LH present in Diclair® play vital role in the initiation of spermatogenesis. The hormone preparation is cheap readily available and does not require cold chain storage (Iheukwumere, 2005).

Haematological and serum biochemical parameters provide valuable information on the health status of animals (Iheukwumere *et al.,* 2006) and also reflect an animal's responsiveness to its internal and external environment (Esonu *et al.,* 2001; Anyaehie and Madubuike, 2004). The effects of such steroid hormones as androgens and estrogens on haematological values are well documented (Iheukwumere *et al.,* 2004). Though studies have been conducted on the haematological parameters of Nigerian domestic chickens(Ikhimioya *et a.*,2000; Iheukwumere*et al.,* 2008), there is no information on the effect of human menopausal gonadotrophin (Diclair®) on such parameters in Harco cocks. Therefore, this study was carried out to evaluate the effect of Diclair® on haematological and serum biochemical parameters of Harco cocks as well as to contribute to knowledge on avian haematology.

II. MATERIALS AND METHODS

Experimental Birds and their Management:

Twenty clinically sound and sexually matured (24 weeks old) Harco cocks purchased from Elgibbor farms in Isuikwuato Local Government Area, Abia State Nigeria, were used for this study. The birds were dewormed and vaccinated soon after purchase. A two-week pre-experimental period was allowed to enable the animals acclimatize. The birds were housed and raised on a deep-litter system. They were fed commercial Grower mash containing 20% CP and 2000 Kcal ME/kg diet twice daily (in the morning and evening). Water was provided *ad libitum.*

Experimental Design

The twenty sexually matured (2 weeks old) Harco cocks were divided into 4 treatment groups, identified as T_1, T_2, T_3, and T_4. Each treatment group consisted of 5 cocks with one cock per replicate in a Completely Randomized Design (CRD), with four levels of Diclair® as treatment. The levels of Diclair® were 0.00ml, 0.09ml, 0.18ml, and 0.27ml represented as T_1, T_2, T_3 and T_4 respectively. T_1 (Treatment 1) which contained no Diclair® served as the control. Diclair treatment was by intramuscular injection. The injection was administered as follows:

Diclair® was supplied in 2 vials each containing FSH 75i.u and LH 75i.u per ml.

Table.1: Doses of Diclair® Administered to Mature Harco Cocks.

Day	Treatment (Diclair® i.u)			
	T_1	T_2	T_3	T_4
1	0.00	0.03	0.06	0.09
2	0.00	0.03	0.06	0.09
3	0.00	0.03	0.06	0.09
Total	0.00	0.09	0.18	0.27

Table.2: Concentration of Diclair® Mature Harco Cocks.

Day	concentration of Diclair® (i.u)			
	T_1	T_2	T_3	T_4
1	0.00	4.50	9.00	13.50
2	0.00	4.50	9.00	13.50
3	0.00	4.50	9.00	13.50
Total	0.00	013.50	27.00	40.50

All treatments were administered intramuscularly on the thigh of each cock using a one ml syringe with 0.01ml graduation. Seven days after Diclair® injection, blood collection and haematological and serum biochemical evaluation were carried out.

Blood Collection and Haematological Analysis:

The cocks were bled one week after Diclair® injections between 9am and 10.30am from wing veins using needle and syringe and aspirated about 5ml of blood from each cock. Two millilitres of each blood sample were poured into Bijou bottles containing ethylene diamine tetra-acetic acid (EDTA) for haematological evaluation. The remaining 3ml of each blood sample were allowed to coagulate to produce sera for blood chemistry analysis.

Blood samples were analyzed within 2 hours of their collection for packed cell volume (PCV) and haemoglobin (Hb). Erythrocyte or red blood cells (RBC) and leucocyte counts were determined as described by Jain (1986). Erythrocyte count was dome in a haemocytometer chamber placed under a light microscope. Packed cell volume was determined by the micro haematocrit method (Jain, 1980) with 75x16mm capillary tubes filled with blood and centrifuged at 3000rpm for 5min. Haemoglobin concentration was also determined by the cyanmethemyoglobin method (Jain, 1986). Total leucocyte count was carried out using a neubaer haematocytometer placed under a light microscope under x 10 magnification, after using Natt and Henricks dilution to obtain a 1:200 blood dilution. Differential leucocyte count was achieved using blood smears stained with Wright's dye and each type of cell (neutrophil, lymphocyte, eosinophil, monocyte and basophil) were counted with a counter.

Blood Chemistry Analysis

The bottles of coagulated blood were subjected to standard methods of serum separation and the harvested sera were used for evaluation of serum biochemical parameters. Urea concentration was determined following method described by Baker and Silverton (1986). Aspertate transaminase, alanine transaminase and alkaline phosphatase activities were determined using spectrophotometric method as described by Rej and Hoder (1983). The standard flame photometry using Gallenkamp analysis was to determine serum calcium ion. Serum total protein was determined by Goldbery refractometer method as described by Kohn and Allen (1995). Albumin and globulin were determined using bromo cresol green (BCG) method as described by Randox (2006).

Data Analysis

http://dx.doi.org/10.22161/ijeab/2.1.54 *ISSN: 2456-1878*

Data collected on haematological and serum biochemical parameters of mature Harco cocks were subjected to one - way analysis of Variance (ANOVA) using the technique of steel and Torrie (1980). Significant treatment means were separated using Duncan's New Multiple Range Test as described by Obi (1990).

III. RESULTS AND DISCUSSION

The results of Diclair® administration on haematology of mature Harco cocks are shown on Table 3.

There were significant differences (P<0.05) among the treatment groups in PCV, HB, RBC, WBC, MCV, MCH and MCHC values. Cocks on T_2 recorded the highest value of 38.40% in PCV and this differed significantly (P<0.05) from cocks on T_1 and T_4 which were similar (P>0.05) to each other in PCV values and also similar (P>0.05) to cocks on T_3. There was no significant difference (P>0.05) between cocks on T_2 and T_3 in PCV values. The PCV values obtained in T_1 (34.00%) and T_4 (32.40%) were within the range of 28 – 37% reported by Simara*ks et al.* (2004) and Iheukwumere *et al.* (2006). However, the PCV values obtained for the whole treatment groups were within the range of (25-45%) reported for birds by Banerjee (2005) and Islam *et al.* (2004).

Cocks on T_2 recorded the highest Hb value of 12.84(g/dl) and thus differed significantly (P<0.05) from cocks on T_1 and T_4 which were similar (P>0.05) to each other in Hb value. There was no significant difference (P>0.05) between cocks on T_2 and T_3 in Hb values. Similarly, there was no significant difference (P>0.05) between cocks on T_1 and T_3. The lowest value in Hb was observed in cocks on T_4 (10.80g/dl). The Hb values obtained in this study were within the normal range of 7.0 – 13.0g/dl reported for birds (Jain, 1993). However, the Hb values obtained in this study were higher than the range of 9.36 ± 0.01 – 9.39 ± 0.00 (g/dl) reported by Iheukwumere *et al*. (2006) for Nigerian indigenous chickens, but lower than the range of 11.00 ± 2.15 – 14.85 ± 1.42 (g/dl) reported by Iheukwumere *et al.* (2008) for Nigerian Local cocks. Haemaglobin concentration of blood has been associated with availability of nutrients to the animal body (Esonu *et al.*, 2001).

Table.1: Effect of Diclair on Haematology of Mature Harco Cocks

Parameters	Treatment (Diclair® i.u)				
	T_1	T_2	T_3	T_4	
	0.00	6.75	13.50	20.25	SEM
PCV (%)	34.00^{b}	38.40^{a}	37.50^{ab}	32.40^{b}	1.28
Hb (g/dl)	11.30^{bc}	12.84^{a}	12.50^{ab}	10.80^{c}	0.41
RBC ($x10^6/mm^3$)	10.14^{b}	9.88^{b}	11.99^{a}	10.14^{b}	0.32
WBC ($x10^3/mm^3$)	6.86^{ab}	6.90^{ab}	7.00^{a}	6.50^{c}	0.05
MCV (fl)	33.40^{b}	39.90^{a}	35.20^{b}	32.00^{b}	1.35
MCH (pg)	11.10^{b}	13.30^{a}	11.80^{b}	10.70^{b}	0.39
MCHC (g/dl)	33.20	33.40	33.30	33.20	0.07

$^{abc:}$ Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

Cocks on T_3 recorded the highest RBC value of 11.99 ($x10^6/mm^3$) and this differed significantly (P<0.05) from cocks on T_1 , T_2 and T_4 which which were similar (P>0.05) to each other in RBC values.The lowest value RBC was observed in cocks on T_1 and T_4 which had 10.14 x $10^6/mm^3$ each. The RBC values obtained in this study were higher than the range of 2-4 ($x10^6/mm^3$) reported by Jain (1993) for birds but within the range of 8 – 11 x $10^6 mm^3$ reported by Simaraks *et al.* (2004). However, the RBC values obtained in this study were lower than the average 14.65 ($x10^6/mm^3$) reported by Kundu *et al.* (1993) and the highest values 13.35 x $10^6/mm^3$ reported by Ameh (2004) and 14.85 ± 2.36 x 10^6 / µl) reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This disparity in the values of RBC may not be unconnected to the differences in breed and nutritional status of the birds (Esonu *et al.*, 2001)

Cocks on T_3 recorded the highest value of 7.00 x $10^3/mm^3$ in WBC and this differed significantly (P<0.05) from cocks on T_4 (6.50x$10^3/mm^3$). There were no significant differences (P>0.05) among cocks on T_1, T_2 and T_3 in WBC values. Cocks on T_1 and T_2were significantly different (P<0,05) from those on T_4. The lowest value in WBC was observed in cocks on T_4 (6.50 x $10^3/mm^3$). The WBC values obtained in this study were lower than the range of 9.30 ± 0.00 – 9.64±0.03 ($x10^3/µl$) reported by Iheukwumere *et al.* (2006) for Nigerian indigenous chickens.

Abnormal production of white blood cells in the blood of animals is usually associated with immune response by animals due to the presence of an antigen (foreign body) in

the body. Elevation of white blood cells suggests infection by microorganisms especially bacterial (Aka *et al.*, 2008; Sowande *et al.*, 2008).

Cocks on T_2 recorded the highest value in MCV 39.90 (fl) differed and this significantly (P<0.05) from cocks on the control treatment (T_1), cocks on T_3 and T_4 which were similar (P>0.05) to each other in MCV values. The lowest value in MCV was observed in cocks on T_4 (32.00fl). The MCV values obtained in this study were lower than the highest value 40.00 ± 7.8(fl) reported by Iheukwumere *et al.* (2008) in Nigerian local cocks and lower than the value 41.00 ± 6.5(fl) reported by Iheukwumere and Herbert (2002) in broiler chickens, but higher than the average 27.32 ± 1.58(fl) reported by Ameh (2004) in Nigerian local cocks. Mean corpuscular volume is an indication of the average volume of blood cells (Lazzaro, 2003).

Cocks on T_2 recorded the highest MCH value of 13.30(pg) and this differed significantly (P<0.05) from cocks on the control treatment (T_1), cocks on T_3 and T_4 which were similar (P>0.05) to each other in MCH values. The lowest value of 10. 70(pg) in MCH was observed in cocks on T_4. The MCH values obtained in this study were lower than the mean value 33.90(pg) reported by Iheukwunere *et al.* (2002) in broiler chickens, and lower than the range of 21.30 ± 2.52 – 33.50 ± 2.13(pg) reported by Iheukwumere *et al.* (2008) in Nigerian local cocks. This disparity in the values of MCV may be attributed to differences in breed, physiological and nutritional status of the birds.

Cocks on T_2 recorded the highest numerical value of 33.40(g/dl) in MCHC. The lowest value of 33.20(g/dl) in MCHC was observed in cocks on T_1 and T_4. The MCHC values obtained in this study were lower than the value 35. 70% reported by Iheukwumere *et al.* (2002) in broiler chickens, but higher than the value 30. 56% reported by Ameh (2004) in Nigerian local cocks. However, the MCHC values obtained in this study were within the normal range of 26.0 – 35.0(g/dl) reported by Banerjee (2005) for chickens and by Islam *et al.* (2004) for local chickens in Bangladesh.

The results of Diclair® administration on differential leucocyte count of mature Harco cocks are shown on Table 2.

There were significant differences (P<0.05) among the treatment groups in neutrophil and lymphocyte values. Cocks onT_2 recorded the highest value of 55.60% in neutrophil and this differed significantly (P<0.05) from cocks on T_1 and T_4 which were similar (P>0.05) to each other and also similar (P>0.05) to cocks on T_3 in neutrophil values. There was no significant difference (P>0.05) between cocks on T_2 and T_4 in neutrophil values. The lowest value of 53.40% was observed in cocks on T_1 and T_4. The neutrophil values obtained in this study were higher than the normal range of 25-30% reported by Banergee (2005) for chickens. Neutrophils have phagocytic and bactericidal capabilities which means that they play an important role in inflammatory condition. They are very important for defense whenever acute infection is present (Banerjee, 2005).

Table.2: Effect of Gonadotrophin (Diclair®) on Differential leucocyte count of Mature Harco Cocks.

	Treatment (Diclair® i.u)				
	T_1	T_2	T_3	T_4	
Parameters	0.00i.u	7.02i.u	14.01i.u	21.00i.u	SEM
Neutrophils (%)	53.40^{b}	55.60^{a}	54.40^{ab}	53.40^{b}	0.51
Lymphocytes (%)	42.60^{b}	41.00^{c}	42.00^{c}	43.00^{a}	0.41
Eosinophils (%)	2.50	2.50	2.50	2.50	0.00
Monocytes (%)	1.00	1.00	1.00	1.00	0.00
Basophils (%)	0.00	0.00	0.00	0.00	0.00

abc: Means within row having different superscript are significantly (P<0.05) different. SEM = Standard error of means.

Cocks on T_4 recorded the highest value of 43.00% in lymphocyte and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_3. Cocks on T_2 and T_3 were similar (P>0.05) to each other in lymphocyte value, but differed significantly (P<0.05) from cocks on the control treatment (T_1). The lowest value of 41.00% in lymphocyte was observe in cocks on T_2. The lymphocyte values obtained in this study were within the normal range of 35-60% reported by Banerjee (2005) for chickens, suggesting that these blood cells can still perform their phagocytic and immune functions.

There were no significant differences (P>0.05) among the treatment groups in Eosinophil and monocyte values. The values recorded for these parameters were 2.50% and 1.00% respectively across the treatment groups. Basophils were not detected among the treatment groups.

The results of Diclair® administration on serum biochemical parameters of mature Harco cocks are shown on Table 3.

http://dx.doi.org/10.22161/ijeab/2.1.54 *ISSN: 2456-1878*

There were significant differences (P<0.05) among the treatment groups in urea, alkaline phosphatase (ALT), glucose, cholesterol, calcium, total serum protein, albumin and globulin values.

Cocks on T_4 recorded the highest value of 29.74(mg/dl) in serum urea and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_3 which were similar (P>0.05) to each other in urea values. The lowest value of 10.20(mg/dl) in urea was observed on cocks on the control treatment (T_1). The urea values obtained in this study were lower than the range of 30.46 ± 2.51 – 54.08 ± 0.11 (mg/dl) reported by Iheukwumere *et al.* (2006) in Nigerian chickens. This disparity in urea values may be attributed to differences in bred and nutritional status of the birds. It has been observed that serum urea content depends on both the quantity and quality of protein supplied in the diet (Iheukwumere and Herbert, 2003).

Table.3: Effect of Gonadotrophin (Diclair®) on serum Biochemistry of Mature Harco Cocks.

Parameters	Treatment (Diclair® i.u) T_1 0.00	T_2 6.75	T_3 13.50	T_4 20.25	SEM
Urea (mg/dl)	10.20[b]	10.80[b]	11.44[b]	29.74[a]	0.95
Alkaline Phosphatase Aspartate (iu/L)	73.60[c]	80.00[b]	80.00[b]	81.00[a]	0.66
Transaminase (iu/L)	0.00	0.00	0.00	0.00	0.00
Alanine ttransaminase (iu/L)	0.00	0.00	0.00	0.00	0.00
Glucose (mg/dl)	151.40[b]	148.60[b]	176.40[a]	132.60[c]	3.17
Cholesterol (mg/dl)	109.60[d]	118.00[c]	120.00[b]	128.00[a]	0.66
Calcium (mg/dl)	8.06[b]	8.10[b]	8.70[a]	8.94[a]	0.18
Total serum protein (g/l)	6.66	6.24	6.30	6.70	0.25
Albumin (g/L)	3.40[b]	4.06[a]	4.16[a]	3.14[c]	0.06
Globulin (g/L)	3.26[b]	2.20[c]	2.14[c]	3.62[a]	0.09

[abc:] Means within row having different superscript are significantly (P<0.05) different.
SEM = Standard error of means.

Cocks on T_4 recorded the highest value of 81.00iu/L in Alkaline phosphatase and this differed significantly (P<0.05) from cocks on T_1, T_2 and T_3. Cocks on T_2 and T_3 were similar (P>0.05) to each other in Alkaline phosphatase value, but differed significantly (P<0.05) from cocks on T_1. The lowest value of 73.60iu/L in alkaline phosphatase was observed in cocks on T_1. The Alkaline phosphatase values obtained in this study were lower than the normal value 482.5u/L reported by Kaneko *et al.* (1997) for chicken. This disparity may not be unconnected to the differences in breed and physiological status of these birds. Alkaline phosphatase assay is useful in the diagnosis of obstructive liver disease (Murray *et al.*, 2003).

Aspartate transaminase and Alanine transaminase were not detected among the treatment groups. An increase in Alkaline phoshatase, Alanine transaminase and Aspartate transaminase values would signify necrosis or myocardial infarction which are all indicators of drug toxicity or harmful chemicals in the body (Nelson and Cox, 2005). In this regard Diclair® can be considered safe for the cocks as the values in the liver enzyme activity of the treatment groups were below the normal value 482.5u/L reported by Kaneko *et al.* (1997) for chicken.

Cocks on T_3 recorded the highest value of 176.40(mg/dl) in serum glucose and this differed significantly (P<0.05) from cocks on the control treatment, cocks on T_2 and T_4. Cocks on T_1 and T_2 were similar (P>0.05) to each other in glucose values, but they differed significantly (P<0.05) from cocks on T_4. The lowest value of 132.60mg/dl in serum glucose was observed in cocks on T_4. The glucose values obtained in this study were within the normal range of 125-200mg/dl reported by Banerjee (2005) for birds. Glucose is one of the metabolites measured as an indicator of the energy status of animals. Normal glucose levels in the cocks indicate adequate synthesis in the liver from propionate metabolism as the major glucose precursor (Sowande, *et al.*, 2008).

Cocks on T_4 recorded the highest value of 128.00mg/dl in serum cholesterol and this differed significantly ($P<0.05$) from cocks on the control treatment (T_1) cocks on T_2 and T_3 which were also significantly different ($P<0.05$) from each other in cholesterol values. The lowest value of 109.60mg/dl in cholesterol was observed in cocks on the control treatment.

The cholesterol values obtained in this study were within the normal range of 52-148mg/dl reported by Banerjee (2005) for birds. This implies that Diclair® injection was safe for the birds, so birds treated with Diclair® injection may not face the risk of myocardial infarction usually associated with high blood cholesterol content and emaciation due to low serum cholesterol (Frandson, 2002).

Cocks on T_4 recorded the highest value of 8.94(mg/dl) in serum calcium and this differed significantly ($P<0.05$) from cocks on T_1 and T_2 which were similar ($P>0.05$) to each other in calcium values. There was no significant difference ($P>0.05$) between cocks on T_4 and T_3 in calcium values. The lowest value of 8.06mg/dl in calcium was observed in cocks on the control treatment (T_1). The calcium values obtained in this study were lower than the mean value 28.4mg/dl reported by Kaneko *et al.* (1997) for chicken. The similarity observed in cocks on T_3 and T_4 indicates probable electrolyte balance in the cocks' body caused by gonadotrophin administration. This observation is in agreement with the report of Iheukwumere *et al.* (2004) in WAD goats.

Cocks on T_4 recorded the highest numerical value of 6.70g/dl serum total protein. The lowest value of 6.24g/dl in serum total protein was observed in cocks on T_2. The serum total protein values obtained in this study were lower than the range of $7.6 \pm 0.27 - 8.2 \pm 0.30$ mg/dl reported by Iheukwumere *et al.* (2006) for Nigerian chickens. This variation in values of serum total protein may not be unconnected to the differences in breed and nutritional status of the birds (Esonu, *et al.*, 2001).

Cocks on T_3 recorded the highest value of 4.16(g/dl) in serum albumin and this differed significantly ($P<0.05$) from cocks on T_1 and T_4 which were also significantly different ($P<0.05$) from each other in albumin value.

There was no significant difference ($P>0.05$) between cocks on T_3 and T_2 in albumin values. The lowest value of 3.14g/dl in serum albumin was observed in cocks on T_4. The serum albumin values obtained in this study were higher than the range of $3.1 \pm 0.27 - 3.5 \pm 0.22$mg/dl reported by Iheukwumere *et al.* (2006) for Nigerian chickens. Low albumin suggests poor clotting ability of blood and hence poor prevention of hemorrhage (Robert *et al.*, 2000).

Cocks on T_4 recorded the highest value of 3.62g/dl in serum globulin and this differed significantly ($P<0.05$) from cocks on T_1, T_2 and T_3. Cocks on T_2 and T_3 were similar ($P>0.05$) to each other in serum globulin value, but differed significantly ($P<0.05$) from cocks on the control treatment (T_1). The lowest value of 2.14 g/dl in serum globulin was observed in cocks on T_3. The serum globulin values obtained in this study were within the range of 2.1 – 3.7g/dl reported for birds by Banerjee (2005). Babatunde and Oluyemi (2006) opined that the higher the value of globulin, the better the ability to fight against disease.This implies that cocks on T_4 which recorded the highest value of 3.62g/dl in globulin had the best ability to resist disease.

IV. CONCLUSION

From the results of this study, it can be concluded that human menopausal gonadotrophin (Diclair®) had no deleterious effect on haematological and serum biochemical parameters of Harco cocks. Though most of the values obtained fall within the normal ranges for chicken, the variations observed suggest the need to constantly monitor blood Profile of Harcococks under Diclair® treatment for spermatogenesis.

REFERENCES

[1] Abu, A.H., Ameh, M. and Iheukwumere, F.C. (2006). Semen quality of Nigerian Local Cocks Treated with Human Menopausal Gonadotrophin (Pergonal®). Livestock Research for Rural Development.

[2] Aka, L.O., Eze, L., Ofor, G.C. and Igbokwe, C.O. (2008). Time dependent postpartum Haematological, Biochemical and Rectal Temperature Changes in West African Dwarf Ewes. Nigerian Society for Animal Production Proceeding 23rd Annual Conference pp. 111-115.

[3] Ameh, M. (2004). Effect of Pergonal® on semen Quality, Haematological values and carcass characteristics of the Nigerian local cocks. M.Sc. Thesis Department of Animal Science and Fisheries, Abia State University, Umuahia, Nigeria.

[4] Anyaehie, A.A. and madubuike, F.N. (2004). Effect of supplementation of High fibre (Maize Cob) full fat Soybean-Based Diets with a Commercial Enzymes Mixture on Performance and Haematological characteristics of Broiler Chicks Proc. 9th Ann. Conf. Anim. Sci. of Nig. (ASAN), Ebonyi State University, Abakaliki Nigeria, 13th – 16th September, 2004, pp. 90-93.

[5] Babatunde, B.B. and Oluyemi, J.A. (2006). Comparative Digestibility of three commonly used

Fibrous Ingredient in Maize, soybean meal and fish Diet by Broiler Chicks *Trop. J. Anim. Sci.*, 3:105-111.

[6] Baker, F.J. and Silverton, R.F. (1986). Introduction to Medical Laboratory Technology 6th Edn. Butter-Worth England.

[7] Banerjee, G.C. (2005). A textbook of Animal Husbandry 8th Edition pp. 124.

[8] Dixon, T.A. and Hopkins, G.J. (1996). Super ovulation in cattle using porcine pituitary gonadotrophin preparation (Plusset Serono) in: Plusset Scientific Literature Serono Veterinary, Rome Italy, pp. 22-23.

[9] Esonu, B.O., Emelalom, O.O., Udedibie, A.B.I., Herbert, U., Ekpor, C.F., Okoli, I.C. and Iheukwumere, F.C. (2001). Performance and Blood Chemistry of Weaner pigs Fed Raw Mucuna bean (Velvet bean) meal. Trop. Anim. Prod. Invest. 4: 49-54.

[10] Frandson, R.D. (2002). Anatomy and Physiology of Farm Animals 3rd Ed. Published by Bialiere Tindal, London pp. 32-54.

[11] Iheukwumere, F.C., Herbert, U. and Ewulu, C. (2002). Effect of Quantitative feed Restriction on Broiler Chickens *J. sustainable Trop. Agric. Res.*, 4:56-60.

[12] Iheukwumere, F.C. and Herbert, U. (2003). Physiological Responses of Broiler Chickens to Quantitative water Restriction, Haematology and serum Biochemistry. *International Journal of Poultry Sciences 2* (2): 117-119.

[13] Iheukwumere, F.C., Herbert, U. and Iloeje, M.U. (2004). Haematological and serum Biochemical values of West African Dwarf Does Following FSH + LH (Pergonal®) Treatment *Int. J. Agric. Rural Dev.*, 5:54-60.

[14] Iheukwumere, F.C. (2005). Super ovulation in Goats In: Afam Anene and Nwaigbo, L.C. (eds). Issues in sustainable Agriculture in Nigeria. Osprey Publication Centre, Owerri, Nigeria, 1 – 9.

[15] Iheukwumere, F.C., A.H. Abu and M. Ameh, (2006). Effect of Human Menopausal Gonadotrophin on Haematological and serum Biochemical parameters of Nigerian Indigenous chickens. *International Journal of Poultry Sciences 5* (7): 632-634.

[16] Iheukwumere, F.C., Abu, A.H. and Ndubuisi, E.C. (2008). Effect of FSH and LH (Pergonal®) Treatment on Haematology, Immune Status and serum Metabolites of West African Dwarf Goats. *Journal of Animal and Verterinary Advances 7* (1): 46-50.

[17] Ikhimioya, I.A., I.T. Arifeniwa, A. Oteku and A. Ahmed, (2000). Preliminary Investigation on the Haematology of Nigerian Indigenous chicken. Proc. 5th Ann. Conf. Anim. Sci. Assoc. Nigeria. Sept. 19-22, Port-Harcoutr, Nigeria pp. 10-12.

[18] Islam, M.S., N.S. Lucky, M.R. Islam, A. Ahadi, B.R. Das, M.M. Rahman and M.S.I. Siddini, (2004). Haematological Parameters of Fayoumi Assil and Local Chickens Reared in Bangladesh. *Int. J. Poultry Sci.*, 3:144-147.

[19] Jain, N.C. (1986). Schalm's Veterinary Haematology, 4th Ed. Lea and Ferbiger, Philadelphia.

[20] Jain, N.C. (1993). Essential of Veterinary Haematology, Lea and Ferbiger, Philadelphia.

[21] Kaneko, J.J., Harvey, J.W. and Bruss, M.I. (1997). Clinical Biochemistry of Domestic Animals 5th Edition. Academic press. San Diego, Carlifonia p. 885-905.

[22] Kohn, R.A. and Allen, M.S. (1995). Enrichment of Proteolytic Activity Relative to Nitrogen in preparation from the Rumen for in vitro studies. *Anim. Food Sci. Tech*. 52 (112): 1-4.

[23] Kundu, A.K.B.P., Mohanty, S.C., S.C. Mishra and M.S. Mistra (1993). Age related changes in the haematology of Guinea fowls. *Ind. J. Pout. Science* 28: 200-207.

[24] Lazzaro, J. (2003). Normal Blood Chemistry for Goats, Sannendoah Dairy Goats. http/www.saanendoah.com/bloodvalues.html//measure.

[25] Murray, R.K., Granner, D.R., Mayes, P.A. and Rodwell, V.W. (2003). Herpers illustrated Biochemistry 26th Edn. McGraw Hill Companies, Inc. U.S.A. pp. 693.

[26] Nelson, D.L. and Cox, M.M. (2005). Lehninger Principles of Biochemistry 4th Edn. W.H. Freeman and Company New York. pp. 119.

[27] Obi, I.U. (1990). Statistical Method of Detecting Differences between Treatment means. Snaap press 2nd Ed. Enugu. Nigeria 24-35.

[28] Randox, (2006). www.Raidox.com/Randox.laboratoryLtd.users'Manual.

[29] Rej, R. and Hoder, M. (1983). Alanine Transaminase. In: Methods of Enzymatic Analysis 3rd Edition H.U. Bergmeyer, I. Berg Meyer and M. grassal (eds). Weinhen Velag.

[30] Robert, K.M., Danyl, K.G., Peter, A.M. and Victor, W.R. (2000). Mayers Biochemistry, 25th Edn. McGraw Hill. New York, pp. 763-765.

[31] Simaraks, S., O. Chinrasri and S. Aengwanich, (2004). Haematological, Electrolyte and serum Biochemical values of the Thai Indigenous chickens (*Gallus*

http://dx.doi.org/10.22161/ijeab/2.1.54 *ISSN: 2456-1878*

domesticus) in North Eastern Thailand-Song Klanakarin *J. Sci. Tec.*, 26:425.

[32] Sowande, O.S., Aina, A.B.J., Oguntona, E.B., Fanimo, A.O., Unaka, V.U., Itassan, T.A., Oseni, M.O. (2008). Performance Blood Chemical Constituents and Mineral Balance of West African Dwarf Sheep Fed preserved Elephant Grass, Layer's Droppings and cassava peel Diet during Dry season. *Nig. J. Anim. Prod. 2008* : 35 (1): 90-102.

[33] Steel, R.G.D. and Torrie, J.H. (1980). Principles and procedures of statistics. A Biometric Approach 2nd Ed. Mc. Graw-Hill Book Co. Inc. New York.

http://dx.doi.org/10.22161/ijeab/2.1.55 *ISSN: 2456-1878*

Kidney Function Test, Weight Gain and Serum Protein Values of Mature Male Turkeys Treated with Gonadotrophin (Diclair®) For Sperm Production

Egu U. N.

Department of Animal Science and Fisheries, Abia State University, PMB 7010, Umuahia, Nigeria

Abstract— *Sixteen sexually matured (12 months old) healthy male turkeys were used to determine the effect of Gonadotrophin (Diclair®) on kidney function, weight gain and serum protein values. The turkeys were divided into 4 treatment groups, identified as T_1 (control) administered with 1.00ml physiological saline (0.00 i.u Diclair®), T_2 , administered with 13.50 i.u Diclair®, T_3,administered with 27.00i.u Dicliar®T4, administered with 40.50 i.u Dicliar(R), with one turkey per replicate in a completely Randomized Design (CRD). The injections were divided into 3 doses each and administered intramuscularly in the thigh for three consecutive days. Blood was collected one week after Diclair® administration. Four turkeys were randomly selected fro-m each treatment groupand bled to collect blood for blood chemistry analysis. The turkey were weighed every week for five weeks and their weight recorded. The result showed that there were significant differences ($P< 0.05$) among the treatment groups in all parameters for kidney function test: chronicle, potassium, sodium, bicarbonate expect creatinine which was similar ($p > 0.05$) among the treatment groups. The results further showed that there were no significant differences ($p > 0.05$) among the treatment groups in initial body weight. However, there were significant differences ($P< 0.05$) among the treatment groups in final body weight and weight gain. Similarly there were significant differences ($P< 0.05$) among the treatment groups in all the serum protein values measure: albumin, globulin, serum total protein as well as albumin/globulin ratio. The results of the study showed that Diclair enhanced kidney function and weight gain without any deleterious effects on serum protein values of the male turkeys.*

Keywords— Diclair®, Kidney function, weight gain, serum proteins male turkeys.

I. INTRODUCTION

Turkeys (*Meleagris gallopara*) are birds that originated in North America, that were domesticated in Europe and are now an important source of food in many parts of the world (Brant, 1998). Turkey occupies an important position next to chicken, duck, guinea fowl and quail in contributing to the most evolving sector which is playing a significant role in augmenting the economic and nutritional status of varied population (Katie and Frazer, 1988). All over the world turkeys are reared for their tasty and high quality meat (probakaran, 2003). Hence they are kept because of the economic service they render (Okendo, 2005) such as eggs, meat, feathers and sometimes pet.

In order to carry out any sustainable improvement in livestock, there should be methods of ensuring the repeatability and multiplication of desired traits in subsequent generations. To get the fullest benefits from the breeding turkenys therefore, a good knowledge of their sperm production is essential as well as their sperm output. In view of the increasing use of livestock for specialized production, there is need for more practical and batter control methods of reproduction.

For several decades natural or synthetic hormones have been used to improve the productive and reproductive potentials of animals. In reproductive management of farm animals, human menopausal gonadotriophin is reputed to be effective in improving semen quality of local cocks (Abu *et al.*, 2006).

Diclair®, also known as Humegon or mentrophim is a human menopausal gonadotrophin lyophilized in vials containing a mixture of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in a ratio 1:1 (Dixon and Hopkins, 1996). Follicle stimulating hormone and LH present in Diclari® play vital role in the initiation of spermatogenesis. The hormone preparation is cheap readily

available and does not require cold chain storage (Iheukwumere, 2005).

It has not been determined if the administration of the hormone preparation for spermatogenesis and semen production would induce any side effects on the kidney function, weight gain and serum protein values of the turkeys. This study was therefore carried out to determine the effect of Diclair® administration on kidney function, body weight gain and serum proteins of mature male turkeys.

II. MATERIALS AND METHODS.

Experimental Birds and their Management

Sixteen healthy sexually matured male turkeys aged 12 months were used for this study. The turkeys were purchased from the local markets and housed in clean pens. Routine management practices were carried out which include deworming, daily observation of birds to identify sick ones, maintaining clean and dry litter and vaccination against diseases. The turkeys were fed Grower Mash. Feed and water were provided *ad libitum* throughout the 28 days duration of the experiment. They were weighed every week and their weights were recorded.

Experimental Design and Drug Administration

Sixteen male turkeys were divided into 4 treatment groups consisting of 4 turkeys per group with one turkey per replicate in a Completely Randomized Design (CRD. These groups were assigned to 4 levels of Diclair® injection as treatments. The levels of Diclair® were 0.00i.u, 20.25i.u, 40.50i.u, and 60.75i.u Diclair® represented as T_1, T_2, T_3, and T_4 respectively. The group which received 0.00i.u Diclair® (T_1) served as the control.

Diclair® was supplied in 3 vials, each containing FSH 75i.u and LH 75i.u. The content of each vial was dissolved in 1ml of physiological saline solution immediately prior to use,resulting in a solution DFSH 75I.U plusDLH 75I.U per ml.

Table.1: Doses of Diclair® Administered to Mature Male Turkeys

	Day	**Treatment Dosage (ml)**		
	T_1	T_2	T_3	T_4
1	0.00	0.03	0.06	0.09
2	0.00	0.03	0.06	0.09
3	0.00	0.03	0.06	0.06
Total	0.00	0.09	0.18	0.27

Table.2: concentration of Diclar® on Mature Male Turkeys

Day	concentration of Diclair® (i.u)			
	T_1	T_2	T_3	T_4
1	0.00	4.50	9.00	13.50
2	0.00	4.50	9.00	13.50
3	0.00	4.50	9.00	13.50
Total	0.00	013.50	27.00	40.50

All treatments were administered intramuscularly in the breast muscle of each turkey using a one ml syring with 0.01 ml graduation

Blood collection and Evaluation of Blood chemistry

The turkeys were bled one week after Diclair(R)injections between 9am and 10.30am from punctured wing vein and aspirated about5ml of blood from each turkey.The blood samples were poured into plain bottles and were allowed to coagulate to produce sera for blood chemistry analysis. The bottles of coagulated blood were subjected to standard methods of serum separation and the harvested sera were used for biochemical evaluation. The standard flame photometry using Gallenkamp analysis was used to determine serum sodium (Na^+) ion and potassium (K^+) ion. While bicarbonate and chloride ions were assayed according to the methods of Baker and Silverton (1986). Creatinine concentration was also determined following methods described by Baker and Silverton (1986). Serum total protein was determined by Goldbery refractometer method as described by Kohn and Allen (1995). Albumin and globulin were determined using bromocresol green (BCG) method as described by Randox (2006).

Body weight measurement

http://dx.doi.org/10.22161/ijeab/2.1.55 *ISSN: 2456-1878*

Body weight of the birds were measured in kilogram using a 20kg weighing scale.

Data Analysis

Data collected on kidney function test, body weight and serum protein values of mature male turkeys were subjected to one-way analysis of variance (ANOVA) using the technique of steel and Torrie (1980). Significant treatment means were separated using Duncant's New Multiple Range Test as described by Obi (1990).

III. RESULTS AND DISCUSSION

The results of Diclair®administration on kidney function of mature male turkeys are shown in table 3.

There were significant difference (P< 0.05) among the treatment groups in all the parameters measured for kidney function: Sodium, potassium, Chloride and carbonate. Serum creatinine was similar(P> 0.05) among the treatment grows.

Turkeys on T_2 recorded the highest value of 147.10 (mmol/L) in serum Sodium and this differed significantly (P< 0.05) from turkeys on T_1, T_3 and T_4 which were also significantly different (P< 0.05) from each other in sodium values. The lowest value in serum sodium was observed in turkey on T_4 (126.13mmol/L). The sodium values obtained in this study were lower than the range of 148-163 (mmol/L) reported by Jain (1993) for birds, and lower than the range of 131.30-136.14 (mmol/L) reported by Iheukwumere *et al.* (2006) in Nigerian indigenous chickens, but higher than the range of 56-59(mmol/L) reported by Iheukwumere *et al.* (2002) This could be attributed to breed and physiological status of the birds. Serum electrolytes play important roles in physiological processes involved in homeostasis.

Turkeys on T_4 recorded the highest value of 5.12 (mmol/L) in serum potassium and this differed significantly from turkeys T_1,T_2 andT_3 which were also significantly different(P<0.05) from each other in potassium values.The lowest value in serum potassium was observed in turkeys on T_1 (4.05 mmol/L). The potassium values obtained in this study were within the range of 4.6- 6.5 (mmol/L) reported by Jain (1993) for birds, but higher than the range of 1.43 $\pm$ 0.02 – 1. 74 $\pm$ 0.15 (mmol/L) reported by Iheukwumere *et al.* (2006) in Nigeria indigenous chickens and higher than the range of 1.55 – 1. 80 (mmio/L) reported by Iheukwumere *et al.* (2002) in broiler chickens. Potassium is excreted in the kidney and elevations of plasma potassium is indicative of under excretion suggesting kidney impairment. When plasma protassium is low, the level of sodium in plasma is elevated. Thus they help in depolarization and repolarization in the nerve cells and muscle cells and in the transmission of impulses in the nerve cells, intracellular and extracellular fluids.

Turkey on T_1 recorded the highest value of 96.13 (mmol/L) in serum chloride and this differed significantly (P< 0.05) from turkeys on T_2, T_3 and T_4 which were also significantly different (P< 0.05) from ach other in chloride values. The lowest value in serum chloride was observed in turkeys on T_4 (81.07 mmol/L). The serum chloridle values obtained in this study were higher than the range of 33.00 – 34.10 (mmol/L) reported by Iheukwumere *et al.* (2002) in broiler chickens, but lower than the range of 144-120 (mmol.L) reported in Thai chickens by simaraks *et al.* (2004) and lower than the range of 130.38 $\pm$ 0.17 – 132. 30 $\pm$ 1.27 (mmol/L) reported by Ihekwumere *et al.* (2006) in Nigerian indigenous chickens.

Turkeys on T_3 recorded the highest value of 22.65 (mmol/L) in serum bicarbonate and this differed significantly (P< 0.05) from turkeys on T_1, T_2 and T_4 which were also significantly different (P< 0.05) from each other in bicarbonate values.The lowest value serum bicarbonate was observed in turkeys on T_1 (21.99 mmol/L). The serum bicarbonate values obtained in this study were higher than the range of 13.44 $\pm$ 0.38 – 15. 60 $\pm$ 0.22 (mmol/L) reported by Iheukwunwre *et al.* (2006) in Nigerian indigenous chickens and higher than the range of 14.80 – 15.60 (mmol/L) reported by Iheukwumere *et al.* (2002) in broiler chickens. Bicar6bonate is used in the buffering system in the blood, extracellular fluid and kidney (Brackett, 2005).

There were no significant differences (P> 0.05) among the treatment groups in serum creatimine. Turkeys on T_2 recorded the highest numerical value of 4.84 (mmol/L) in serum creatinine. The lowest numerical value of 1.35 (mmol/L) in serum creatinine was observed in turkeys on T_4. The serum creatinine values obtained in this study were higher than the range of 1 – 2 mg/dl reported for birds (Reece and swenson, 2004; Banerjee, 2005), but lower than the range of 18.00 – 18.50 mg/100ml reported by Ihekwumere *et al.* (20002) in broiler chickens. Creatinine measurement is used almost exclusively in the assessment of kidney function. The rate of production of creatinine is constant and elevations of plasma creatinine are indicative of under excretion suggesting kidney impairment.

http://dx.doi.org/10.22161/ijeab/2.1.55 *ISSN: 2456-1878*

Table.3: Effect of Diclair® on Kidney function of Mature Male Turkeys

Parameters	Treatment of (Diclair® i.u)				
	T_1 0.00	T_2 13.50	T_3 27.00	T_4 40.50	SEM
Sodium (mmol/L)	134.33^{c}	147.10^{a}	145.13^{b}	126.13^{d}	0.45
Potassium(mmol/L)	4.05^{d}	4.63^{b}	4.17^{c}	5.12^{a}	0.05
Chloride (mmol/L)	96.13^{a}	82.10^{c}	82.97^{b}	81.07^{d}	0.21
Bicarbonate (mmol/L)	21.99^{d}	22.17^{c}	22.63^{a}	22.46^{b}	0.02
Creatinine (mmol/L)	1.42	4.84	1.52	1.35	1.81

$^{abcd:}$ Means within row having different superscript are significantly (P< 0.05) different. SEM = standard error of means

Table.4: Effect of Diclar® on Body Weight Gain of Mature Male Turkeys

parameters	Treatment Diclar® i.u)				
	T_1	T_2 13.50	T_3 27.00	T_4 40.50	SEM
InitialbodyWeigh(kg)11.20		11.23	11.23	11.30	0.09
Final body weight (kg)	13.33^{b}	13.37^{b}	13.53^{b}	14.03^{a}	0.16
Body Weight gain (kg)	2.13^{b}	2.14^{b}	2.30^{b}	2.73^{a}	0.14

$^{ab:}$ Means within row having different superscripts are significantly(P<0.05) different.SEM = Standard error of means.

Table.5: Effect of Diclair® on Serum Protein Values of Mature Male Turkeys

parameters	Treatment Diclar® i.u)				
	T_1 0.00	T_2 13.50	T_3 27.00	T_4 40.50	SEM
Albumin (g/L)	3.20^{c}	4.43^{a}	4.32^{b}	3.12^{C}	0.03
Globulin (g/L)	2.61^{a}	1.67^{d}	2.36^{ab}	1.97^{c}	0.00
Globulin/Abumin ratio	0.82^{a}	0.38^{b}	0.56^{ab}	0.63^{ab}	0.09
Serum total protein (g/L)	5.73^{c}	6.13^{b}	6.67	5.17^{a}	0.03

$^{abcd:}$ Means within row having different superscript are significantly (P< 0.05) different. SEM = Standard error of means.

The results of Diclair® administration on body weight gain of mature male turkeys are shown in Table 4.There were significant differences (P< 0.05) among the treatment groups in final body weight and weight gain. However, there were no significant differences (P> 0.05) among the treatment groups in initial body weight.

Turkey on T_4 recorded the highest valve of 14.03kg in final body weight and this differed significantly (P< 0.05) from turkeys on T_1, T_2 and T_3 which were similar (P> 0.05) to each other in body weight.The lowest value of 13.33kg in final body weight was observed inturkeys on T_1.

Turkeys on T4 recorded the highest value of 2.73kg in body weight gain this differed significantly (P<0.05) from turkeys on T_1, T_2 and T_3 which were similar (P>0.05) to each other body weight gain. The lowest value of 2.13kg in weight gain was observed in turkeys on T_1.

The observation in this study that the group that received the highest dose of Diclair® recorded the highest final body weight and weight gain suggest that 40.50 i.u/ turkey within 3 days given in this study could have increased metabolism and efficient utilization of nutrients that resulted in increase final body weight and weight gain.

http://dx.doi.org/10.22161/ijeab/2.1.55 *ISSN: 2456-1878*

The results of Diclair®administration on serum protein values of mature male turkeys are shown in Table 5. There were significant differences (P< 0.05) among the treatment groups in all the serum proteins measured: albumin, globulin, serum total protein as well as globulin/albumun ration.

Turkeys on T_2 recorded the highest value of 4.43g/L in serum albumin and this differed significantly (P< 0.05) from turkeys on T_1, T_3 and T_4. Turkeys on T_1 and T_4 were similar (p > 0.05) to each other in serum albumin values, but they differed significantly (P< 0.05) from those on T_3. The lowest value in serum albumin was observed in turkeys on T_4 (3.112g/L). The serum albumin values obtained in this study were within the range of 3.1±0.27–3.5 ± 0.22 (mg/dl) reported by Iheukwumere *et al.* (2005) for Nigeria chicken. Low albumin suggests poor clotting ability of blood and hence poor prevention of haemorrhage (Robert *et al.*, 2000).

Turkeys on T_1 recorded the highest value of 2.61g/L in serum globulin and this differed significantly (P< 0.05) from turkeys on T_2, T_3andT_4 which were also significantly difference(P<0.05) from each other in globulin values. The lowest value in serum globulin was observed in turkeys on T_2 (1.67g/L).

The serum globulin values obtained in this study were within the range of 2.1 – 3.7 g/dl reported for birds by Banerjee (2005). Babatunde and Oluyemi (2006) opined that the higher the value of globulin, the better the ability to fight against diseases.

Turkeys on T_1 recorded the highest globulin/albumin ratio of 0.82 and this differed significantly (P< 0.05) from turkeys on T_2 which were similar (P> 0.05) to turkeys on T_3 and T_4 in globulib/albumin ratio There were on significant differences (P>0.05) among turkeys on T_1, T_3 and T_4 in globulin/albumin ratios. The lowest globulin/albumin ratio of 0.38 was observed in turkeys on T_2

Turkeys on T_3 recorded the highest value of 6.67 (g/l) in serum total protein and this differed significantly (P< 0.05) from turkeys on T_1, T_2 and T_4 which were also significantly different (P< 0.05) from each other in serum total protein. The lowest value of 5.17g/l in serum total protein was observed in turkeys on T_4 . The serum total protein values obtained in this study were lower than the range of 7.6 ± 0.2.27 – 8.2 ± 0.30 mg/dl reported by Iheukwumere *et al.* (2006) in Nigerian chickens. The variation in values of serum total protein may not be unconnected to the differences in breed and nutritional status of the birds (Esonu *et al.*, (2001). Serum total protein is the protein retained in an animal's body (Esonnu *et al.*, 2001; Kaneko *et al.*, 1997). Blood protein content has been shown to depend on the quality of dietary protein (Esonu *et al.*, 2001; Iheukwumere *et al.*, 2006).

IV. CONCLUSION

From the results of this study, it can be concluded that Diclair® improved kidney function and body weight gain of mature male turkeys at the level of 40.50 i.u without any deleterious effects on serum protein values.

REFERENCES

[1] Abu, A.H., M. Ameh, and F.C. Iheukwumere, (2006). Semen quality of Nigeria Local cocks treated with human menopausal gonadotrophin (Pergonal®). Livestock Research for Rural Development

[2] Babatunde, B.B. and J.A. Oluyemi, (2006) Comparative Digestibility of Three commonly used Fibrous ingredients in Maize, Soybean Meal and fish Diet by Broiler Chicks *Trop J. Anim Sci*, 3:105-111.

[3] Baker, F.J. and R.F. Silvertop, (1986). Introduction to Medical Technology 6th Edn. Butter- Worth England.

[4] Banerjee, G.C. (2005). A text book of Animal Husbandry 8th Edition pp.124

[5] Brackett, B. G. (2005). Physiology of Domestic Animals Twelfth Edition William O. Reece Editor PP. 670-691

[6] Brant, A.W. (1998). A brief history of turkey, Word's Poultry Science 44:365 – 373

[7] Dixon, T.A. and G.J. Hopkins, (1996). Super Ovulation in Cattle using Porcine Pituitary gonadotrophin Preparation (Plusset Serono) in Plusset Sci0entific Literature Serono Veterinary Rome, Italy, PP.22-23

[8] Esonu, B.O., O.O. Emelalom, A.B.T. Udedibi0e, I.C. Okoli, and F.C. Iheukwumere, (2001). Performance and blood chemistry of Weaner Pgs fed raw mucuna Bean (Velvet bean) meal. Trop. Anim. Prod. Invest, 4:49-54

[9] Iheukwumere, F.C., U. Herbert, and C. Ewulu, (2002). Effect of Quantitative feed Restriction on Broiler Chickens *J. Sustainable Trop Agric Res*, 4:56 – 60

[10] Iheukwumere, F.C. (2005). Super Ovulation in Goats in: Afam Anene and Nwaigbo, L.C (eds). Issues in Sustainable Agriculture in Nigeria. Osprey Publication Centre Owerri, Nigeria, 1-9

[11] Iheukwumere, F..C., I.C. Okoli, G.A. Anyanwu, and B.O. Esonu, (2005). Growth Performance, haematological and Serum Biochemical constituents

of grower Rabbits Fed *Microseisms puberula*, Hook – Euphorbiaceae. Animal Research Advances 1 (1): 24-31.

[12] Iheukwumere, F.C., A.H. Abu, and M. Ameh, (2006). Effect of Human Menopausal Gonadotrophin on Haematology and Serum biochemical Parameters of Nigerian Indigenous Chickens. *International Journal of Poultry Science 5* (7): 632-634

[13] Jain, N.C.(1993). Essential of Veterinary Haematology, Lea and Ferbiger Philadelphia

[14] Kaneko, J.J., J.W. Harvey, and M.I. Bruss, (19997). Clinical Biochemistry of Domestic Animals 5th Edition. Academic Press San Diego, Carlifona P. 885-905

[15] Katie, T, and A. Frazer, (1998). The Complete book of raising Livestock and Poultry Macmillan Publishers Ltd.

[16] Kohn, R.A. and M.S. Allen, (1995). Enrichment of Proteolytic Activity Relative to Nitrogen in Preparation from the Rumen for in Vitro studies, Anim. Food Sc. Tech. 52 (112): 1-4

[17] Obi, I.U., (1990). Statistical Method of Dtecting Differences between Treatment Means. Snaap Press 2nd Ed. Enugu, Nigeria 24-35.

[18] Okeudo, N.J (2005). Emperical Studies of Living condition of domestic animals in Nigerian, results from Nigerian in U.C Amalu and Gottwal, F.(eds). Studies of sustainable Agriculture and Animal Science in Sub Sahara Africa. Peter Lang, Europals Cher Verlay der Wissen Shaften. Germany.

[19] Probakaran, R. (2003). Good Practices in Planning and Management of integrated Commercial Poultry Production South Asia FAO Animal Production and health paper – 259, pp.71-86

[20] Randox (2006). WWW. Randox. Com/Randox. Laboratory Ltd. Users' Manual

[21] Reece, W.O. and M.J. Swenson, (2004). The Composition Function of Blood in Reece, W.O (ed) ducks.

[22] Robert, K.M., K.G. Daryl, A.M. Peter, and W.P. Victor, (2000). Mayers Biochemistry, 25th Edn. Mc Graw Hill. New York, pp. 763 – 765.

[23] Simaraks, S.,O. Chinrasri, and S. Aengwanich, (2004). Haematological, Electrolyte and Serum Biochemical Values of the Thai Indigenous Chickens (*Gallus domestica*) in Nort eastern Thailand. Song Klanakarin J. Sci. Tec, 26: 425-430.

[24] Steel, R.G.D. and J.H. Torrie, (1980). Principles and Procedures of Statistics. A Biometric Approach 2nd Ed. Mc. Graw – Hill Book Co. Inc. New York.

http://dx.doi.org/10.22161/ijeab/2.1.56 ISSN: 2456-1878

Production of Biodiesel using waste temple oil from Shani Shingnapur temple (Dist. Ahmednagar), Maharashtra, India using chemical and biological methods

Madhumeeta Chadha[1], Priyanka Desai[1], Ashwini Tambe[2], Sulabha B.Deokar[1], Kshitija Mutalik[1*]

[1]Department of Biotechnology, NowrosjeeWadia College, Affiliated to SavitribaiPhule Pune University, India
[2]Department of Zoology, NowrosjeeWadia College, Affiliated to SavitribaiPhule Pune University, India

Abstract*—In India, due to various mythological and religious reasons hundreds of devotees pour oil over the idols in Hanuman or Maruti and Shani temples. The oil once poured cannot be reutilized and was ultimately wasted. These waste temple oil from Shani Shingnapurwas used to produce biodiesel. Immobilized Pseudomonas aeruginosa was used to catalyze transesterification of waste temple oil. The cells of P.aeruginosa were immobilized within the sodium alginate. Biodiesel production and its applications were gaining popularity in recent years due to decreased petroleum based reserves. Biodiesel cost formed from waste temple oil was higher than that of fossil fuel, because of high raw material cost.To decrease the cost of biofuel, waste temple oil was used as alternative as feedstock. It has lower emission of pollutants; it is biodegradable and enhances engine lubricity. Waste temple oil contains triglycerides that were used for biodiesel production by chemical and biological method.Transesterification reaction of oil produces methyl esters that are substitutes for fatty acid alkyl biodiesel fuel. Characteristics of oil were studied such as specific gravity, viscosity, acid number, saponification number.Parameters such as temperature,oil: methanol ratio were studied and 88%, 96% of biodiesel yield was obtained with effect of temperature and oil: methanol ratio on transesterification reaction. Withaddition ofNaOH or KOH to fatty acids which formed salt known as soap,which is excellent emulsifying and cleaning agents.*

***Keywords*— Biodiesel, catalyst, immobilization, trasnesterification.**

I. INTRODUCTION

Biodiesel is defined as a fuel composed of mono-alkyl esters of long chain fatty acids derived from vegetable oils or animal fats[24].A mono-alkyl ester is the product of the reaction of a straight chain methanol, such as methanol or ethanol, with fat or oil (triglyceride) to form glycerol (glycerin) and the esters of long chain fatty acids. Biodiesel is "a substitute for, or an additive to diesel fuel that is derived from the oils and fats of plants and animals". Increasing uncertainty about global energy production and supply, environmental concerns due to the use of fossil fuels, and the high price of petroleum products are the major reasons to search for alternatives to petro-diesel. Moreover, biodiesel fuel has become more attractive because of its environmental benefits due to the fact that plants and waste temple oils and animal fats are renewable biomass sources. Biodiesel represents a largely closed carbon dioxide cycle (approximately 78%), as it is derived from renewable biomass sources. Compared to petroleum diesel, biodiesel has lower emission of pollutants,it is biodegradable and enhances the engine lubricity and contributes to sustainability. Use of (unprocessed) waste temple oil in the compression ignition engines is reported to cause several problems due to its high viscosity. Biodiesel which is used as an attractive alternative fuel, is prepared by transesterification of waste temple oils with an methanol in presence of a catalyst. The use of waste temple oil as biodiesel feedstock reduces the cost of biodiesel production since the feedstock costs constitutes approximately 70-95% of the overall cost of biodiesel production. Hence, the use of waste temple oil should be given higher priority over the edible oils as biodiesel feedstock. [6] Small amount of biodiesel can be used to add in low sulphur formulation of diesel to increase the lubricating capacity that is lost when sulphur is removed.

1.1 Petrodiesel is an hydrocarbon mixture, considered to be a fuel oil and 18% denser than gasoline. It contains higher quantities of sulphur,reduction in level of sulphur is required in diesel fuels. Higher concentration of sulphur in diesel are harmful for environment. To control the diesel particulate emission, used nitrogen oxide adsorbers to reduce

http://dx.doi.org/10.22161/ijeab/2.1.56 *ISSN: 2456-1878*

emissions. Forlubrication of engine addition of sulphur is important. Lowering the content of sulphurreduces lubricity of the fuel.

1.2 Advantages of biodiesel

Renewable fuel, obtained from waste temple oils or animal fats.low toxicity, in comparison with diesel fuel degrades more rapidly than diesel fuel, minimizing the environmental consequences ofbiofuel spills.Lower emissions of contaminants like carbon monoxide, particulate matter, polycyclicaromatic hydrocarbons, aldehydes.It lowers health risk, due to reduced emissions of carcinogenic substances. No sulphur dioxide (SO_2) emissions occur. It may be blended with diesel fuel at any proportion; both fuels may be mixed during thefuel supply to vehicles.Excellent properties as a lubricant.It is the only alternative fuel that can be used in a conventional diesel engine, withoutmodifications. Used cooking oils and fat residues from meat processing may be used as raw materials.

1.3 Use of Biodiesel all over the world

Many international automobile companies have started manufacturing cars that are compatible with biodiesel. These cars run on various blends of biodiesel. The companies include Chevy, Volkswagen, Mercedes-Benzes. Recent news suggests that, various railway trains present in United Kingdom have started to use biodiesel as its fuel source. Disney land has also started employing biodiesel for running of its internal tracts. United States was the first country to run entire flight duration of an airplane solely on biodiesel. This has encouraged other nations to followsuit.

1.4 Source used for biodiesel production

The source of oil used for production of biodiesel is 'waste temple oil'collected from‘Shani’ templefromShaniShingnapur, Taluka.Newasa, District.Ahmednagar,Maharashtra, India havingLatitude 19.381°N,Longitude 74.85°E.

1.5 Chemical composition of biodiesel

It consist of alkyl usually methyl esters. It has combustion properties similar to regular diesel. Chemical formula for diesel fuel is $C_{12}H_{26}$. Biodiesel is derived from waste temple oils such as triglycerides which are esters of glycerol with fatty acids.

Classification of biodiesel: According to the various sources used in the production of biodiesel, the following classes exists as:

Primary: Waste temple oil

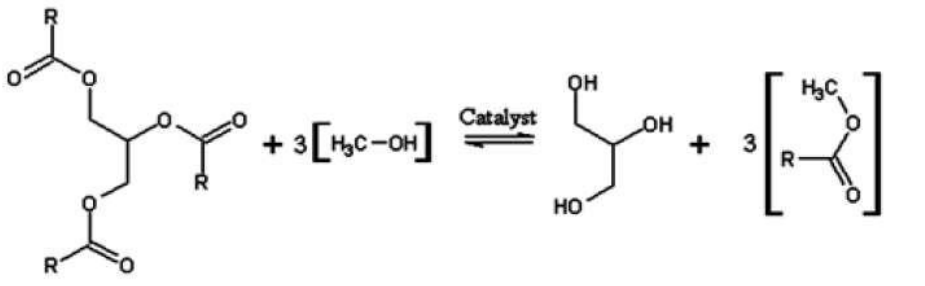
Secondary: Feedstock- sunflower, canola, soya bean

Tertiary: Algae

1.6 CHEMICAL METHOD

Transesterificationrefers to the reaction of an ester group with anmethanol that has a structuredifferent to that of the original methanol moiety of the ester, such that a new ester group is formedin which the original methanol moiety is exchanged with that of the reacting methanol. In the case ofthe transesterification of triglycerides of fatty acids (waste temple oil) with methanol (classical/biodiesel production process), the three ester groups of a triglyceride molecule in which threefatty acid moieties are attached to a single methanol moiety (i.e. glycerol) react with threemolecules of methanol to yield three molecules of esters each containing single fatty acid andmethanol moieties and one molecule of glycerol. Therefore, the general chemical name ofbiodiesel produced by the transesterification of waste temple oil is fatty acid methyl esters.Methanol is commonly used in industrial biodiesel production as a result of its relatively lowcost and easy availability. The classical reaction protocol for the transesterification of triglycerideswith methanol using homogeneous catalysts such as Sodium methoxide requires mixing andstirring the reagents in a batch reactor. At the end of the reaction, the non-polar phase containingthe ester and the polar phase containing glycerol and methanol are separated to recover theproducts, catalyst, and the excess of methanol.

The tranesterification reaction is given below [11].



1.6.1 Parameters affecting biodiesel production:

It is necessary to mention some important process variables of biodiesel production fromwaste temple oils such as the methanol/oil ratio, reaction time, mixing intensity, temperature andcatalysts used.

a. Temperature and time

Typically, the transesterification reaction is complete within around 1 hr using a methanol/oil Molar ratio of 6:1 at a reaction temperature of 60°C.

b. Oil : methanol ratio

The molar ratio of methanol to triglycerides is an important variable that affects the yield ofbiodiesel in the transesterification reaction. Most systems for transesterificationrequire anmethanol/triglyceride molar ratio of 6:1. The excess of methanol with respect to the reactionStoichiometry is needed to shift the reaction equilibrium to the right (product side).

c. Catalyst

The selection of an appropriate catalyst is of fundamental importance for the design of asustainable transesterification process.

Homogeneous alkaline catalysts, such as sodium hydroxide and potassium hydroxide, are mostcommonly used in industrial transesterification processes for biodiesel production, mainlybecause they are able to efficiently promote the reaction at relatively low temperatures. **Heterogeneous catalysts** have the general advantage of being reusable and easy to separate from the reaction products (generally cleaner process). In addition and more specifically, they do notform soaps, are more selective to biodiesel (purer product), and simplify the glycerol purification.A lower methanol-to-oilratio, easier product recovery, and higher environmental compatibility than chemical catalysts(homogeneous or heterogeneous).Furthermore, fats present in oils/fats can be completely converted into alkyl esters using enzyme catalysts. Notably, new immobilization technology indicates that enzyme catalysts can become cost-effective as compared to chemical processing.However, the production cost of lipases, which is the most investigated and promising class ofenzyme catalysts for transesterification, is still significantly higher than that of alkaline catalysts.Enzyme-based technology is still at a stage of intensive research and process optimization.

1.7 BIOLOGICAL METHOD

It has been shown that the enzymatic production of biodiesel is possible byusing either extracellular or intracellular lipases. The choice of the method should be based onthe balance between simplified upstream operations (intracellular) and high conversions(extracellular). Both types can be immobilized for use without a need for downstream operations.Moreover, immobilized enzymes are generally more expensive than chemical catalysts. [12]

Domain	Bacteria
Phylum	Proteobacteria
Class	Gamma proteobacteria
Order	Pseudomonadales
Family	Pseudomonadaceae
Genus	*Pseudomonas*
Species	*aeruginosa*

Microorginsm used- *Pseudomonas aeruginosa*

Immobilization of cells

It is used for intact or disintegrated dead cells that contain active enzymes or resting or growingcells.This technique is used especially with eukaryotic cells where the whole metabolicmachinery is often required for their specific application.

1.7.1. Methods of cell immobilization

a) Carrier-free immobilization: Immobilization of a given biomass onto a preformed carrier surface. Immobilization of a given biomass during the course of carrier formation (e.g., bypolymerization).Immobilization by controlled growth of an inoculum or by germination of immobilizedspores.

b) Alginate:It is extracted from seaweed and is a linear copolymer of *b*-d-mannuronic acid and *a*-l guluronic acid linked by 1,4-glycosidic bonds. It forms a gel in the presence of multivalent ions, usually calcium or aluminum. The controlled entrapment of cells is simple and generally nontoxic. Various cell types can be immobilized with negligible loss of viability.

Advantages of cell entrapment method:

1.Entrapment is simple and proceeds under very mild conditions.

2.Prepolymers do not contain toxic monomers.

3.The network structure of the gels can be adapted as required.

4.Optimalphysicochemical gel properties can be achieved by selecting suitable.

II. MATERIALS AND METHODS

2.1 Collection of waste temple oil:

The waste temple oil was collected in sterile plastic bottle from ShaniShingnapurtemple and trasported to the laboratory for further study.

2.2 Characteristics of oil and its calculations

a.Specific gravity:-The specific gravity was determined with a specific gravity bottle .The temperature at which the specific gravity was determined 30ºC/30ºC. The specific gravity was calculated by the formulae:

Specific gravity at 30ºC / 30ºC = A-B/C-B

where A = weight in g of the specific gravity bottle with oil at 30ºC,

B = weight in g of the specific gravity bottle, and

C= weight in g of the specific gravity bottle with waster at 30ºC

The specific gravity of the fuels at 15.6 °c was also employed to find the specific gravity at other temperatures by using the ATSM D1250 petroleum measurement tables (1953).

b.Viscosity of oil: Viscosity was measured by Oswald viscometer from the following formula

Viscosity (η) = $n_w t_s * d_s / T_{w*} d_w$

Where η_s = viscosity of the sample in cp at room temperature

η_w = viscosity of the waster in cp at room temperature

t_s = time of flow of sample (vol=v) in sec.

t_w = time of flow of waster (vol=v) in sec.

d_s = density of sample in g/l at room temperature

d_w= density of waster in g/l at room temperature.

c.Acid number: 2 g of oil sample were dissolved in 25 ml of methanol (ethanol) and added 2 to 3 drops of 1 % phenolphthalein indicator, titrated against by 0.1 N NaOH.

Acid number = N EV/W

d.Saponification value: It was also calculated by the formula :

Saponification value = 56.1N/W

where B = volume in ml of standard hydrochloric acid required for the blank

S = volume in ml of standard hydrochloric acid required for the sample

N = normality of the standard hydrochloric acid,

and W = weight in g of the material taken for the test

w g of the sample requires x mg KOH

1 g of the sample requires x/w mg KOH

Results of all characteristics of oil were noted down.

2.3. Methods for biodiesel formation:

2.3.1. Chemical method:

a. Titration

1g NaOH / 1 lit solution in burette was taken .Titration was done in a conical flask, that contained 10ml isopropyl methanol, 1ml waste temple oil, 1-2 drops phenolphthalein indicator with constant shaking .When the solution changes from colorless to faint pink, it indicated end point of the reaction .The burette reading was noted down.Burette reading was divided by 10 and then 3.5 was added to obtain the grams of NaOH which was required for conversion of 1 liter of waste oil temple oil into biodiesel.

b. Pre- treatment of waste temple oil

The oil was filtered to remove debris such as leaves, flowers, dirt etc. Heating of the oil up to 50-60ºC, was done to reduce the viscosity.

c. Transesterification reaction

In a bottle NaOH was added to methanol, closed the bottle and shaken well, to dissolve the entire NaOH into methanol. This reaction was exothermic and was accompanied by warming the bottom of the bottle. It was resulted in the formation of sodium methoxide. This mixture was then added to the pre- treated waste temple oil.The flask was kept on a magnetic stirrer for 2 hrs at 500-600 rpm to mix the oil with the Sodium methoxide properly.

d. Separation process

The entire contents of the flask were poured into a separating funnel after 2 hrs.The apparatus was left undisturbed overnight. The next day 2 layers were observed.First layer wasGlycerol - the bottom layer (by product) and second layer was biodiesel- the upper layer(honey colour)

Glycerol was poured off in flask and biodiesel was stored separately.

e. Soap formation:

The amount of glycerol formed was measured. It was heated at 65-70ºC in a boilingwater bath to remove traces of methanol. 1.2g of NaOH was dissolves in 10 ml of warm water and added to the above mixture. Heated the mixture for further 20 min with continuous stirring .Dye was added and mixed properly.The contents were poured into a tray and covered to allow solidification for 2-3 days .It was followed by curing for 4 days to ensure both sides were dried properly before usage.

2.3.2. Biological method

a.Bacterial culture:

Pseudomonas strains were collected from NCIM, Pune during this work.Nutrient agar plates and nutrient agar slants were prepared.The culture was maintained on agar slant and stored at 4ºC in refrigerator.Streaking of *Pseudomonas aeruginosa* on the agar plates and slants

was done and incubated for 2-3 days at 25ºC.The inoculate was grown aerobically at 25ºC in an shaker incubator at 200 rpm. Centrifugation was done at 1200 rpm for 20 minutes and active cells were isolated and further used for immobilization method.

b.Cells immobilization by entrapment method: The sodium alginate entrapment of cells was performed according to the standard method. Alginate solution with a concentration range of 0.5 - 10% was used for the cell immobilization and was prepared by dissolving sodium alginate in boiling water and autoclaved at 121ºC for 15 min. Both alginate slurry and cell suspension was mixed and stirred for 10 min to get a uniform mixture the alginate/ cell mixture which was extruded drop by drop into a cold sterile 0.2 M $CaCl_2$ solution through a sterile 5 ml pipette and kept for curing at 4ºC for 1 h. The beads were hardened byresuspending into a fresh chilled $CaCl_2$ solution for 24 h at 4ºC with gentle agitation. Finally these beads were washed with distilled waster to remove excess calcium ions and unentrapped cells. When the beads are not being used, they are preserved in 0.86% sodium chloride solutions in the refrigerator.Methanolysis was carried out by adding methanol, 3ml hexane and immobilized whole cells to waste temple oil.Thismixture was kept in an shaker incubator at 200 rpm for 48 hrs at 25ºC.Reaction was then stopped by removing the beads by filtration. The contents were poured in a separating funnel and leaved overnight. Glycerol and biodiesel were separated into two distinct layersand collected biodiesel which was stored separately.

2.3.3. Optimum parameters of transesterification reaction for production of biodiesel.

a.Effect of temperature on the transesterification reaction: Effect of temperature on the transesterification reaction was examined at the temperature range from 50ºC to 65ºC. Four conical flasks containing 50 ml of waste temple oil, 3 ml hexane, methanol (1:6 molar ratio of oil/methanol) and 3g beads of immobilized cell concentration. These flasks incubated at four different temperatures respectively 50ºC, 55ºC, 60ºC and 65ºC at 100 rpm for 48 hrs.

b.Effect of oil / methanol molar ratio on the transesterification reaction: Oil/ methanol molar ratios were effect on the yield of biodiesel, because to shift the transesterification reaction in forward direction. It is necessary to use either an excess amount of methanol or to remove one of the products from the reaction or mixture. Experiments were carried out with different molar ratios of 1:1, 1:2, 1:3(oil to methanol) at constant levels of 50 ml waste temple oil, 3 ml hexane and 3g immobilized cell concentration in a conical flasks. This reaction mixture incubated at optimum conditions such as temperature 60ºC, pH 7.0 and reaction time 48 h. Fig2 shows that the increasing the molar ratio, the yield of biodiesel was found to be increasing.

2.3.4. Laboratory tests:-Performed for biodiesel obtained by chemical and biological method.

a. Methanol test:

One part of biodiesel was added to 9 parts of methanol by volume.Results were noted down.

b. Firewall test:

Foam formation was observed at the top of test tube after addition of 2ml of biodiesel.Results were observed.

c. Flammability test:

Biodiesel was placed in petri plate and elit fire to it and observed.

d. Clarity test:

2-3 drops of biodiesel obtained was poured on a newspaper.Results were seen.

III. RESULTS AND DISCUSSION

Fig.1: Waste temple oil sample

3.1 Characteristics of waste temple oil value

Saponification number	12.70 mg KOH/g oil
Specific gravity at 30ºC	0.91236
Viscosity of oil	49.520 mm^2 /s at 303 K
Acid number (with ethanol)	9.7 mg KOH/g oil

3.2 Chemical method for Biodiesel Production

Biodiesel was obtained by chemical method using transesterification reaction. After separation process, Biodiesel and Glycerol were obtained.

http://dx.doi.org/10.22161/ijeab/2.1.56 *ISSN: 2456-1878*

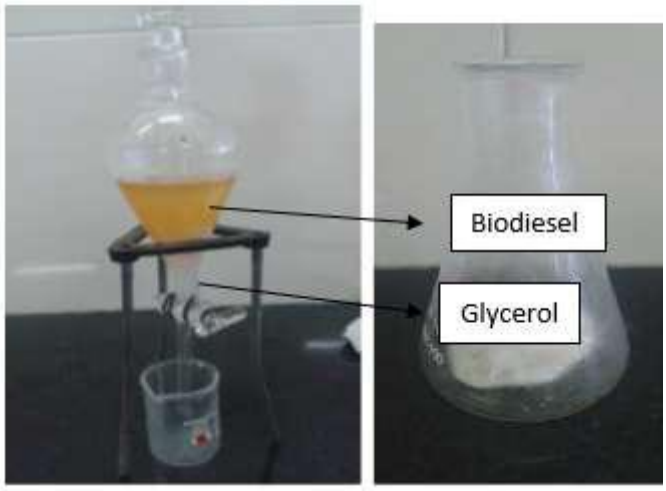


Fig.2: Biodiesel obtained by chemical method *Fig.3: Glycerol obtained by Chemical method*

Using glycerol obtained from separation process, soap was formed.

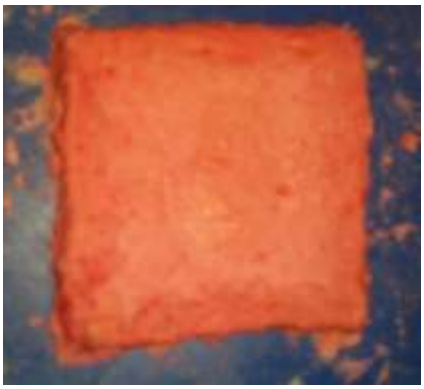


Fig.4: Formation of Soap

3.3 Biological method of biodiesel formation:

In present work experiments were carried out to growth of culture, *Pseudomonasaeruginosa* produced from NCIM,Pune and maintained it on agar slant, cell immobilized by entrapment method.

Fig.5: Immobilized Pseudomonasaeruginosa beads by calcium alginate method

3.4 Effect of temperature on transesterification reaction-The highest percentage of conversion of oil into biodiesel was observed at 60ºC and thereafter decreases due to denature of the enzyme. Biodiesel yield found to be 88%.

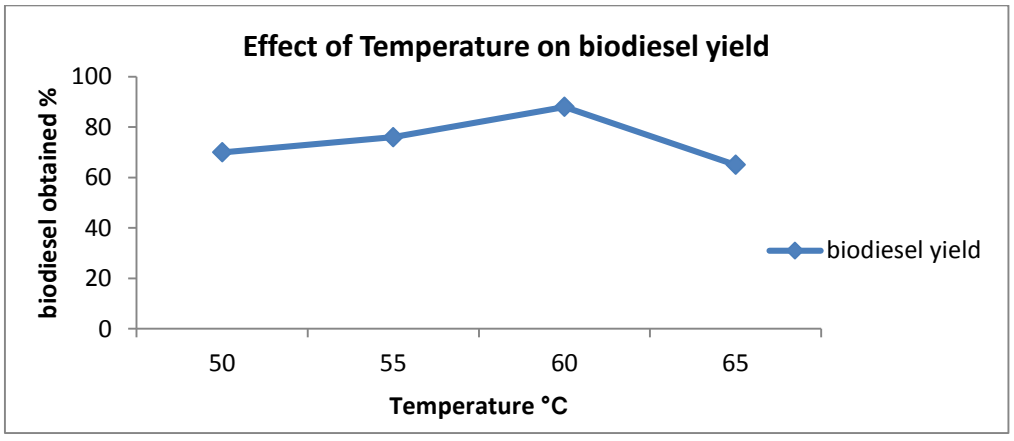


Fig.7: Effect of temperature on biodiesel yield

3.5 Effect of oil/methanol molar ratio on transesterification reaction-Maximum yield were obtained at molar ratio of 1:2. Further yield of biodiesel was found to be decreasing with increasing molar ratio beyond 1:2. It was due to the inhibition of excess methanol reduces the enzyme activity.

http://dx.doi.org/10.22161/ijeab/2.1.56 *ISSN: 2456-1878*

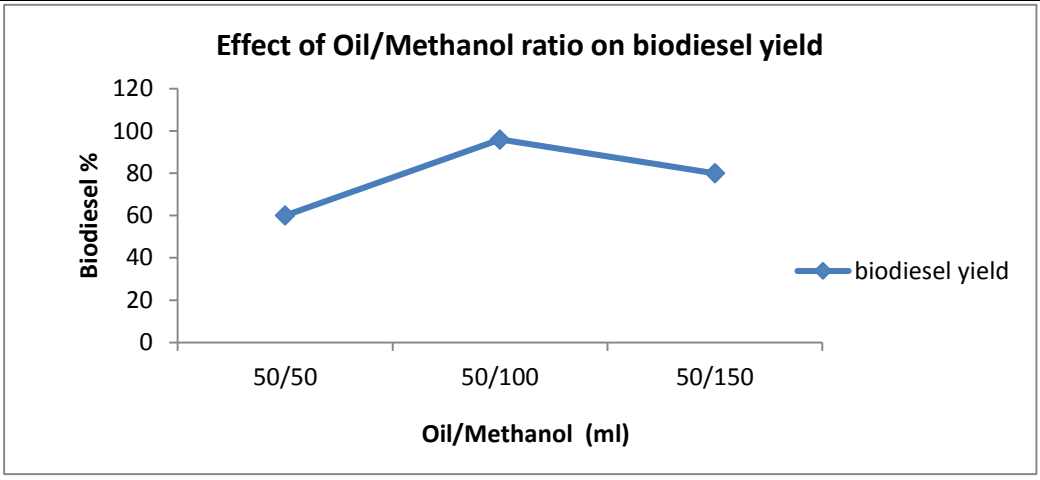


Fig.8: Effect of Oil/Methanol on biodiesel yield

From the above data one concludes that 1:2 ratio of Oil: Methanol is best suited for maximum biodiesel yield which was obtained 96%.

3.6 Laboratory test:

Test	Result
Methanol test	No precipitation
Firewall test	Impure with glycerol
Flammability test	Flammable
Clarity test	No debris in biodiesel

Methanol Test- Since no precipitation was formed it indicated that biodiesel obtained was not converted into any other product.

Firewall Test- This indicated that the biodiesel obtained was not 100% pure and contained small amount of glycerol contamination.

Flammability Test- Resulted in combustion.This was achieved to prove the flammability property of obtained biodiesel .

Clarity Test- Since the newspaper could be read easily it satisfied the visibility or clarity test.It also indicated that there were no debris present in the obtained biodiesel.

IV. DISCUSSION

Biodiesel can be obtained by chemical method and biological method.Transesterification was carried out using waste temple oil and methanol. Immobilized beads were obtained by cell entrapment method. Characteristics of waste temple oil were studied. Conversion of oil to biodiesel by transesterification reaction and its effects like temperature, oil : methanol ratio on the production of biodiesel yield were obtained 88% and 96% respectively.Whereas, DevendraPratap Singh, Hemant Kumar (2013) got 76% biodiesel yield from Jatropha oil. Using Soxhelt extraction Bobade S.N and Khyade V. B (2012) obtained 31% yield of *Pongamiapinnata*seeds. One of the Biodiesel byproduct soap was formed. Laboratory tests such as methanol,firewall, flammibility and clarity tests were performed to analyze the quality of biodiesel. It is renewable alternative fuel which has low toxicity in comparison with diesel fuel, degrades more rapidly minimizing the environmental consequences of biodiesel spills.

V. CONFLICT OF INTEREST

We all authors do not have any conflict of interest.

VI. ACKNOWLEDGEMENT

We are greatfull to our Principal Dr. K. S.Venkataraghvan,NowrosjeeWadia College,Pune and Department of Biotechnology for providing laboratory facilties.

REFERENCES

[1] Ashwini. R. Tambe, R. M. Pawar, S. B.Deokar.Evaluation of antibacterial and antifungal activity of various concentrations of Azadirachtaindica (Neem) against human pathogenic bacterial and fungal strains.Asian Journal of Multidisciplinary studies.4(3),2016:61-66

[2] A.R.Tambe, S.B.Deokar, R.M.Pawar. Evaluation of Antimicrobial activity and Phytochemical Analysis of Zingibrofficinale (Ginger) Rhizome Extract.International Journal of Allied Practice,Research and Review.3(6),2016:1-9

[3] Ashwini.R.Tambe, KshitijaS.Mutalik, Sulabha. B.Deokar. Study of Antimicrobial potential and phytochemical analysis of Oscimum sanctum (Tulsi) leaves extract. International Journal for Science and Research in Technolog.2(7),2016:250-256

[4] Ahmed ElSawy, MichaelBaswell.Automation of a Biodiesel Processor from Waste Vegetable Oil Using Programmable Logic Controller. American Society for Engineering Education, 2012
[5] Arjun B. Chhetri , K. Chris Watts , M. Rafiqul Islam. Waste Cooking Oil as an Alternate Feedstock for BiodieselProduction.*Energies.1*,3-18 (2008)
[6] Arumugam Sivasamy, KienYooCheah, Paolo , Francis Kemausuor, Sergey Zinoviev, StanislavMiertus. Catalytic Applications in the Production of Biodiesel from Waste temple Oils.ChemSusChems 2009.2:278-300
[7] ASTM-IP Petroleum Measurement Tables. 1953. Prepared jointly by American Society for Testing Materials and the Institute of Petroleum. ASTM Designation: D1250, IP Designation:200.
[8] ASTM D1298-99e2. 2003. Standard Test Method for Density, Relative Density (specific gravity), or API Gravity of Crude Petroleum and Liquid Petroleum products by Hydrometer Method.
[9] ASTM data series publication. 1975. Viscosity index tables for celsius temperatures. (Ds39B), (05-039020-12)
[10] B.D. Singh Biotechnology: Expanding Horizons, Kalyani Publishers,2012
[11] Bobade S. N, Khyade V. B. Detailed study on properties of Pongamiapinnata (Karanja) for the production of Biodiesel. Research Journal of Chemical Sciences.2 (7),2012: 16-20
[12] Cappucino.J.G, Sherman N. Microbiology A Laboratory Manual.Pearson 7th edition.2005.13-16
[13] DevendraPratap Singh, Hemant Kumar Production of Biodiesel using Immobilized *Pseudomonas fluorescens*.International Journal for Research and Development in Engineering (IJRDE), Vol 2 , Issue-2, Nov-Dec
[14] E.R. Ram Rattan, Dr. Manoj Kumar Biodiesel (a renewable alternative fuel) production from mustard oil and its performance on domestic small diesel engines .International Referred Research Journal, RNI-RAJBI 2009/30097;3 (29) 2012
[15] Emad A. Shalaby, Nour Sh. El- Gendy. Two steps alkaline transesterification of waste cooking oil and quality assessment of produced biodiesel .International Journal of Chemical And Biochemical Studies(2012) 1:30-35
[16] Hanric Muharka ,sadar Wahjudi , nanik Hendrawati, Profiyanti Hermien, Zakijah Irfin. Comparison Of The Characteristics Of CPO Oil And Waste Cooking Oil To The Influence Of Stirring Speed, Temperature And Reaction Time In The Transesterification. *International Journal Of Engineering And Science. Vol.2, Issue 6 (2013), 32-36*
[17] Indian standard methods of sampling and test for Butterfat's ,Fifthedition,December,(1995)
[18] Owolabi R.U, AdeiumoA.l. ,Aderibingbe A.F. Biodiesel: Fuel for the Future (A Brief Review) International Journal of Chemical and Biochemical Studies (2012)
[19] PatniNeha, BhomiaChintan, DasguptaPallavi, TripathiNeha. Use of Sunflower and Cottonseed Oil to prepare Biodiesel by catalyst assisted Transesterification .Research Journal of Chemical Sciences 3(3):42-47 (2013)
[20] Prafulla D. Patil, Veera Gnanaeswar Gude, Harvind K.Reddy, Tapasway Muppaneni ,Shuguang Deng. Biodiesel Production from Waste Cooking Oil Using Sulphuric Acid and Microwave Irradiation Processes.Journal of Environment Protection, 3 :107-113 (2012)
[21] Sandhya B. Walke, Ranjana p. Bhdne, Sulabha B deokar, Shantaram L. Bonde.Kinetics of Rapid Chlorination of Imidazole by N-Chlorosuccinimide in Aqueous medium and study of Antimicrobial Activity of its ChloroDerivative.Int.J.Chem.Pharm.Sci., 2016,4(2):61-69
[22] S. D. Romano and P. A. Sorichetti, Dielectric Spectroscopy in Biodiesel Production and Characterization, Green Energy and Technology, DOI: 10.1007/978-1-84996-519. 4(2),Springer-Verlag London Limited 2011
[23] Tambe Ashwini, MinazInamdar, QusaiIndonesiawala, KshitijaMutalik, SulabhaDeokar. Synthesis of Microbial Biodegradable Starch-based Bioplastic.Asian Journal of Multidisciplinary Studies.4(9),2016
[24] Vicente. G.M, Martinez and J.Arcil, 2007.Optimization of integrated biodiesel production, Part 1.A study of biodiesel purity and yield. Biores.Technol.,98:1742-33

http://dx.doi.org/10.22161/ijeab/2.1.57 *ISSN: 2456-1878*

Effects of Fungicides for Non Target Fungi *Alternaria cassiae*

Silvia Patricia Carraschi[1*], Claudinei da Cruz[2], Aritana Gil Basile[3], Robinson Antonio Pitelli[3]

[1]Universidade de Araraquara. Av. Maria Antonia Camargo de Oliveira, 170 - Vila Suconasa, Araraquara - SP, 14807-120, Brasil.

[2]Centro Universitário da Fundação Educacional de Barretos (Unifeb), Barretos (SP).

[3]Núcleo de Estudos e Pesquisas Ambientais em Matologia (NEPEAM) da UNESP, Campus Jaboticabal (SP).

Abstract**—** *The fungicides are used to control of pathogenic fungi in several tilth but they can affect negatively the microorganisms diversity of soil. The aim of this research was to evaluate the toxicity and environmental risk of tebuconazoles: captan, tebuconazole and the mixture chlorothalonil + propamocarb hidrochloride for fungi Alternaria cassiae. Each fungicide were performed three experiments in completely randomized design with three repetitions and the growth was evaluated daily. Inhibition concentration (IC50;7d) of tebuconazole was 3.49 mg L^{-1}, the captan was 47.36 mg L^{-1} and of mixture chlorothalonil + propamocarb hidrochloride, 64.04 mg L^{-1}. Tebuconazole is classified as moderately toxic and sensitivity, captan, low toxicity and sensitivity and the mixture, non toxic and insensitive but only captan showed possibility of adverse effect for A. cassiae.*

Keywords**— soil, microorganism, xenobiotics, pesticide.**

I. INTRODUCTION

The fungicides have been using control pathogenic fungi of plants and some of them are non specific and can affect the abundance of non target microorganisms (Stenersen 2004; Zhang *et al*., 2016). Besides, as azoles can affect the ergosterol biosynthesis, may be studied its effects for non target organisms present in the application area (Dijksterhuis *et al*., 2011).

As one of them, Tebuconazole is a systemic triazole classified by USEPA as carcinogenic for human beings (Konwick *et al*., 2006; Hu *et al*., 2007) and toxic for aquatic organisms (Yu *et al*., 2013). Captan is from carboximide group, non systemic, wide range and inhibits the spores germination, growth and the oxygen uptake (Boran *et al*., 2012). Propamocarb is a carbamate practically non toxic and 97% is degraded by aquatic microorganisms in 35 days and chlorothalonil is a derivative of benzoic acid, considered moderately toxic and is degraded in two metabolits in two weeks (Teather *et al*., 2001).

The soil microbiota is responsable by decomposition of organic residues and by nutrient cycling and exercises influency in the organic matter transformation and in the carbon and mineral nutrient stocking (Zilli *et al*., 2003). Fungi are part of this microbiota and they are affected negatively by chemical intake according to Botelho e Monteiro (2011) who verified that inseticides, herbicides and maturers used in the manegement of sugar cane cause toxic effects for *Metarhizium anisopliae* and *Beauveria bassiana*.

Alternaria spp. is an ascomyce from Dematiaceae Family, Order Hyphomycetes and produces conidia as dictyospores (Rotem, 1994). *Alternaria cassiae* has wide geographic distribution in the tropical and subtropical region, as Asia (Jurair and Khan, 1960), North America (Walker and Boyette, 1982) and South America (Figueiredo *et al*., 1992). This fungi has a big importancy in the agroecosystem dynamic, especially, in the biological control of *Senna obtusifolia* (L.) (Pitelli *et al*., 2007) and it can be a good option as bioherbicide (Boyete *et al*., 2012). Besides, *Alternaria* is natural inhabitant of soil submited at organic or conventional manegement and for this it is exposed at pesticides (Prade *et al*., 2007).

Therefore, the evaluation of the pesticides effect for non target fungi is very important because the soil with unbalance fungal content can presents problems with decomposition and mineralization rate, quantity and nutrient variety, water retention capacity and erosion resistance (Stahl e Parkins, 1996).

In this context the ecotoxicology has been using as a tool to estimate the effects for non target organisms, comunities, populations, animals or vegetables, terrestrial or aquatics (Cairns e Niederlehner, 1995). According to Zagatto e Bertoletti (2008) the ecotoxicology can evaluate the damage occurred in the several ecosystems after the exposure and also to predict the future impacts of chemicals.

Thus, the risk evaluations may be more necessary to contemplate the principal agriculture patrimony, the soil

health and fertile. The aims of this research were to estimate the toxicity of captan, tebuconazole, clorotalonil + chloridrate propamocarb and to evaluate the environmental risk for fungi *Alternaria cassiae*.

II. MATERIAL AND METHODS

Cultivation of fungi

The stain of *A. cassiae* was from Laboratory of Biological Control of Weed (CENARGEN/EMBRAPA), Brazil.

For the cultivation it was used the medium PDA (potato-dextrose-agar) that after autoclaved at 121ºC, during 15 min, was spilled in plates and expected until solidificaton. After, it was disposed a disk from a fungi colony (6.5 mm diameter) in the center of plate, from the growth active region of colony. The plates were incubated in at 25ºC and photoperiod of 12 hours.

Toxicity of fungicides for fungi

The fungicides were captan (Captan® SC), tebuconazole (Orius® 250EC) and the mixture clorotalonil + chloridrate propamocarb (Tattoo® C).

First of all, the sensibility of fungi was evaluated with the reference substance potassium dicromate, being IC50;7d, 65.97 ± 9.48 mg L^{-1} (coefficient of variation 14.38%).

Previous tests were performed and it was determinade the interval of concentrations that caused zero and 100% of inhibition in the fungi growth according IBAMA (1987), with some adaptations.

In the definitite assays the concentrations used were: captan, 1.0, 10.0, 50.0, 90.0, 130.0 and 170.0 mg L^{-1}; tebuconazole, 1.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg L^{-1}; and clorotalonil + chloridrate propamocarb, 25.0, 50.0, 100.0, 150.0, 200.0, 250.0 and 300.0 mg L^{-1} and the control. Three definitive tests were performed each one in completely randomized design with three replicates each concentration.

The fungicides were added in the medium, spilled in the plates and after solidification a disk 6.5 mm diameter of *A. cassiae* was disposed centrally. The plates were sealed and kept in the 25ºC, and photoperiod of 12 hours, for seven days.

The evaluation of growth halo was daily according two axis perpendicular. The inhibition concentration (IC50;7d) were estimated by Trimmed Spearman-Karber software (Hamilton *et al*., 1977).

The toxicity was classified according to Edgington *et al*., (1971): IC50 < 1.0 mg L^{-1}: high toxicity and sensitivity; 1.0 < IC50 < 10.0 mg L^{-1}: moderate toxicity and sensitivity; 10.0 < IC50 < 50.0 mg L^{-1}: low toxicity and sensitivity and IC50 > 50.0 mg L^{-1}: non toxic and insensitive.

The environmental risk was estimated according to Urban e Cook (1986) which use the estimated environmental concentration (EEC), which is the higher and lower concentration each fungicide recommended and IC50;7d each fungicide found in the toxicity tests *in vitro*. Thus, these data resulte in a quotient (Q): without adverse effect ($Q \leq 0.1$); possibility of adverse effect ($0.1 \leq Q \leq 10$) and probability of adverse effect ($Q > 10$).

III. RESULTS

IC50;7d of tebuconazole was 3.49 mg L^{-1}; captan, 47.36 mg L^{-1} and of mixture clorotalonil + chloridrate of propamocarb, 64.04 mg L^{-1} (Table 1) and they were classified as moderately toxicity and sensitivity; low toxicity and sensibility and non toxic and insensitive, respectively.

Table.1: Inhibition Concentration 50% (IC50;7d) (mg L^{-1}), upper limit (UL) and lower limit (LL) of fungicides for A. cassiae.

		Captan	
Tests	**LL**	**IC50;7d**	**UL**
1	48.44	60.00	74.57
2	37.81	44.98	53.53
3	25.99	37.00	52.79
Average	**37.41**	**47.36**	**60.29**
		Tebuconazole	
Tests	**LL**	**IC50;7d**	**UL**
1	2.95	3.98	5.38
2	2.95	4.14	5.81
3	1.33	2.37	4.23
Average	**2.41**	**3.49**	**5.14**
		Clorotalonil + Chloridrate de Propamocarb	
Tests	**LI**	**CE50;7d**	**LS**
1	51.06	66.73	87.22
2	52.58	80.04	121.84
3	30.59	45.37	67.32
Average	**44.74**	**64.04**	**92.12**

LL: lower limit; UL: upper limite; IC50 – inhibition concentration 50%

Captan caused 8% of inhibition with 1.0 mg L^{-1} and 81% with 170.0 mg L^{-1}. Tebuconazole caused 26% with 1.0 mg L^{-1} and 83% with 50.0 mg L^{-1}, but there was a stabilization trend from 20.0 mg L^{-1}. Mixture, caused 36% with 25.0 mg L^{-1} and 73%, 300.0 mg L^{-1} (Figure 1).

http://dx.doi.org/10.22161/ijeab/2.1.57 *ISSN: 2456-1878*

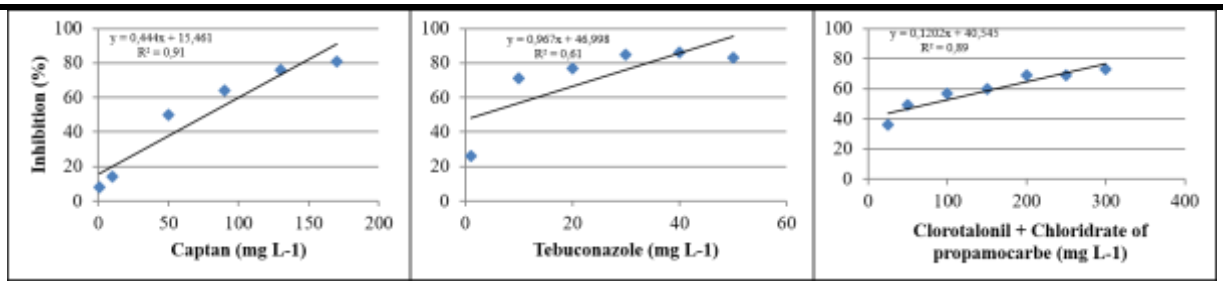


Fig.1: Relation of concentration effect of fungicides for Alternaria cassiae.

In relation to environmental risk, captan has possibility of adverse effect, therefore tebuconazole and the mixture don´t cause any adverse effect for fungo *A. cassiae*, independent of the dosage, according to Urban and Cook (1986) (Table 2).

Table.2: Environmental risk of fungicide for Alternaria cassiae.

Fungicides	**EEC (mg m^{-2})**	**Q=EEC/IC50**	**Classification**
Captan	12.00	0.25	PAE
	16.80	0.35	PAE
Tebuconazole	0.075	0.02	AAE
	0.150	0.04	AAE
Chlorothalonil + Chloridrate Propamocarb	1.80	0.03	AAE
	2.70	0.04	AAE

ECC: estimate environmental concentration; PAE: possibility adverse effect (0.1≤Q≤10); AAE: any adverse effect (Q≤0.1)

IV. DISCUSSION

The influence of fungicides for soil microorganisms depends of many factors between the physical properties, biochemical soil and fungicide concentration (Vyas, 1988). Fungicide effects for microorganisms envolve modification of availability and transformation of nytrogen, as nitrification and desnitrification (Chen *et al*., 2001) and consequentely in the soil quality. Then, the soil quality affects your potential, productivity and global sustainable of the agroecosystem and become necessary your study to secure the decisions for the better use of this resource (Sposito and Zabel, 2003).

Tebuconazole was the more toxic for *A. cassiae* (3,49 mg L^{-1}) and its toxicity is associated with inhibition of ergosterol synthesis, one component of fungal membrane and responsable by fluidity regulation, activity and distribution of integral proteins and control of celular cycle (Bard *et al*., 1993). This characterize the ergosterol biosynthesis way as essential for fungal growth (Alcazar-Fuoli *et al*., 2008). Tebuconazole also was toxic with low concentrations for *Colletotrichum gloeosporioides*, IC50 < 1.0 mg L^{-1}, which causes diseases in papaya (Tavares and Souza, 2005) and it showed high patogenecity *in vitro* for *Lasiodiplodia theobromae*, IC50 of 0.42 mg L^{-1} (Locatelli *et al*., 2015).

Captan offers risk for *A. cassiae* and it can unbalance the fungal content in the soil. Others agrochemicals used in the sugar cane (insecticides aldicarbe and fipronil, herbicides diuron and clomazone+ametryn and maturers etil-trinexapac and sulfometurom-metilic) were toxic for fungi *Metarhizium anisopliae* and *Beauveria bassiana* afecting the biological control by these fungi (Botelho and Monteiro, 2011).

Tebuconazole was more toxic that others but it doesn´t cause risk for this funghi because the EEC is much lower that IC50. According Carraschi *et al* (2015), the closer the recommended dose of the insecticide thiameothoxan is from the lethal concentration, the greater the possibility of it causing an adverse effect for a bioindicator. This relation also was verified with capatan and the mixture for *A. cassiae*.

Beside the fungi, the bacterial biomas also can be affected by agrochemicals use. According Widenfalk *et al*. (2008) captan doesn´t cause negative effects in the microbial biomass using the dosage permited and according to Milenkovski *et al*. (2010) only was observed some effect when used larger doses than found in the environment. Tebuconazole decrease the bacterial biomass in the litter after chronic exposure (six weeks, 20.0 µg L^{-1}) (Artigas *et al*., 2012). About clorotalonil, Xiaoqiang *et al*. (2008) described that the inhibitory effect by clorpirifós for soil organisms is increased when it is associated with clorotalonil.

V. CONCLUSION

Tebuconazole causes moderately toxicity, captan, low toxicity and the mixture is considered non toxic for *A. cassiae*, the funghi non target, but only captan represents the possibility of adverse effect for this organism.

ACKNOWLEDGMENTS

At CNPq process MS/CNPq 131410/2006-7.

REFERENCES

[1] Alcazar-Fuoli, L., Mellado, E., Garcia-Effron, G., Lopez, J. F., Grimalt, J.O., Cuenca-Estrella, J.M. and Rodriguez-Tudela, J.L. 2008. Ergosterol biosynthesis pathway in *Aspergillus fumigatus*. Steroids. **73**(3): 339-347.

[2] Artigas, J., Majerholc, J., Foulquier, A., Margoum, C., Volat, B., Neyra, M. and Pesce, S. 2012. Effects of the fungicide tebuconazole on microbial capacities for litter breakdown in streams. Aqu Toxicol. **122**: 197-205.

[3] Bard, M., Lees, N.D., Turi, T., Craft, D., Cofrin, L., Barbuch, R., Koegel, C. and Loper, J.C. 1993. Sterol synthesis and viability of erg11 (cytochrome P450 lanosterol demethylase) mutations in *Saccharomyces cerevisiae* and *Candida albicans*. Lipids. **28:** 963–967.

[4] Boran, H., Capkin, E., Altinok, I. and Terzi, E. 2012. Assessment of acute toxicity and histopathology of the fungicide captan in rainbow trout. Experim Toxicol Path. **64(**3): 175-179.

[5] Botelho A.A.A., Monteiro, A.C. 2011. Toxicidade de agrotóxicos usados no cultivo da cana-de-açúcar para fungos entomopatogênicos no solo. Pesticidas: Rev Ecotoxicol e Meio Ambi. **21**: 73-84.

[6] Boyette C.D., Bryson, C.T., Hoagland, R.E. and Weaver, M.A. 2012. Effects of simulated rainfall on disease development and weed control of the bioherbicidal fungi *Alternaria cassiae* and *Colletotrichum truncatum*. Weed Technol. **26**(1): 117-121.

[7] Cairns, J.Jr. and Niederlehner, B.R. 1995. Ecological Toxicity Testing. *Lewis Publishers*, Boca Raton, USA, pp. 240.

[8] Carraschi S.P., Florencio, T., Garlich, N., Silva, A. F., Marques, A.M., Cruz, C. and Ranzani-Paiva, M.J.T. 2015. Ecotoxicology of drugs used in fish disease treatment. J Environ Chem Ecotoxicol. **7(**3): 31-36.

[9] Chen, S.K., Edwards, C.A. and Subler, S. 2011. Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. Soil Biol Bioch. **33**(14): 1971-1980.

[10] Dijksterhuis, J., van Doorn, T., Samson, R. and Postma, J. 2011. Effects of seven fungicides on non-target aquatic fungi. Water, Air, Soil Poll. **222(**1-4,): 421-425.

[11] Edgington, L.V., Khew, K.L. and Barron, G.L. 1971. Fungitoxic spectrum of benzimidazoles compounds, Phytopathol. **61**: 42-44.

[12] Figueiredo, G., Fontes, E.G., Teixeira, C.A.B. and Pais, J.S. 1992. Levantamento e seleção de patógenos para controle biológico de fedegoso (*Senna obtusifolia*). Fitopatol Brasil. **17**: 169.

[13] Hamilton, M.A., Russo, R.C. and Thurston, R.V. 1977. Trimmed Spearman-Karber method for estimating medial lethal concentrations in toxicity bioassays. Environ Sci Technol. **7**: 714-719.

[14] Hu, M.L., Jiang, M., Wang, P., Mei, S.R., Lin, Y.F., Hu, X.Z., Shi, Y., Lu, B. and Dai, K. 2007. Selective solid-phase extraction of tebuconazole in biological and environmental samples using molecularly imprinted polymers. Analyt Bioanaly Chem. **387**(3): 1007-1016.

[15] IBAMA. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. 1987. Avaliação da toxicidade aguda para peixes. Parte D. 3. Manual de testes para avaliação de ecotoxicidade de agentes químicos**.** Brasília, DF, pp. 23.

[16] Jurair, A.M.N. and Khan, A.A. 1960. New epecies of *Alternaria* on *Cassia holsericea*. Fresem. Pak J Scient Industr Res. **3**: 71-72.

[17] Konwick, B.J., Garrison, A.W., Avants, J.K., Fisk, A.T. 2006. Bioaccumulation and biotransformation of chiral triazole fungicides in rainbow trout (*Oncorhynchus mykiss*). Aquat Toxicol. **80**(4): 372-381.

[18] Locatelli, T., Freire, M.D.G.M., de Souza, C.L.M. and Dias, V.M. 2015. Toxicidade de fungicidas no crescimento e germinação in vitro do fungo *Lasiodiplodia theobromae*. Biol & Saúde. **5**(17);. 9-22.

[19] Milenkovski, S., Bååth, E., Lindgren, P.E. and Berglund, O. 2010. Toxicity of fungicides to natural bacterial communities in wetland water and sediment measured using leucine incorporation and potential denitrification. Ecotoxicol. **19**(2): 285-294.

[20] Pitelli, R.L.C.M., Penariol, M., Pitelli, A.M.C.M. and Pitelli, R.A. 2007. Host specificity of a brazilian isolate of *Alternaria cassiae* (Cenargen CG593) under greenhouse conditions. Phytoparasit. **35**(2):123-128.

[21] Prade, C.A., Matsumura, A.T., Ott, A.P. and Porto, M.L. 2007. Diversidade de fungos do solo em sistemas agroflorestais de citrus com diferentes tipos

de manejo no município de Roca Sales, Rio Grande do Sul, Brasil. Bioci. **15(**1): 73-81.

[22] Rotem, J. 1994. The Genus Alternaria: Biology, Epidemiology and Pathogenicity. St. Paul, Minnesota, pp. 326.

[23] Sposito, G. and Zabel, A. 2003. The assessment of soil quality. Geoderma. **114**: 143-144.

[24] Stahl, P.D. and Parkins, T.B. 1996. Relationship of soil ergosterol concentration and fungal biomass. Soil Biol Biochem. **28**: 847-855.

[25] Stenersen, J. 2004. *Chemical pesticides mode of action and toxicology*. CRC press. pp. 296.

[26] Tavares, G.M. and Souza, P.E.D. 2005. Efeito de fungicidas no controle in vitro de Colletotrichum gloeosporioides, agente etiológico da antracnose do mamoeiro (*Carica papaya* L.). Ciênci Agrotecnol. **29**(1): 52-59, 2005.

[27] Teather, K., Harris, M., Boswell, J. and Gray, M. 2001. Effects of Acrobat MZ® and TattooC® on Japanese medaka (*Oryzias latipes*) development and adult male behavior. Aqu Toxicol. **51**(4): 419-430.

[28] Urban, D.J. and Cook, N.J. 1986. Hazard evaluation division – Standard evaluation procedure ecological risk assessment. Washington, USEPA Publication 540/9-86-001, pp. 96.

[29] Vyas, S.C. 1988. Soil microorganisms and their activities. In: Vyas, S. C. (Ed.) Non-target effects of agricultural tebuconazoles. Boca Raton: CRC Press, p. 131-160.

[30] Walker, H.L. and Boyette, C.D. 1986. Biocontrol of sicklepod (*Cassia obtusifolia*) in soybeans (*Glycine max*) whit *Alternaria cassiae*. Weed Sci. **33**: 212-215.

[31] Widenfalk, A., Bertilsson, S., Sundh, I. and Goedkoop, W. 2008. Effects of pesticides on community composition and activity of sediment microbes–responses at various levels of microbial community organization. Environ Pollu. **152**(3): 576-584.

[32] Xiaoqiang, C.H.U., Hua, F.A.N.G., Xuedong, P.A.N., Xiao, W.A.N.G., Min, S.H.A.N., Bo, F.E.N.G. and Yunlong, Y.U. 2008. Degradation of chlorpyrifos alone and in combination with chlorothalonil and their effects on soil microbial populations. J Environ Sci. **20**(4): 464-469.

[33] Yu, L., Chen, M., Liu, Y., Gui, W. and Zhu, G. 2013. Thyroid endocrine disruption in zebrafish larvae following exposure to hexaconazole and tebuconazole. Aqua Toxicol. **138**: 35-42.

[34] Zagatto, P.A. and Bertoletti, E. 2008. Ecotoxicologia aquática – princípios e aplicações. São Carlos: RIMA, pp. 478.

[35] Zhang, M., Xu, Z., Teng, Y., Christie, P., Wang, J., Ren, W., Luo, Y. and Li, Z. 2016. Non-target effects of repeated chlorothalonil application on soil nitrogen cycling: the key functional gene study. Sci Total Environ. **543**: 636-643.

[36] Zilli, J.É., Rumjanek, N.G., Xavier, G.R., Costa Coutinho, H. L. and Neves, M.C.P. 2003. Diversidade microbiana como indicador de qualidade do solo. Cadernos de Ciênci Tecnol. **20**(3): 391-411.

http://dx.doi.org/10.22161/ijeab/2.1.58 *ISSN: 2456-1878*

A Review Study on Fluoride Toxicity in Water and Fishes: Current Status, Toxicology and Remedial Measures

Rajinder Kaur, Amita Saxena, Munish Batra*

Department of Fisheries Resource Management, College of Fisheries, GBPUA&T, Pantnagar, India
*Department of Veterinary Pathology, College of Veterinary and Animal Sciences, G. B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand, India

***Abstract*—** *Fluoride is widely distributed in nature in many forms and its associated compounds have been used extensively but its limit in water is exceeding the permissible level. Excess of fluoride (>1.5 mg/l) in drinking water is harmful to the health. Fluoride toxicity is increasingly becoming a matter of great concern as many countries in the world have been declared as endemic for fluoride. This makes it imperative for scientists to focus on the precise toxic effects of fluoride on various soft tissues. Fluoride is toxic to all the system and causes oxidative stress in various tissues. When fluoride is ingested, approximately 93% is absorbed into the bloodstream. Contamination of drinking water due to fluoride is a severe health hazard problem. A good part of the material is excreted but the rest is deposited in the bones and teeth and is capable of causing a crippling skeletal fluorosis, non-skeletal fluorosis and dental fluorosis. There are various treatment technologies for removing fluoride from groundwater but these methods are very expensive. Besides using the water treatment techniques, various plants are having therapeutic properties to reduce the fluoride toxicity which is a cost effective to cure the fluoride induced toxicity.*

Keywords*— *Fluoride, Water, Fluorosis, Fishes, Defluoridation, Medicinal plants.

I. INTRODUCTION

Water is the most abundant and essential life supporting component. But now days, most of the countries in the world are facing the problem of drinking water. In India, drinking water is found to be contaminated at many places by different kinds of pollutants such as fluorides, iron and nitrates etc. Pure water is scarce and not easily available to all living beings including human being, birds, animals as well as plants. Deprived sections of the society consume contaminated water and become sick periodically, often results in the outbreak of epidemics. The water may be contaminated by natural sources or by the industrial effluents. One such contaminant is fluoride.

The fluorides belong to the halogen group of minerals and are natural constituents of the environment. Fluoride is an essential element for life which is mainly found in ground water when it is derived by the solvent action of water on the rocks and the soil of the earth's crust. It is the most electronegative of all chemical elements and is never encountered in nature in the form of element. It is seventeenth in the order of frequency of occurrence of the elements and represents about 0.06% to 0.09% of the earth's crust **(Wedepohl, 1974)**. World Health Organization (WHO) and IS: 10500 recommended that the permissible limit of fluoride content in drinking water is 1.0 to1.5 ppm. At low concentration, fluoride deficiency can occur but at high concentrations of fluoride some deleterious effects can arise. In relation to drinking water it is generally believed that too little (<0.5 mg/l) or too high (>1.5mg/l) can affect bone and teeth structure **(Edmunds and Smedley, 1996 and 2003)**. Among the water quality parameters, fluoride ion exhibits unique properties as its optimum concentration in drinking water is advantageous to health and in case of exceeding concentration above the permissible limit affects the health **(Venkata *et al.*, 1995)**. High fluoride concentration in the ground water and surface water in many parts of the world is of great concern. The main source of fluoride in ground water is fluoride-bearing rocks such as cryolite, fluorite, fluorspar, fluorapatite and hydroxylapatite **(Meenakshi *et al.*, 2004)**. The content in ground water is a function of many factors such as solubility and availability of fluoride minerals, pH, temperature and velocity of flowing water and concentration of calcium and bicarbonate ions in water. **(Agarwal *et al.*, 1977; Chandra *et al.*, 1981).** Fluoride enters the body through water, drugs, food, industrial exposure, etc. but among the other souces, drinking water is the major source (75%) of daily intake. Because of the strong electronegative charge, fluoride is attracted towards the positively charged calcium in teeth and bones. It causes many health problems such as dental fluorosis, skeletal fluorosis, teeth

mottling and deformation of bones in human beings **(Susheela *et al.*, 1993)**. Excessive fluoride concentration affects both plants and animals. It is oftenly considered as a "two edged sword" because deficiency of fluoride intake leads to dental caries while excessive consumption leads to dental and skeletal fluorosis. So the fluoride is harmful in both the cases i.e., deficiency and excess. **Kharb and Susheela (1994)** reported that fluoride has also affected the soft tissues such as muscles and ligaments. Fluorosis is an important clinical and public health problem in several parts of the world. Global existence of fluorosis is reported to be about 32% **(Varier, 1996)**.

The conventional method of defluorination includes: adsorption, ion-exchange and reverse osmosis **(Amor *et al.*, 1998; Hichour *et al.*, 1999, 2000)**. The ion-exchange and reverse osmosis process are relatively expensive than adsorption. Therefore, still adsorption is considered to be the most viable method to defluoridate the water. In this process, contaminated water is passed through an adsorbent bed, where fluoride can be removed by ion-exchange, physical or surface chemical reaction with adsorbent. As this method is easy to operate and cost-effective, adsorption is still widely accepted as an efficient pollution removal technique. Various publications are available on effective fluoride removal methods using low cost materials. Several tested materials include activated carbon, activated alumina, amorphous alumina, bleaching earth, calcite, charcoal, zeolite clay and red mud etc **(Rubel, 1983; Yang *et al.*, 1999; Li *et al.*, 2001; Wang and Reardon, 2001; Christopher *et al.*, 2004)**. The materials like bentonite, lignite, charfines, kaolinite, and nirmali seeds were also investigated for the removal of fluoride **(Srimurali *et al.*, 1998).** Plant materials are also reported to accumulate fluoride and hence act as defluoridating agents and also Vitamin C is an efficient source to reduce fluoride.

II. PERMISSIBLE LIMITS FOR FLUORIDE CONCENTRATION IN DRINKING WATER (VARADAJAN AND PURANDARA, 2008)

- Bureau of Indian Standards (BIS)-0.6 to 1.2 mg/l
- World health Organization (WHO-1984) for drinking water-1 to 1.5 mg/l
- Indian Council of Medical Research (ICMR-1975)-1 mg/l
- World Health Organization (WHO) European Standards- 0.7 to 1.7 mg/l related to temperature.

III. SCENARIO OF FLUOROSIS AT GLOBAL LEVEL

Fluoride content is high in various parts of the world and it causes adverse effects to the living beings. Fluorosis is a health problem of global community as 23 nations across the world are facing this problem **(Basha *et al.*, 2010)**. Especially, it is prevalent in the third- world countries where most of the people are dependent on drinking water containing fluoride **(Madhusudhan *et al.*, 2009)**. The problem of dental fluorosis is a major issue in many countries like China, India and Mexico **(Pendrys and Katz, 2001)**. Apart from these nations, Argentina, Algeria, Egypt, Iran, Australia, Iraq, Japan, Jordan, Kenya, Libya, Morocco, New Zealand, Pakistan, South Africa, Syria, Tanzania, Thailand, Turkey and United States of America are under the threat of fluorosis. Approximately 6% of the total population of Mexico is affected by fluorosis **(Valdez *et al.*, 2011)**. The epidemiological studies that have been conducted in China have shown that about 330 million people are exposed to high fluoride content and among them about 42 million people are suffering from fluorosis **(Wang *et al.*, 2004)**. High fluoride concentrations in groundwater are found in the Africa, Australia, China, Ghana, India, Kenya, Sri Lanka, Tanzania and USA besides other countries in different continents **(Jagtap *et al.*, 2012)**.

IV. FLUORIDE LEVEL IN INDIAN WATER

Fluoride content is above the permissible levels of 1.5ppm occur in 14 Indian states, namely, Andhra Pradesh, Bihar, Rajasthan, Gujarat, Haryana, Karnataka, Uttar Pradesh, Kerala, Madhya Pradesh, Maharashtra, Orissa, Punjab, Tamil Nadu and West Bengal affecting about 69 districts. It has been found that 65 per cent of India's villages are at fluoride risk **(Kumar and Shah, 2006)**.

In India, about 25 million people are affected by fluorosis, especially in the states of Andhra Pradesh, Bihar, Delhi, Gujarat, Haryana, Jammu Kashmir, Kerala, Madhya Pradesh, Maharashtra, Punjab, Rajasthan and Tamil Nadu. In India, about 66.62 million people are at the risk of fluorosis **(Susheela, 2007)**. According to the recent survey by International Water Management Institute (IWMI) in north Gujarat, the results showed that 42 per cent of the people covered in the sample survey (28,425) were affected; while 25.7 per cent and 6.2 per cent were affected by dental fluorosis and muscular skeletal fluorosis respectively. 10 per cent were affected by both the types of fluorosis.

V. GENERAL MECHANISM OF TOXICITY

A review by **Barbier *et al.* (2010)** has sketched a number of cellular processes in which fluoride can have negative effects. Effects that have been identified through different experimental studies include alteration of gene

expression, disruption of enzyme activity (mostly inhibition), inhibition of protein synthesis and secretion and generation of reactive oxygen species (ROS).

Fluoride disrupts the activity of enzyme by binding it to the functional amino acid groups that encircles the enzyme's active centre. This consists of the enzyme inhibition of the glycolytic pathway and the Krebs cycle **(Barbier *et al.*, 2010)**. At micromolar and millimolar concentrations, fluoride can act as an anabolic agent and promote the cell proliferation and an enzyme inhibitor respectively. This is illustrated from the study of **Mendoza-Shulz *et al.* (2009).** There is an example of phosphatases, which play an important role in the ATP (cellular energy) production cycle and cellular respiration. Fluoride interrupts the signalling pathways which are involved in cell proliferation and apoptosis and then cause the inhibition of protein synthesis and secretion **(Barbier *et al.*, 2010)**. It has been found that fluoride has association with oxidative stress which can lead to the reduction of mitochondrial fitness and also degrades the cellular membranes. The increase of oxidative stress leads to an increase in the expression of genes responsible for stress response **(Barbier *et al.*, 2010)**.

VI. TOXICOLOGICAL EFFECTS OF FLUORIDE ON FISH

Fluoride is present in the environment as the stable form of the super reactive element fluorine. Fluorine is the seventeenth most plentiful element in the earth's crust, with fluoride detectable in almost all minerals. The main minerals are Cryolite (Na_3AlF_6), Fluorspar (CaF_2) and Fluorapatite ($Ca_{10}F_2(PO_4)_6$). Naturally, through the weathering of alkalic and silicic igneous and sedimentary rocks, primarily shales, as well as from emissions of volcanic eruption, fluoride enters the aquatic system. It has been found that in freshwater there is a concentration less than 1.0 mg/l and in natural water, its concentrations may exceed even 50.0mg/l **(McNeely *et al.*, 1979)**. To judge on the potential environmental impacts of fluoride, it is important to firstly gather the current available information about its impact on the homeostasis within organisms. However, the evidences from the studies conducted till now are not conclusive whether fluoride is essential for any other biological function or not **(Government of British Columbia, 1990)**. The most common disorder associated with the excessive fluoride level is fluorosis. This condition is related to the retention of excess fluoride content within the body and its harmful integration into biochemical pathways, often as a replacement for calcium **(Barbier *et al*., 2010)**.

6.1 Effect on behaviour

Behavioural alterations can be considered as sensitive indicators of environmental stress. Many studies have been done to observe the behavioural changes in aquatic organisms due to exposure of pollutants **(Shaikh, 1999)**. Fluoride induced changes in the behaviour of fresh water fishes have been reported from different experiments **(Aziz *et al.*, 2014)**. Delay in trout migration has been reported by **Neuhold and Sigler (1960)** at measurable level of fluoride. **Manna *et al.*, 2007** observed the adverse effect due to fluoride toxicity includes enzyme inhibition, collagen breaks down, gastric damage and disruption of the immune system. **Bajpai *et al.*, (2009)** have also reported the behavioural abnormalities on the exposure of sodium fluoride to the experimental fishes include erratic swimming, fast breathing, loss of schooling behaviour and secretion of large amount of mucus on body of *Heteropneustis fossilis*. **Narwaria and Saksena (2012)** reported that the behavioural responses due to sodium fluoride toxicity include body position, habit, food sensitivity, rate of operculum opening and swimming movements. However the accumulation and increased mucus secretion in the fluoride exposed fish may be an adaptive and protective response to avoid the absorption of the applied toxicant by the overall body surface **(Das and Mukherjee, 2003; Yilmaz *et al.*, 2004)**. Due to fluoridicated toothpaste, changes in the behaviour of *Clarias batrachus* and *Catla catla* were reported by **Sahu *et al.* (2014)** and **Verma *et al.* (2015)** respectively. The behavioural changes in feeding, swimming movement, body orientation, opercular activity, gulping activity, mucus secretion and body coloration were observed.

6.2 Effect on growth and development

During the study of growth parameters, physical variables are taken into consideration such as length, weight, volume etc. Exposures to trace elements and fluoride affect the developmental stages of aquatic organism **(Thurberg *et al.*, 1975). Ellis *et al.*, (1948)** reported delay in hatching time when the fish eggs were subjected to 1.5ppm fluoride level. **Shi *et al.*, (2009 a, b)** have reported the significant increase in fluoride concentration in bone, gill, cartilage and skin of Siberian sturgeon when exposed to lethal dose of fluoride. **Tripathi *et al.* (2005)** found that the higher concentrations of Fluoride inhibit the growth of fishes such as weight, length and of fingerlings of *Heteropneustis fossilis*. According to **Bajpai and Tripathi (2010)** lipid and protein act as growth bioindicator against fluoride pollutant in *Heteropneustis fossilis*. These biomolecules gets reduced in the body tissues after the chronic exposure of fluoride. Hence, resulted in the depletion of the appropriate growth and development of fish. **Agniwanshi *et al.*, (2014)** studied the effect of sodium fluoride on body weight gain and gonadosomatic index in freshwater catfishes and revealed a statistically significant effect of different doses of sodium fluoride on gonadosomatic index (GSI) and

body weight gain in both species, *i.e., Clarias batrachus* and *Heteropneustes fossilis* which is irrespective of phase of annual reproductive cycle. It has also been noticed the concentration of sodium fluoride is having inverse relationship with the body weight as the increase in the fluoride content results in decrease in body weight gain. Further, in both the sexes i.e., male and female of *Clarias batrachus* and *Heteropneustes fossilis* GSI were found to decrease in most of the groups maintained in different concentrations of sodium fluoride as compared to control group.

6.3 Effect on Chromatophores

The various studies have been conducted indicating the negative effect of pollutants in fishes but very little information is available about their effect on pigmentation. Chromatophores are reponsible for the change of colour in fishes at the time of courtship, protection, mating and reproduction **(Fujii, 2000)**. The study on effect of fluoride on coloration in *Heteropneustis fossilis* and *Channa punctatus* respectively found that continuous exposure of sodium fluoride resulted in altered size, shape and dispersion quality of chromatephores in the skin of *Heteropneustis fossilis*. Chromatophore numbers were increased while their size was reduced and shape of chromatophores become stellate in comparisons to reticulate chromatophore of control group **(Tripathi *et al.*, 2005; Bajpai *et al.*, 2012)**.

6.4 Effect on Reproductive System

Fluoride adversely affects the structure and mobility of sperm causes alteration in the level of reproductive hormones. **Shingadia and Agharia (2001)** observed histoarchitectural changes in the testis and ovary of larvivorous fish because of fluoride exposure. In testis, it caused degeneration of seminiferous tubules and their epithelium due to denudation and vacuolization of cells, atrophy of spermatocyte and hyperplasia of sertoli cells where as in ovary, it caused hyperplasia of germinal epithelial and involution of ova, decreased frequency of oocyte maturatin and cytoplasmic vaculolation. **Kasirsagar *et al* (2011)** reported damaged oocyte, disorganization of ooplasm, inhibition of ovarian development and empty space of follicle in fresh water fish *Rita rita* due to the fluoride induced toxicity.

6.5 Effect on Haematology

Saxena *et al.* (2001) reported significant decrease in TEC, Hb, PCV, MCV, MCH and MCHC with increase in the concentration of fluoride in *Channa punctatus*. Gupta *et al.* (2002) also found that fluoride caused decrease in TEC, Hb, PCV, ESR while TLC was increased in *Channa punctatus* and *Labeo rohita*. **Kumar *et al.*, (2007)** reported significant decrease in the content of RBC, Hb, PCV and carrying capacity of oxygen by blood in *Clarias batrachus*. Time and dose dependent decrease in RBC, WBC count and Hb was observed by **Kamble and Velhal (2010)** at different concentrations of fluoride *i.e.*, 100ppm, 200pm and 300ppm. The results indicated immunological suppression. Study on fluoride toxicity in *Clarias batrachus* by **Guru *et al.*, 2014** has shown clumping of RBCs and clumping becomes prominent at higher fluoride concentrations. The TEC, Hb, PCV, MCV, MCH and MCHC level was progressively decreased with an increase in the concentration of fluoride.

6.6 Effect on serum and tissue biomolecules

Studies by **Chitra *et al.*, 1983** and **Kumar *et al.*, 2007** have shown that fluoride affects the certain biomolecules and enzymes in different tissue of fresh water fishes. Fluoride mainly affects the cholesterol, glucose, protein, lipid and glycogen level as all these biomolecules play a pivotal role in survival, growth and reproduction of fishes. **Dousset *et al.*, (1987)** and **Gikunju *et. al.* (1992)** have reported an increase in the cholesterol level in liver, muscle and testis of fishes due to fluoride doze. Alteration in the level of these biomolecules can results in reduction of fish growth and population. Calcium (Ca) and magnesium (Mg) act as second messenger for replication, transcription and translation. Fluoride reduces the absorption of both Ca and Mg from fish gut **(Machoy, 1995)**. **Kumar *et al.*, 2007** conducted an experiment to study the fluoride-induced biochemical changes in different tissues such as muscle, liver, and testis of fresh water catfish. There was significant decrease in the glycogen content in muscle and testis at the lower concentration but at the same time it was increased in all the three tissues at the higher concentration. **Aziz *et al.*, 2013** have found that fluoride increased the alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) level in gills of fresh water fish *Oreochromis mossambicus*. The exposure of sodium fluoride (NaF) to the larvivorous fish (*Poecilia reticulate)* revealed stress on respiratory metabolism and led to decrease in Succinic dehydrogenase and Lactate dehydrogenase (LDH) enzymes which are involved in carbohydrate metabolism and this decrease might have led to metabolic shift from aerobic to anaerobic mode of respiration during the toxic phase of fluoride induced stress **(Shingadia and Agharia, 2013)**.

6.7 Genotoxicity and Cytotoxicity in fish

It has been reported that on exposure to high content of fluoride, it inhibits the cell proliferation, growth and induced apoptosis. **Jha (2004)** has seen DNA and cytogenetic alterations in aquatic organisms impaired enzyme function or general metabolism, abnormal

development, immunotoxicity, cytotoxicity, reduced growth, survival and reproduction potency. According to some reports, fluoride caused chromosomal aberration and DNA damage in mammalian cells. **(Joseph *et al.*, 2000; Podder *et al.*, 2008). Tripathi *et al.* (2009)** reported that the chromosomal aberrations increased with the increase in fluoride dose in *Clarias batrachus*. Results of the research experiments have shown that fluoride influences the different singling pathways which are involved in cell proliferation and apoptosis **(Barbier *et al.*, 2010)**. Cytotoxic and genotoxic studies in fish are demonstrating the sensitivity of these organisms. Micronuclei test (MNT), Comet assay and Chromosomal aberration test (CAT) are the most commonly and widely used methods to prove genotoxicity in fish **(Garg and Sharma, 2012)**.

6.8 Effect on Histology

Tripathi *et al.* (2006) reported the effect of fluoride on vertebral coloumn of *Channa punctatus* and its results revealed that fluoride caused decrease in diameter of neural canal and increase of bone density. **Bhatnagar *et al.*, 2007** visualized the fluoride-induced histopathological changes in gill, intestine and kidney of fresh water fish, *Labeo rohita*. In the fluoride exposed group, with increasing severity with the time, the gill tissue developed clubbed lamellae, lamellar hyperplasia and mucoid metaplasiad. The kidney showed renal architectural damage in the form of shrunken glomeruli, shrunken lumen of renal tubules, increased capsular space and vacuolated cytoplasm. The intestine exhibited flattening and fusion of villi and a cracked clay appearance. All these changes were not seen in the control group.

Haque *et al.*, 2012 observed severe vacuolation in the gastric epithelium and disruption in the tubular gastric glands of the stomach occurred. In the stomach, there was loss of microridges with vigorous mucus secretion, degeneration of epithelial cells and disarrangement of mucosal folds. Changes in the intestine include degeneration of villi with severe necrosis in absorptive columnar epithelial cells and also the fusion of cells at the basal region was visible. The disruption of primary and secondary mucosal folds resulted in reducing the absorptive luminal surface area. The centrolobular area of the liver exhibited focal necrosis. The zymogen granules gets scattered in the hepatopancreatic acinar cells and these changes resulted into the cell's degranulation and vacuolation. Kidney showed disruption of Bowman's capsule and degradation in the epithelial cell lining of renal tubules, particularly in the proximal tubules**.**

Bajpai *et al*. (2012) found that on exposure to fluoride, primary and secondary lammelar epithelium become swelled and clubbing on the tip of secondary lamellae of gills, shortening, and fusion of secondary lamellae, hyperplasia and hypertrophy in chloride cells of gills. **Kahirsagar *et al.* (2011)** and **Shingadia and Agharia (2013)** observed the fluoride induced histopathological alteration in ovary and testis of freshwater fish *Rita rita*. **Shingadia (2014)** observed the loss of structural integrity of mucosal folds, degeneration of mucosal epithelium, vacuolation and decrease in number and rupture of goblet cells. The flask shaped goblet cells became spherical with decrease in their number & ruptured epithelial cell lining. Columnar cells of villi formed homogenous mass due necrosis of intestinal tissue. Serosa of intestine was also ruptured. Thinness of circular muscle increased causing obliteration of sub-mucosa, which showed disruption with atrophy. **Yadav *et al.* (2014)** conducted a study to observe the alterations on fluoride exposure and its results showed that fluoride caused vacuolization, pyknotic nuclei, disruption and rupture as well as hypertrophy and hyperplasia of hepatocyte in *Hetropneustes fossilis*.

6.9 Effect on Genobiotics

National Cancer Institute Toxicological Program categorizes fluoride to be a suspicious carcinogen. On the basis of studies done, it was suggested that fluoride is one of the most damaging environmental pollutant and is deliberated as mutagenic agent, genotoxic and neurotoxic. It may induce mutagenic, genotoxic and neurotoxic effects in aquatic organisms **(Bhatnagar and Regar, 2005; Azmat *et al.*, 2007; Tripathi *et al.*, 2009)**.

VII. REMEDIAL MEASURES

Chronic fluoride intake in absence/non-availability of pure drinking water is the prime cause of fluorosis. Long term intake of fluoride is also known to cause physiological disturbances in carbohydrate and lipid metabolism and cause oxidative stress. There are no remedial measures for fluorosis other than using water purification techniques. Following are the reviews of role of medicinal plants in reducing/ ameliorating the oxidative stress caused due to fluoride intake.

7.1 Defluoridation using water purification techniques

The high fluoride levels in drinking water and its impacts on human and animal health have increased the importance of defluoridation studies **(Chidambaram *et al.*, 2003)**. Defluoridation was reported by adsorption **(Raichur and Basu, 2001)**, chemical treatment **(Reardon and Wang, 2000)**, ion exchange **(Singh *et al.*, 1999)**, membrane separation **(Dieye *et al.*, 1998)**, electrolytic defluoridation **(Mameri *et al.*, 2001)** and electro dialysis **(Hichour *et al.*, 2000)** etc. Among various processes, adsorption was reported to be an effective, environmentally friendly and economical one **(Mohan *et***

***al.*, 2007)**. The advantages of biosorption are very well known, the contaminants in water are removed by getting concentrated onto a disposed of **(Volesky, 2007).** Biosorption offers advantages of high efficiency in dilute effluents and no requirements of nutrient. Recently substantial interest was seen on the biosorbent material's applications for the removal of various pollutants and it also provides a cost-effective solution for the water management **(Volesky and Holan, 1995)**. "Adsorption is a mass transfer process in which a constituent in the liquid or gas phase is accumulated on solid or liquid phase and separated from its original environment" **(Crittenden *et al.*, 2005)**. The adsorption process has more advantages than other methods to remove pollutants from the water and wastewater, as it is having more simplified design of adsorption unit, negligible amount of sludge production and low investment costs **(Malakootian *et al.*, 2008)**. The uptake of anions has become a growing concern in the field of biosorption **(Kratochvil and Volesky, 1998)**. Investigators reported various types of adsorbents namely activated alumina **(Ghorai and Pant, 2004)**, tita-nium-rich bauxite **(Das *et al.*, 2005)**, synthetic resins **(Meenakshi and Vishwanathan, 2006)**, manganese oxide-coated alumina **(Maliyekkal *et al.*, 2006),** carbon nanotubes **(Li et al., 2003),** fish bone charcoal **(Killedar and Bhargave, 1993)**.

Mariappan *et al.* (2003) studied defluoridation technique using poly aluminum hydroxy sulphate (PAHS). The results of the study showed that the floc formation and settling are quick and volume of resulting sludge is very less. **Sanjaykumar (2002)** used various indigenous chemicals and minerals to study the defluoridation methods. The study concluded that alum can be used as an effective defluoridation agent if alum dose, alkalinity of water, water pH and colloidal concentration are optimized. **Muthuganesh *et al.*, (2003)** used poly aluminum chloride (PAC) to study fluoride removal techniques and compared it with the most commonly existing 'Nalgonda technique'. The results from the study indicated that PAC can be an effective coagulant for fluoride removal with higher removal efficiency of about 65% -75% with less detention time. **Bhargava and Killedar (2006)** used fishbone charcoal prepared from fishbone in coastal areas and condluded that the removal of fluoride was found to be function of contact time, pH, initial fluoride ion concentration and adsorbent (fishbone charcoal) dose. **Ganguly (2006)** used boiler bottom ash as an adsorbent material to separate fluoride content from the water.

7.2 Therapeutic effects of medicinal plants in reducing fluoride toxicity in water

Fluoride is toxic to all the system and cause oxidative stress in various tissues. All over the world, research is going on different plant species to study their principles and potential. Plant-based dietary therapies are considered to have higher potential for therapeutic applications as there can be minimum or no side effects with their use. Research in the area of nutrition is now being mainly focused on formulating 'healing diets' which can improve the overall health efficiently. There are various plants and plant based products having higher efficacy in reducing the oxidative stress due to fluoride, as fluorosis is considered as both endemic and global spanning in several continents. Medicinal plants play important role in amelioration of fluoride toxicity. Nutritional interventions like high intake of vitamin C, vitamin D and calcium helps to reduce the problem of fluorosis. *Emblica officinalis* (G), *Mangifera indica* (L), *Limonia acidissima* (L), *Averrhoa carambola* (L) and leaves of *Tamarindus indicus* are effective to used against the fluoride toxicity The fruits and leaves of these plants are well known for their medicinal use as it contained phytosterols, saponins, polyphenols, flavonoids, ascorbic acid and fibers **(Narasimhacharya and Vasant, 2012).**

Murugan and Subramanyam (2002) studied the use of Aloe Vera (Indian aloe) a medicinal plant and concluded that at neutral pH the defluoridation was maximum. **Prabavathi (2003)** studied defluoridation techniques by using lignite rice husk and rice husk powder as adsorbent by varying pH, concentration of fluoride, weight of adsorbent and contact time. **Jamode *et al.* (2004)** used fresh leaves chosen based on their crude fiber content and tress were obtained from Pipal (*Ficus religliusa*), neem (*Azadirachta indica*) and khair (*Acacia catechu* Willd) for the uptake of fluoride ion from the fluoridated water. During the study by using adsorption method, it was found that various parameters such as contact time, pH, adsorbent dose, size and type of adsorbents and initial fluoride ion concentrations affect the fluoride removal efficiency at optimum conditions. **Gopal and Elango (2007)** used activated carbon developed from leaves of *Agave sisalana* by batch process. Maximum adsorption of fluoride ion was observed in the pH level of 6.76, optimum dosage of 5g/l and optimum contact time was observed to be 40 minutes. Up to 86% level, the defluoridation can be achieved using this process.

Biological materials such as leaves of neem (*Azadirachta indica),* peepal (*Ficus religiosa)* and khair (*Acacia catechu)* and tamarind gel and seeds have been used to defluoridate water. Various herbal or natural products are being increasingly investigated for their role in minimising the effects of fluoride toxicity for e.g., supplementation of tamarind fruit pulp increased urinary excretion of fluoride while decreasing the retention of fluoride in bone. The bark and seed extracts of *Moringa oleifera* and *Terminalia arjuna* have also been shown to

decrease the fluoride induced toxicity. Additionally, plant metabolites such as a 43 kD protein isolated from *Cajanus indicus*, quercetin and curcumin have been shown to ameliorate the fluoride induced oxidative stress and also improve the functions of kidney, liver and erythrocytes. Additionally, administration of black berry juice and black tea were found to be useful in reducing the effects of fluoride **(Narasimhacharya and Vasant, 2012)**. **Pandey *et al.* (2012)** used biomass of *Tinospora cordifolia* for the sequestration of fluoride from drinking water. **Ramanjaneyulu *et al.* (2013)** used tamarind shell and papal leaf powder to remove fluoride from the drinking water. The effect of controlling parameters of adsorption like dose and pH of adsorbent, contact time and initial sorbate concentration for fluoride removal efficiency was studied and also found the optimum values for maximum uptake. At pH 2, tamarind fruit shell and papal leaf powder exhibited highest fluorine removal efficiency about 85% and 79% respectively. The medicinal plants like Amla (*Emblica officinalis*), lemon (*Citrus lemon*) and Tomato (*Lycopersicon esculentum*) are good source of antioxidants and played an important role in ameliorating the harmful effects of fluoride water **(Sharma *et al.*, 2014)**.

7.3 Role of Vitamin C in ameliorating fluoride toxicity

Vitamin C can be used as an effective fluoride ameliorating agentt as it is an excellent source of electrons, therefore, it forms free radicals by donating electron and then it can quench the fluoride ion reactivity and mitigate the harmful effects of fluoride water by increasing its urinary excretion and decreasing its retention in the body.

Shanmugam and Reddy (2015, 2016) evaluated the protective e toxicity in fishes and the results showed that Hb, RBC, PCV, M intoxicated fishes where as WBC level was increased. Howeve returning back all the haematological parameters near to normal lev (TG, TC, VLDL and LDL,) and liver markers (SGOT and SGPT) enzymes. Whereas, the level was decreased in NaF treated fishes. However, Vitamin C effectively reduced the elevation in lipid metabolic profiles and liver marker enzymes in NaF treated fishes.

Yadav *et al.*, 2014 conducted an experiment to evaluated the oxidative stress biomarkers in the freshwater fish, *Heteropneustes fossilis*(Boch) exposed to sodium fluoride. There was increase in LPO and in response to this the antioxidant defense mechanisms were induced. The effect of chronic exposure of fluoride on LPO, enzymatic and non-enzymatic antioxidant in liver and ovary. SOD, CAT and GST levels were increased significantly while the GPx and GSH level decreased significantly and non-significantly respectively.

VIII. CONCLUSION

In many developing countries, drinking water is found to be contaminated with fluoride sources and its chronic ingestion can causes a lot of severe problems to all the living organisms not only the aquatic organisms (including both plants and animals) but also the terrestrial organisms, birds as well as human beings who are using this fluoride contaminated water for drinking purpose. Fluoride toxicity causes fluorosis of various types such as skeletal, dental and non-skeletal fluorosis. To prevent this toxicity, best method is "water defluoridation" but most of the defluoridation techniques are expensive and not within reach of millions of people across the globe. So we can use focus on using various plants and plant products to ameliorate the effect of fluoride, as the medicinal plants are easily available or one can grow it in nearby areas which is available for plantation.

IX. FUTURE PERSPECTIVE

Further research is required to discover some cost effective and eco-friendly methods of defluoridation. So that such methods can reach up to common people. Various plants are known to have therapeutic effect in fluoride toxicity but many more are yet to discover. There is need to understand the molecular mechanisms of plant and plant products in reversing the adverse effects of fluoride intoxicated tissues which will help in understanding their beneficial effects in a better way and also bridging the gap between the existing researches.

REFERENCES

[1] Agarwal, V., Vaish, A. K. and Vaish, P. (1977). Ground water quality: Focus on fluoride and fluorosis in Rajasthan, *Curr. Sci*, 739: 743- 746.

[2] Agniwanshi, S., Shedpure, M. and Jain, N. (2014). Effect of sodium fluoride on body weight gain and gonadosomatic index in freshwater catfishes. *Journal of Industrial Pollution Control,* 30(2): 339-343.

[3] Aziz, F. R. A. (2013). "Skin permeability in NaF induced alteration of metals and liver marker enzymes in edible fish.

[4] Aziz, F. R. A. and Jabeen, F. (2014). A behavioral, morphological and growth rate alterations in fresh water fish *Tilapia mossambica* under fluoridation, Keenjhar Lake, Thatta, Sindh, Pakistan. *International Journal of Advanced Research*, 376-382.

[5] Azmat R. Talat R. and Ahmed K., 2007. "The Length-weight relationship, condition factor and impact of fluoride concentration in *Johnius belangerii* of Arabian Sea". *Journal of Environmental Toxicology*, 1: 138-143.

[6] Bajpai, S., Tewari, S. and Tripathi, M. (2009). "Evaluation of acute toxicity levels and behavioural responses of *Hetropneustes fossilis*

(Bloch) to sodium fluoride". *Aquaculture*, 10(1): 37-43.

[7] Bajpai, S. and Tripathi, M. (2010). "Retardation of growth after fluoride exposure in catfish, H*eteropneustis fossilis* (Bloch)". *Bioresources for Rural Livelihood*, 67 -173.

[8] Bajpai, S., Tewari, S. and Tripathi, M. (2012). Impact of fluoride on structural changes in gills of Indian cat fish, *Hetropneustes fossilis* (Bloch) after acute exposure Trend. *Bioscience*, 4(2): 165-168.

[9] Barbier, O., Arreola-Mendoza, L. and Del Razo, L. M. (2010). Molecular mechanisms of fluoride toxicity. Chemico-Biological Interactions 188:319-333..

[10] Basha, P. M., Madhusudhan, N., Rai, P., Ahmed, F. and Prasad, G. R. (2010). Effect of maternal fluoride exposure on developing CNS of rats: Protective role of Aloe vera, Curcuma longa and Ocimum sanctum. *Ind J Exp Bio.*, 48: 830-836.

[11] Bhargava, D. S. and Killedar, D. J. (2006). Fluoride adsorption on fishbone charcoal. *Journal of IAEM*, 33(3): 179-183.

[12] Bhatnagar, C., Bhatnagar, M. and Regar, B. C. (2007). Fluoride-induced histopathological changes in gill, kidney, and intestine of fresh water teleost, *Labeo rohita. Fluoride*, 40(1): 55–61.

[13] Bhatnagar, C. and Regar, B. C. (2005). Neurodegenerative effect of Fluoride (NaF) on the brains of fresh water teleost, *Labeo rohita*. *Indian Journal of Environmental Sciences*, 9: 15-19.

[14] Chandra, S. J., Thergaonkar, V. P. and Sharma, R. (1981). Water quality and dental fluorosis, *Ind. J. Pub*, 115(25): 47-51.

[15] Chidambaram, S., Ramnathan, A. L. and Vasudevan, S . (2003). "Fluoride removal studies in water using natural materials," *Water SA*, 29(3): 339-343.

[16] Chitra, T., Reddy, M. M. and Rao, J. V. R. (1983). "Levels of muscle and liver tissue enzymes in *Channa punctatus* (Bloch) exposed to NaF". *Fluoride*, 16:48-51.

[17] Christopher, J., Kenneth, G., Ishida, P. and Richard, M. (2004). Bold testing of drinking water treatment: Co- polymers for compatibility with polyamide reverse osmosis membranes. 9*th World Filtration Congress*, 18-22 April 2004, New Orleans, 1-10.

[18] Crittenden, J., Trussell, R., Hand, D., Howe, K. and Tchobanoglous, G. (2005). "Water Treatment: Principles and Design," John Wiley and Sons, New York.

[19] Das, B. K. and Mukerjee, S. C. (2003). "Effect of pyrethroid on the behavior of *Heteropneustes fossilis*". *Comp. Biochem. Physiol. C. Toxicol. Pharmacol*., 314(1): 109-112.

[20] Das, N., Pattanaik, P. and Das, R. (2005). "Defluoridation of drinking water using activated titanium-rich bauxite," *Journal of Colloid and Interface Science*, 292: 6-45.

[21] Dieye, A., Larchet, C., Auclair, B. and Mar-Diop, C. (1998). "Elimination des Fluorures Parla Dialyse Ionicque Croisee," *European Polymer Journal*, 34(1):67-75.

[22] Dousset, J. C., Rioufol, C., Philibert, C. and Bourbon, P. (1987). "Effects of inhaled HF on cholesterol, carbohydrate and tricarboxylic acid metabolism in guinea pigs". *Fluoride*, 20:137-141.

[23] Edmunds, W. M. and Smedley, P. L. (1996). Ground water geochemistry and health: An overview. *In*: Environmental geochemistry and health (Eds: J.D. Appleton, R. Fuge, G.J.H. Mc call). Special Publication 113. London: *Geological Soceity*. 91-105.

[24] Edmunds, W. M. and Smedley, P. L. (2003). Fluoride in natural waters occurrence, controls and health aspects. *In*: Medical geology. New York (Ed.: O.Selenus). Academic Press.

[25] Ellis, M. M., Westfall, B. A. and Ellis, M. D, (1948). "Determination of water quality". Fish and Wild

[26] Fujii, R. (2000). "The regulation of motile activity in fish chromatophores." *Pigment Cell Res*., 13(5): 300-309.

[27] Ganguly, S., Mukharjee, S. N. and Misra, A. K. (2006). Removal of Fluoride from aqueous phase using boiler bottom ash as an adsorbent. *Journal of Indian Water Works Association*, 47-56.

[28] Garg, R. K. and Sharma, R. (2012). "Genotoxicity assessment using micronucleus assays in Sperata

[29] Ghorai, S. and Pant, K. (2004). "Investigations on the column performance of fluoride adsorption by activated alumina in a fixed-bed," *Chemical Engineering Journal*, 98(1-2): 165-173.

[30] Gikunju, J. K., Githui, K. and Maitho, T. E. (1992). "Fluoride levels in bore-hole water around Nairobi".

[31] Gopal, V. and Elango, K. P. (2007). Adsorption of fluoride from aqueous solution by active carbon. *Journal of Indian Water Works Association*, 183-191.

[32] Government of British Columbia, Ministry of Environment: Environmental Protection Division (1990) Ambient Water Quality Criteria for Fluoride: Overview report. Government of British Columbia, Victoria, British Columbia, Canada.

[33] Guru, S. K., Rajesh, B. and Kumar, M. B. (2014). "Fluoride induced alterations in erythrocyte and related parameters of an Air breathing fish, *Clarias punctatus* (Bloch). *Biolife*, 2(4):1371-1375.

[34] Haque, S., Pal, S. and Mukherjee, A. K. (2012). Histopathological and ultra-microscopic changes induced by fluoride in soft tissue organs of the air breathing teleost, *Channa punctatus* (Bloch). *Fluoride*, 45(3 Pt 2): 263-273.

[35] Hichour, M., Persin, F., Molenat, J., Sandeaux, J. and Gavach, C. (1999). Fluoride removal from diluted solu- tions by Donnan dialysis with anion-exchange membranes. *Desalination*, 122: 53-62.

[36] Hichour, M., Persin, F., Sandeaux, J. and Gavach, C. (2000). "Water defluoridation by donann dialysis and electro dialysis," *Separation and Purification Technology*, 18(1): 1-11.

[37] Hichour, M., Persin, F., Sandeaux, J. and Gavach, C. (2000). Fluoride removal from waters by Donnan dialysis. *Sepa- ration and Purification Technology*, 18: 1-11.

[38] Jagtap, S., Yenkie, M. K., Labhsetwar, N. and Rayalu, S. (2012). Fluoride in drinking water and defluoridation of water. *Chem Rev*., 112: 2454-2466.

[39] Jamode, A. V., Sakpal, V. S. and Jamode, V. S. (2004). Defluoridation of water using inexpensive adsorbents. *Journal of Indian Institute of Science*, 84: 163-171.

[40] Jha, A. N. (2004). "Genotoxicological studies in aquatic organisms: An over view. "Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 552(12): 1-17.

[41] Joseph, S. and Gadhia, P. K. (2000). "Sister chromatid exchange frequency and chromosome aberrations in residents of fluoride endemic regions of South Gujarat". *Fluoride*, 33(4):154-8.

[42] Kamble, N. A. and Velhal, V. V. 2010. "Study of sodium fluoride toxicity on hematological parameter of catfish (*Clarias batrachus* , Linn)". *Fluoride*, (1): 37-41.

[43] Kasirsagar, S. S., Kamble, S. V. and Rao, R. K. (2011). Effect of fluoride on histopathological changes in ovary of fresh water fish, *Rita rita*. *Bionano Frontier*, 4(2):276-278.

[44] Kharb, P. and Susheela, A. K. (1994). Fluoride ingestion in excess and its effect on organic and certain inorganic constituents of soft tissues. *Medical Science Research*, 22: 43-44.

[45] Killedar, D. J. and Bhargava, D. S. (1993). "Effects of Stirring Rate and Temperature on Fluoride Removal by Fishbone Charcoal," *Indian Journal of Environmental Health*, 35(2): 81-87.

[46] Kratochvil, D. and Volesky, B. (1998). "Advances in the biosorption of heavy metals," *Trends in Biotechnol*ogy, 16(7): 291-300.

[47] Kumar, M. D. and Shah, T. Groundwater pollution and contamination in India: The emerging challenge. Vallabh Vidyanagar, Gujarat, India: IWMI-TATA Water Policy Research Program. 14p.

[48] Kumar, A., Tripathi, N. and Tripathi, M. (2007). "Fluoride induced biochemical changes in fresh water cat fish (*Clarias batrachus*, Linn)". *Fluoride*, 40(1): 37-41.

[49] Li, Y. H., Wang, S., Cao, A., Zhao, D., Zhang, X., Xu, C., Luan, Z., Ruan, D., Liang, J., Wu, D. and Wei, B. (2001). Adsorption of fluoride from water by amorphous alumina supported on carbon nanotubes. *Chemical Physics Letters*, 350: 412-416.

[50] Li, Y. H., Wang, S., Zhang, X., Wei, J., Xu, C., Luan, Z. and Wu, D. (2003). "Adsorption of fluoride from water by aligned carbon nanotubes," *Materials Research Bulletin*, 38(3): 469-476.

[51] Machoy, M. A. (1995). "Fluoride magnesium interaction". *Fluoride*, 28:175.

[52] Madhusudhan, N., Basha, P. M., Begum, S. and Ahmed, F. (2009). Fluoride induced neuronal oxidative stress amelioration by antioxidants in developing rats. *Fluoride*, 42(3): 179-187.

[53] Malakootian, M., Almasi, A. and Hossaini, H. (2008). "Pb and Co removal from paint industries effluent using wood ash," *International journal of Environmental Science and Technology*, 5(2): 217-222.

[54] Maliyekkal, S., Sharma, A. and Philip, L. (2006). "Manganese- oxide-coated alumina: a promising sorbent for defluoridation of water," *Water Research*, 40(19): 3497-3506.

[55] Mameri, N., Lounici, H., Belhocine, D., Grib, H., Prion, G. F. and Yahiat, Y. (2001). "Defluoridation of sahara water by small electro coagulation using bipolar aluminium electrodes," *Separation and Purification Technology*, 24(1-2): 13-119.

[56] Manna, P., Sinha, M. and Sil, P. C. (2007). "A 43 kD protein isolated from the herb *Cajanus indicus* L.

[57] Mariappan, P., Yegnaraman, V. and Vasudevan, T. (2003). Defluoridation of water using Alumina-Gibbsite. *Indian Journal of Environmental Protection*, 23(9): 975-985.

[58] McNeeley, R. N., Neimanis, V. P. and Dwyer, L. (1979). Water quality source book. A guide to water quality parameters. Inland Waters

Directorate, Water Quality Branch, Environment, Canada.

[59] Meenakshi, S. and Viswanathan, N. (2006). "Identification of selective ion exchange resin for fluoride sorption," *Journal of Colloid and Interface Science*, 308(2): 438-450.

[60] Meenakshi, V. K., Garg, K., Renuka and Malik, A. (2004). Ground water quality in some villages in Haryana, India: Focus on fluoride and fluorosis, *Journal Hazardous Material*, 106: 85-97.

[61] Mendoza-Schulz, A., Solano-Agama, C., Arreola-Mendoza, L., Reyes-M.rquez, B., Barbier, O., Del Razo, L. M. and Mendoza-Garrido, M. E. (2009). The effects of fluoride on cell migration, cell proliferation, and cell metabolism in GH4C1 pituitary tumour cells, *Toxicol. Lett*. 190: 179–186.

[62] Murugan, M. and Subramanian, E. (2002). Application of aloe vera (Indian Aloe) – A plant material for defluoridation. *Indian Journal of Environmental Protection*, 22(9): 1034-1039.

[63] Muthuganesh, I., Vinodhini, V., Padmapriya, G., Sathiyanarayanan, K and Sabumon, P. C. (2003). An improved method for defluoridation. *Indian J Environ Health*, 45(1):65-72

[64] Narasimhacharya, A. V. R. L. and Vasant, R. A. (2012). Medicinal plants as antioxidants in fluoride induced oxidative stress. *Research Signpost*, 267-287.

[65] Narwaria, Y. S. and Saksena, D. N. (2012). "Acute toxicity bioassay and behavioural responses induced by sodium fluoride in freshwater fish *Puntius sophore* (Bloch)". *Fluoride*, 45(1): 712.

[66] Neuhold J. M. and Sigler W. F. (1960). "Effect of sodium fluoride on carp and rainbow trout". *Trans Am and Molecular Biology*, 40(3): 382-395.

[67] Pandey, P. K., Pandey, M. and Sharma, R. (2012). Defluoridation of water by a biomass: Ti*nospora cordifolia. Journal of Environmental Protection*, 3: 610-616.

[68] Pendrys, D. G. and Katz, R. V. (2001). Risk factors for enamel fluorosis in optimally fluoridated children born after the US manufacturers' decision to reduce the fluoride concentration of infant formula. *American Journal of Epidemiology*, 148:967-974.

[69] Podder, S., Chattopadhyay, A., Bhattacharya, S. and Ray, M. R. (2008). "Differential in vivo genotoxic effects of lower and higher concentrations of fluoride in mouse bone marrow cells". *Fluoride*, (4): 301-307.

[70] Prabavathi, N.T., Ramachandramoorthy, R., Edison, R., Kavitha, B., Sivraj, C. and Srinivasan. (2003). Drinking water of salem district-estimation of fluoride and its defluoridation using lignite rice husk and rice husk powder. *Indian journal of Environmental Protection*, 23(3): 304-308.

[71] Raichur, A. M. and Basu, M. J. (2001). "Adsorption of Fluo-ride onto Mixed Rare Earth Oxides," *Separation and Pu-rification Technology*, 24: 121- 127.

[72] Ramanjaneyulu, V., Jaipal, M., Yasovardhan, N. and Sharada, S. (2013). Kinetic studies on removal of fluoride from drinking water by using tamarind shell and pipal leaf powder. *International Journal of Emerging Trends in Engineering and Development*, 3(5): 146-155.

[73] Reardon, E. J. and Wang, Y. (2000). "Limestone reactor for fluoride removal from waste waters," *Environmental Sci-ence & Technology*, 34(15): 3247- 3253.

[74] Rubel, J. F. (1983). The removal of excess fluoride from drinking water by the activated alumina method. In: Shupe, J.L., Peterson, H.P. and Leone, N.C. Eds., Fluoride effects on vegetation animals and humans. Paragon Press, Salt Lake City, 345-349.

[75] Sahu, U., Choubey, K. and Gangadhar. (2014). Behavioral changes in *Clarias Batrachus* due to fluoridicated toothpaste. *Shrinkhala*, 2.

[76] Sanjaykumar, Gopal, K. and Seth, P. K. (2002). Indigenous minerals for defluoridation of water, *Indian journal of Environmental Protection*, 22(4): 361-372.

[77] Shaikh, F. I. (1999). "Chronic toxic effects of heavy metals on some physiological aspect of

[78] Shanmugam, K. R. and Reddy, S. (2016). Effect of vitamin c on haematobiochemical parameters in sodium fluoride (NaF) intoxicated *Channa punctatus* fishes. *Indo American Journal of Pharmaceutical Research.*

[79] Shanmugam, K. R. and Reddy, S. A. (2015). Protective effect of vitamin c in sodium fluoride (NaF) induced toxicity in fishes: a study with reference to lipid metabolic profiles and liver markers. *Indian Journal of Fundamental and Applied Life Sciences.*

[80] Sharma, J. D., Bhinda, S., Sharma, P. K., Kumari, M. and Boriwal, R. (2014). Therapeutic efficacy of medicinal plants to mitigate fluorosis. *Journal of Global Biosciences*. 3(5): 802-807.

[81] Shi X., Zhuang P., Zhang L., Feng G., Chen. L., Liu J. andWang R. (2009b). Growth inhibition of Siberian Sturgeon (II) from dietry and waterborne fluoride. Research Report. *Fluoride*, 137-141.

[82] Shi, X., Zhuang, P., Zhang, L., Feng, G., Chen, L., Qu, L. and Wang, R. (2009a). "The bioaccumulation of

[83] Shingadia, H. U. (2014). Chronic revelation to sodium fluoride induces pathohistological alterations in intestine of *Poecilia reticulata* peter. *Bionano frontier*, 2: 293-296.

[84] Shingadia, H. U. and Agharia, E. R. (2013). "Sodium fluoride induced alteration in dehydrogenase and acetyl cholinesterase activity in vital tissues of *Poecilia reticulata* peters". *Life Science Leaflet*, 122 -128.

[85] Singh, B., Sen, P. K. and Maunder, J. (1999). "Removal of fluoride from spent pot liner leachate using ion exchange," *Water Environment Research*, 71(1): 36-42.

[86] Srimurali, M., Pragathi, A. and Karthikeyan, J. (1998). A study on removal of fluorides from drinking water by ad- sorption onto low-cost materials. *Environmental Pollution*, 99: 285-289.

[87] Susheela, A. K., Kumar, A., Betnagar, M. and Bahadur, M. (1993). Prevalence of endemic fluorosis with gastro-intestinal manifestations in people living in some north-Indian villages, *Fluoride*, 26: 97-104.

[88] Susheela, A. K. (2007). A treatise on fluorosis. Fluorosis Research and Rural development foundation, New Delhi, 133p.

[89] Thurberg, F. P., Cable, W. D., Dawson, N. A., Mac Innes, D. R. and Wehzleff, D. R. 1975. "Respiratory response of larval , juvenile and adult surf clams , Spisula solidissima, In; Respiration of marine organisms Life Service Research Report 9, U.S. Department of Interior, Government Printing Office, Washington, D.C., 122.

[90] Tripathi N., Bajpai S., and Tripathi M. (2009). "Genotoxic alterations induced by Fluoride in Asian catfish,*Clarias batrachus* (Linn.)". *Fluoride*, 42(4): 292-296.

[91] Tripathi, A., Tripathi, N., Kumar, A. and Tripathi, M. (2006). Effect of fluoride on vertebral coloumn of a fresh water fish *Channa punctatus*. *J.Appl. Biosci*., 32(2): 164 -167.

[92] Tripathi, M., Tripathi A. and Gopal, K. (2005). "Impact of fluoride on pigmentation of a freshwater fish" *Channa punctatus*. .*J.Appl. Biosci*., 31(1): 35-38.

[93] Valdez, L., Fregozo, C. S., Beltran, M. L., Coronado, O. G. and Vega, M. I. (2011). Effects of the fluoride on the central nervous system. *Neurologia*, 26(5): 297-300.

[94] Varadajan, N. and Purandara, B. K. (2008). Fluoride contamination in ground water of malaprabha sub basin, *Journal of environ science and engg*., 50(2): 121-126.

[95] Varier, P. S. (1996). Indian medicinal plants: A compendium of 500 species, Vol. 1-5. Orient Longman Ltd., Madras.

[96] Venkata Mohan, S., Ramanaiah, S., Rajkumar, B. and Sarma, P. (2007). "Biosorption of fluoride from aqueous phase onto algal spirogyra io1 and evaluation of adsorption kinetics," *Bioresource Technology*, 98(5): 1006-1016.

[97] Venkata, M. S., Nikhila, P. and Reddy, S. J. (1995). Determination of fluoride content in drinking water and development of a model in relation to some water quality parameters, *Fresenius Envir Bull*, 4, 297-302.

[98] Verma, A., Solanki, M. S. and Shrivastava, S. 2015. Behavioral changes in *Catla catla* due to fluoridated toothpaste. *Indian streams research journal*, 5 (7).

[99] Volesky, B. (2007). "Biosorption and Me," *Water Research*, 41(18): 4017-4029.

[100] Volesky, B. and Holan, Z. R. (1995). "Biosorption of heavy metals," *Biotechnology Progress*, 11(3): 235-250.

[101] Wang, Y. and Reardon, E. J. (2001). Activation and regen- eration of a soil sorbent for defluoridation of drinking water. *Applied Geochemistry*, 16: 531-539.

[102] Wang, A., Xia, T., Ru, R., Yuan, J., Chen, X. and Yang, K. (2004). Antagonistic effect of selenium on oxidative stress, DNA damage, and apoptosis induced by fluoride in human hepatocytes. Fluoride, 37: 107-116.

[103] Wedepohl, K. H. (1974). Hand book of geochemistry. Springerverlage berlin. (Ed.: Heidelberf). New York. 2: 9.

[104] Yadav, S. S., Kumar, R. and Tripathi, M. (2014). Effects of fluoride exposure on some enzymatic and histopathological changes in the liver of *Heteropneustes fossilis* (Bloch). *International Journal of Fauna and Biological Studies*, 1(5):80-84.

[105] Yang, M., Hashimoto, T., Hoshi, N. and Myoga, H. (1999). Fluoride removal in a fixed bed packed with granular cal- cite. *Water Research*, 33: 3395-3402.

[106] Yilmaz, M., Gul, A. and Erbasli, K. (2004). "Influence of water hardness on cadmium toxicity to Salmogairdneri (Rush)". Bull. Environ. Contam.*Toxicol*.,56 (4): 575-582.

http://dx.doi.org/10.22161/ijeab/2.1.59 *ISSN: 2456-1878*

Resource Use Conflicts and Biodiversity Conservation in Jozani Ecosystem, Zanziba

Zella Adili Y., Juma Said M., Kalumanga Venance E

[1]Department of Economic Studies, The Mwalimu Nyerere Memorial Academy, P.o.Box 307, Zanzibar, Tanzania.
Mobile: +255787260448/719369242
[2]Ministry of Agriculture, Natural Resources, Livestock and Fisheries, P.o.Box 159, Zanzibar, Tanzani
Mobile: +255777496310/688937989
[3]Department of Gender Studies, The Mwalimu Nyerere Memorial Academy, P.o.Box 307, Zanzibar, Tanzania.
Mobile: +255754565770/712227545

***Abstract*—** *Resource Conflicts are the major challenge to the responsible Institutions in the management and conservation of biodiversity in Zanzibar due to the existence of multiple and interactive reasons that lead to conflicts. This paper intends to reveal the less known current status of resource conflicts in the management of biodiversity in Jozani ecosystem, Zanzibar. The study employed descriptive survey research design of the causal comparative research design to collect data from 280 respondents which constitute the study population. Descriptive statistics such as percentages, mean, frequency, standard deviation and Pearson correlation were used for data analysis. The outcome of the study showed that there is significant relationship existed between resource conflicts and the management of biodiversity conservation in Jozani ecosystem. The study has implications for environmental policy makers. The study concludes by asserting that unemployment, poverty and scarcity of environmental resources are the major causes of conflict, therefore the call is directed to policy makers to strengthen efforts on resolving conflicts by establishing overall strategies such as establishment of participatory community-based approaches to natural resource management, conflict resolution capacity building measures among the stakeholders, amendment of Laws and expansion of employment to reduce direct relying on using natural resource assets for livelihood.*

Keywords*— *Resource conflicts, biodiversity conservation, Jozani ecosystem.

I. INTRODUCTION

1.1 Background information

Management and conservation of biodiversity established on the basis of three key objectives: conservation of biological diversity, sustainable use of its components, and fair and equitable sharing of the benefits arising from the use of genetic resources (The United Nations Convention on Biological Diversity (CBD), 1992). These efforts are now problematic in their application because of competition for those resources resulted to conflicts at national and global level.

Resource conflicts have been major threats for sustainable management and conservation of biological diversity since time immemorial (Ruckstuhl, 1999). Currently it is recognized as one of critical and complex problem areas that have implications on the conservation of ecosystems in global environment and development discourse (CBD, 2016). Increasing resource competition at the global environment brings about social disparity and conflicts. These types of conflicts greatly impacted environmental quality, linked to human activities (UNEP, 2016). The effectiveness and peaceful management of natural resources depends on the ability to identify resource conflicts and adopt strategies that prevent disagreement from becoming intractable disputes (WCPA, 2016). Resource conflicts and insecurity are caused by a number of transnational problems which once seemed quite distant, like environmental degradation, natural resource depletion, rapid population growth and refugee flows which poses a threat to prosperity and have security implications for both present and long-term development policies. Thus, embraced environmental component after recognizing risks including massive population flight, desertification, large-scale ecosystem damage, biodiversity loss, pollution and climate change because these can unbalance international stability (Mulongoy and Gidda, 2008).

Risks associated with resource conflict in the world generally come into sharp focus when considering the potential impacts of global trends, such as climate change, population, urbanization and food crises. A number of global assessment reports, have all emphasized the liability to climate change impacts which includes degradation and competition of resource that stimulate war (UNEP-WCMC and IUCN, 2016). At present, the majority of literatures in the world concerned with environmental issues consider resource conflicts as one among serious problems for biodiversity conservation. Current discussions on the social impacts of climate change, for example, emphasize

the risk of negative causes toward social conflict in poor countries as a consequence of trends toward scarcity of resource, increased population movements, and thus greater competition over natural resources (CBD, 2016).

In many African countries which having many biodiversity species indicates that, resource conflicts are caused by competition of scarcity of resources and human made disturbance of ecosystems (FAO, 2016). This scarcity of resources has resulted from overuse of resources, growing human populations and the level of resource consumptions, which eventually lead the destruction of biological resources due to human competition and conflicts. Several headquarters of tropical forests in DRC are now taken over by military, including Kivunga National Park, Kahuzi-Biega National Park and the Okapi Wildlife Reserve (WCPA, 2016). Liberia conflict forced rural people to hunt duikers (*Cephalophus spp*), pygmy, hippos (*Choeropsis liberiaensis*), forest elephants and chimpanzees for foods (Wolkomir and Wolkomir 1992).

In East African countries the increasing of human-wildlife conflict are highly contributed by changing of land use in areas surrounding protected areas, which bring about difficulties for community based conservation to succeed. These areas were experiencing expansion of small holder cultivation in wildlife dispersal areas. The situation has been reported to reduce animal home ranges, leading to increase human wildlife interaction, which may degenerate into human wildlife conflict (Kaswamila, 2009).

In Tanzania, human problems constraining Wildlife Sector are responsible for increasing of resource conflicts. Wildlife Conservation Authority is accused for marginalizing people, denying people access to traditional and legitimate rights, property damage and risk to human life through attack by wild animals and disease transmission (UNEP, 2016). In broad sense, the primary causes of resource conflict are demographic, economic, institutional and technological (UNEP, 2016). Again the report of (CBD, 2016) revealed that the habitat loss in Tanzania was a serious problem for different ecosystems (CBD, 2016).

Community competitions of using natural resources in Jozani Chwaka Bay National Park area stimulate the emergence of resource conflicts. People use resources in an unsustainable and highly destructive way (ZRG, 2015). This situation results into high destruction of antelope's habitat and other biological diversity species in Jozani National Park.

1.2 Problem statement

In recent decades, there are an increasing trend of destruction of biodiversity particularly in forestry, land and wildlife that endanger efforts of management and conservation of biodiversity in Jozani ecosystem especially Jozani Chwaka Bay National Park area because of resource conflicts between communities themselves, park management and community, and between individuals. This situation has severe implication in efforts of management and conservation of biodiversity in Jozani Chwaka Bay National Park (JCBNP), Zanzibar. Since the declaration of Jozani National Park in 2004, more than 68 resource conflicts associated with competition and scramble of resources between the communities themselves and institutions responsible for management of National Park have been reported. The Zanzibar Land Tenure Act No. 12 of 1992 has created land problems and conflicts especially in the Protected Areas where the Act does not recognize land tenure rights at the community level. This creates the scramble for demanding land for cultivation which resulted into resource conflicts. Communities living around Jozani National Park are directly relying on using natural resource assets to ensure their livelihoods which result into community resource conflicts as individuals are competing over declining level of existing resources. This study therefore investigated how communities and government Institutions could address and analyze resource conflicts issues so as to minimize the escalation of biodiversity in Jozani ecosystem.

1.3 Objectives of the study

1.3.1 Main objective

The main objective of this study was to examine the current status of resource use conflicts and the management and conservation of biodiversity in Jozani ecosystem, Zanzibar.

1.3.2 Specific objectives

i. To establish the level of resource conflicts in the study area.
ii. To establish the level of the management of biodiversity conservation in in the study area.
iii. To examine the causes of resource conflict on the management of biodiversity conservation in the study area
iv. To determine the results of resource conflicts on the management of biodiversity conservation in the study area.
v. To establish the relationship between resource conflicts and the management of biodiversity conservation in the study area.

1.4 Scope of the Study

The geographical scope of this study focused on Jozani Chwaka Bay National Park, Zanzibar. Jozani forest lies about 35 km South-East of Stone Town in the South of the Island and lies between the Chwaka Bay in the North and

Uzi Bay. The Jozani Chwaka Bay National Park is a 50 square kilometer National Park located on the Island of Zanzibar Tanzania. The Park is situated between the villages of Pete, Jozani and Kaebona to the North; and Kiongoni, Kinduni, Chuchumile and Kisomanga to the West and Northwest, and Mapopwe village is within the National Park. The Park extends to the Ufufuma and Chwaka village to the North and Northeast respectively. On the Eastern side of the Park is Jozani Charawe road.

The theoretical scope of this study focused on conflicts theory, in the area of conflict resolution and management such as 'human need theory' Burton (1987), 'scarcity based theory' by Hobbes (1996), 'unmet needs theory' by Maslow (1971), 'statist theory' propounded by (Kahl, 2006) and population theory which is known as "Malthusian theory" propounded by Malthus (1798).

The content scope of this study focused on the relationship between resource use conflicts and the management and conservation of biodiversity in Jozani National Park area, Zanzibar. On other hand the study focused on identifying the level of resource conflict, levels of the management of biodiversity conservation, causes of resource conflicts, and results of resource use conflicts in the management of biodiversity conservation and provides appropriate methods and techniques for controlling them.

II. METHODOLOGY

2.1 Research design

This research used Descriptive survey research design of the causal comparative research design because researcher intended to examine the causes of conflicts and relationship between resource conflicts and the management and conservation of biodiversity in Jozani National Park, Zanzibar as explained much by Kothari (2008).

2.2 Research population

The study population includes 66 Jozani Park Authority which is government officials as the main stakeholders, 30 NGOs dealing with Jozani environmental conservation and 278 local leaders of the community living around Jozani National Park as shown in the table 1.

Table.1: Total population of the study

Participants	Population	%
JPA government officials	66	17.94
JECA	30	8.15
Jozani Local Leaders	278	73.91
Total	**368**	**100**

2.3 Sample size

Sample size of this study was 280 respondents as shown in the Table 2.

Table.2: Sample size of the Study

Participants	Population	%
JPA	66	23.58
JECA	30	10.71
Jozani Local Community	184	65.71
Total	**280**	**100**

2.4 Sampling procedure

Purposive sampling procedure was used to Government Officials and Non-government Organizations such as JPA and JECA. Simple random sampling procedure was also used in selecting participants within Community Leaders because every one of the targeted population had equal chance to be selected.

2.5 Research instruments

Non-standardized questionnaire for data collection was used as it provides a convenient way of gathering information from respondents. The questionnaire includes questions relating to demographic background and other research objectives. i.e. constitute the level of resource use conflicts, the level of management of biodiversity, causes of resource conflicts and the results of resource conflicts in the management of biodiversity conservation. The instrument was used to government officials, Non-government organization and local communities relating to management of biodiversity conservation. Also, Likert scale was employed to rank questions ranges from Strongly Agreed to Strongly Disagreed.

2.6 Data Analysis

The researcher analyzed quantitative data using descriptive statistics such as percentages, mean,

frequency, standard deviation, tables and bar charts by using Statistical Package for Social Science (SPSS). Also correlation co-efficient was used to establish relationship between resource conflicts and the management of biodiversity conservation in Jozani National Park area, Zanzibar.

III. FINDINGS AND DISCUSSIONS

3.1 Demographic information

According to finding in this study as shown in table 3, 71.1% (199) of the respondents were male and 28.9% (81) were female. 34.6% (97) of the respondents range from 20 – 30 years of age, 36.8 (103) of the respondents range from 31 – 40 years of age, 17.1% (48) of the respondents range from 41 – 50 years of age and 11.4% (32) of the respondents range were above 50 years of age. The table demonstrates further that, the majority of the respondents had secondary education with a percentage 43.9% (123) while master level had the least respondents with a percentage 0.7% (2) of the total respondents. This is because the government of Zanzibar offers free education up to secondary education. The Table 3 also indicate that 27.9% (78) of the respondents are working in the Public sectors while 72.1% (202) of the respondents are working in the Private sectors. People use self-employment as a private employment because low level of employment opportunities.

Table.3: Background of information

Categories	Frequency	Percentage
Gender		
Male	199	71.1
Female	81	28.9
Total	280	100
Respondent's age (in years)		
20 -30	97	34.6
31 – 40	103	36.8
41 – 50	48	17.1
Above 50	32	11.4
Total	280	100
Respondent's qualification		
Certificate	82	29.3
Secondary	123	43.9
Diploma	13	4.6
Degree	6	2.1
Master	2	.7
None	54	19.3
Total	280	100
Respondent's work place		
Public sector	78	27.9
Private sector	202	72.1
Total	280	100
Respondent's work experience		
1 – 5	35	12.5
5 – 10	70	25.0
Above 20	175	62.5
Total	280	100

3.2 The level of resource conflicts on the management of biodiversity conservation in Jozani National Park area, Zanzibar

3.2.1 Interpretation of Likert Scale

4.45 - 5.00 Strongly Agreed
3.45 – 4.40 Agreed
2.45 – 3.40 Neutral/Undecided
1.45 – 2.40 Disagreed
0.5 – 1.40 Strongly Disagreed

Table 4 illustrates the mean, Interpretation and Standard Deviation of the level of resource conflicts on the management of biodiversity conservation in Jozani

National Park, Zanzibar. The low Standard Deviation shown in the table such as 0.46 means that the respondents strongly agreed to the opinion as per information they provid that the level of resource conflicts are increased because of unemployment. This is because communities living around Jozani National Park are directly relying on using natural resource assets to ensure their daily survival and livelihoods which result into resource competition and conflicts

At the same time the highest Standard Deviation shown in the table was 1.13 imply that the respondents also agreed to opinion of information provided that increasing of multiple actors in the management and conservation of resources makes the level of resource conflicts to be high.

Table.4: The mean, Interpretation and Standard Deviation of the level of resource conflicts on the management of biodiversity conservation

Item	Mean	Interpretation	St. Deviation
Increasing of scarcity of resources make the level of resource conflicts to be high.	4.0 0	Agree	1.10
The level of resource conflicts are increased because of rural hardship	4.68	St. Agreed	.66
The level of resource conflicts are increased because of poverty	4.82	St. Agreed	.49
The level of resource conflicts are increased because of unemployment	4.83	St. Agreed	.46
Lack of existence of resource rights such as land tenure rights at the community level makes the level of resource conflicts to be high	4.69	St. Agreed	.56
The demand of resources makes the level of resource conflicts to be high	4.40	Agreed	.61
Increasing of multiple actors in the management and conservation of resources makes the level of resource conflicts to be high	3.58	Agree	1.13
Poor management system of land and other natural resources makes the level of resource conflicts to be high	4.20	St. Agreed	.99

3.3 The level of the management of biodiversity conservation in Jozani National Park area, Zanzibar

Table 5 illustrates the mean, Interpretation and Standard Deviation of the level of the management of biodiversity conservation in Jozani National Park area, Zanzibar. The low Standard Deviation was 0.42 means that the respondents strongly agreed to the opinion as per information provided that the level of management of biodiversity conservation become is low because of poverty. This is because people are directly relying on using natural resource assets to ensure their daily survival and livelihoods and hence set back the management and conservation of biological biodiversity.

At the same time the highest Standard Deviation was 1.36 imply that the respondents disagreed with the point that the level of biodiversity conservation is low and unachievable because of unclear, precise and achievable objectives.

Table.5: The mean, Interpretation and Standard Deviation of the level of management of biodiversity conservation

Item	Mean	Interpretation	St. Deviation
The level of management of biodiversity is low because of illegal harassment of habitats and their	4.42	St. Agreed	.72

environment such as poisoning, shooting and trapping			
The level of management of biodiversity is low because of climate change	3.65	Agreed	1.10
The level of management of biodiversity is low because of lack of enough funds	4.26	St Agreed	1.26
The level of management of biodiversity is low because of lack of enough high skilled staffs	4.19	St. Agreed	1.30
The level of management of biodiversity is low because of long term period of biodiversity conservation	3.38	St. Agreed	1.33
Poor policy and law enforcement and governance in the department and communities, reduce the level of proper management of biodiversity conservation	3.81	St. Agreed	1.37
The level of management of biodiversity conservation is low because of human interaction and conflicts	4.35	Agree	.56
Changing of land uses in area surrounding protected areas has made the level of management of biodiversity conservation become low	4.27	Agreed	.79
The level of management of biodiversity become low because of poverty	4.76	St. Agreed	.42
The level of management of biodiversity become low because of hunger	4.71	St. Agreed	.49
The level of management of biodiversity become low because of diseases	3.06	Disagreed	1.45
The level of management of biodiversity become low because of illiteracy	4.63	St. Agreed	.64
The level of management of biodiversity become low because of lack of gender participation	3.24	Agreed	1.34
The level of management of biodiversity become low because of unclear, precise and achievable objectives	2.93	Disagreed	1.36
The level of management of biodiversity become low because of lack of careful evaluation of the cost and benefit of the projects	3.40	Agreed	1.34
The level of management of biodiversity become low because of lack of strong participation of the communities	3.60	St. Agreed	1.34

http://dx.doi.org/10.22161/ijeab/2.1.59 *ISSN: 2456-1878*

3.4 The causes of resource conflicts on the management of biodiversity conservation in Jozani National Park area, Zanzibar

According to the table 6 illustrate the mean, Interpretation and Standard Deviation of the causes of resource conflicts on the management of biodiversity conservation in Jozani National Park area, Zanzibar. The low Standard Deviation was 0.62 means that the respondents strongly agreed to the opinion as per information provided that scarcity of environmental resources are the causes of resource conflicts. This is caused by population pressure and human consumption over the environmental resources that lead to environmental degradation and poverty.

At the same time the highest Standard Deviation was 1.06 imply that the respondents were neutral to opinion of information provided that climate change are fundamental causes of resource conflicts. That means that they were neither agreed nor disagreed to the information provided in the questionnaire.

Table.6: The mean, Interpretation and Standard Deviation of the causes of resource conflicts on the management of biodiversity conservation

Item	Mean	interpretation	St. Deviation
Human activities for demanding material needs such as foods, living spaces, health maintenances and supply of energy are the causes of resource conflicts	4.39	St. Agreed	.73
Absence of democracy and good governance that limit the capacity of the individual justice, rights to information and participation in environmental decision making are the causes of resource conflicts	3.48	Agreed	1.20
The scarcity of environmental resources are the causes of resource conflicts	4.57	St. Agreed	.62
Population growth is the causes of resource conflicts	4.70	St. Agreed	.82
The implication and bias of law and policies to rural communities are the causes of resource conflicts	4.13	St. Agreed	1.13
Climate change are fundamental causes of resource conflicts	3.53	Neutral	1.06
Conflicting of interests between the community and conservation authority are the causes of resource conflicts	4.35	St. Agree	.79
Illegal exploitation of natural resources, continue to be one of the contributing factor for resource conflicts	4.43	St. Agreed	.65
Lack of proper distribution of revenues from tourism, hunting and other source of revenues are considered to be the causes of resource conflicts	4.16	St. Agreed	1.12
Vulnerability of women's in physical, social, economical and political affairs are the causes of resource conflicts	3.36	Neutral	1.29

3.5 The results of resource conflicts on the management of biodiversity conservation in Jozani National Park area, Zanzibar

Table 7 illustrates the mean, Interpretation and Standard Deviation of the results of resource conflicts on the management of biodiversity conservation in Jozani National Park area, Zanzibar. The low Standard Deviation was .59 shows that the respondents strongly agreed to the opinion as per information provided that resource conflicts lead to the destruction of forestry.

Table.7: The mean, Interpretation and Standard Deviation of the results of resource conflicts on the management of biodiversity conservation

Item	Mean	Interpretation	St. Deviation
Resource conflicts lead to the destruction of ecosystems	4.09	St. Agreed	1.10
Resource conflicts lead to the habitat destruction	4.10	St. Agreed	1.05
Resource conflicts made people to use natural resources in an unsustainable and destructive way	4.63	St. Agreed	.64
Resource conflicts lead to destruction of forestry	4.71	St. Agreed	.59
Resource conflicts lead to the destruction of animal meat	4.68	St. Agreed	.60
Resource conflicts lead to the destruction of resources and pollution	3.98	St. Agreed	1.09

Table 8 indicates that there is a significant relationship between resource conflicts and the management of biodiversity conservation in Jozani National Park Area, Zanzibar. This is shown by P value of 0.355 which indicate a significant relationship since it is above 0.000.

Table.8: The relationship between resource conflicts and the management of biodiversity conservation in Jozani National Park Area, Zanzibar

Correlation

Variables Correlated	R. values	P. values	Interpretation
Resource Conflicts Vs The Management of Biodiversity Conservation	0.355	0.000	Significant Correlation

IV. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Mostly all natural resources are part of environment and the conflicts associated with resource use are environment in nature. Environmental conflict is a common issue worldwide and it linked to political, economic, social, and ecological context of the world. Scarcity of environmental resources which are considered to be important source of conflicts can be important factors leading to tension and clashes in the many societies because resource scarcity has its greatest social impact when these factors interact. This study therefore concluded by emphasize that poverty and unemployment were the major causes of resource conflicts and poor management and conservation of biodiversity in Zanzibar.

4.2 Recommendations

Therefore, the call is directed to the government to intensify efforts on conflict management plan by establishing overall strategies for managing conflict such as establishment of participatory community-based approaches to natural resource management and conflict resolution. Establishment of conflict resolution capacity building measures among the stakeholders such as training negotiation skills, facilitation skills, mediation skills, communication skills, leadership skills and

awareness about rising the process of consensus-building. Laws should be also amended to avoid doubtful of land tenure rights in the management and conservation of biodiversity conservation at the community level. Again the economic situation of the country also needs to be restoring so as to enhance the standard of living of the citizens and emancipate societies in directly relying on using natural resource assets to ensure their daily survival and their livelihoods. Finally government and corporate bodies should create conducive employment opportunities to the people so as to reduce the high level of poverty and unemployment.

REFERENCES

[1] Article (1) of the United Nations Convention of Biological Diversity, (1992).
[2] Burton, J.W (1987). Resolving deep-rooted conflict. A handbook. University press of America.
[3] CBD, (2016). Technical Series 84: Update on climate geoengineering in relation to the convention on Biological Diversity: Potential impacts and regulatory framework. Secretariat of Convention of Biological Diversity. Montreal.
[4] FAO, 2016. Mainstreaming ecosystem services and biodiversity into agricultural production and management in East Africa: Technical Guidance. Rome
[5] Hobbes, T., (1996). Leviathan. Rev. student. Ed. R. Tuck. Cambridge, MA: Harvard University Press.
[6] Kahl, C.H. (2006). States, scarcity, and civil strife in the developing world. Princeton, NJ: Princeton University Press.
[7] Kaswamila, A., 2009. Food Impacts of Game on Household Security: A Case of Serengeti District. REPOA. Dar es salaam.
[8] Kothari C.K (2008). Research Methodology, Methods and Techniques. Review Second Edition. Published by New Age International (P) limited.
[9] Malthus, T.R., (1798): An Essay on the Principle of Population. J. Johnson, in St. Paul.s Church-Yard, London, UK.
[10] Maslow, A. (1971). The farther reaches of human nature. New York: The Viking Press.
[11] Mulongoy, K.J. and S.B. Gidda (2008). The Value of Nature: Ecological, Economic, Cultural and Social Benefits of Protected Areas. Secretariat of the Convention on Biological Diversity, Montreal. 30pp
[12] Ruckstuhl, S. (1999). Renewable Natural Resources, Practical Lessons for Conflict Sensitive Development.
[13] Section (3) of the Zanzibar Land Tenure Act No. 12 of 1992.
[14] UNEP, 2016. UN biodiversity report identifies risks and uncertainties of novel strategies to tackle climate change. Secretariat of CBD. Montreal.
[15] WCPA, (2016). Protected Planet Report: How protected areas contribute to achieving global targets for biodiversity. UNEP-WCMC. Cambridge.
[16] ZRG (2015). Zanzibar biodiversity, climate changes and energy crisis; implementation of Zanzibar environmental policy. Government printer, Zanzibar.

http://dx.doi.org/10.22161/ijeab/2.1.60 *ISSN: 2456-1878*

Assessment of the Effectiveness of Chilling Method in Mitigating Human-Elephant Conflicts in Western Serengeti, Tanzania

Mdee Norah .V[1], Zella Adili .Y[2]

[1]Tanzania Wildlife Research Institute, Mahale/Gombe Research Centre, P.O. Box 1053, Kigoma, Tanzania
Tel. +255-28-2802072/ +255-784-687137/+255-758-506207

[2]Department of Economic Studies, The Mwalimu Nyerere Memorial Academy (MNMA)-Zanzibar, P.O Box 307 Zanzibar, Tanzania
Tel: +255 24 2250315; +255 787 260448

Abstract— *This paper reveals the less known effectiveness of Chilling method in mitigating human elephant conflicts carried out in Western Serengeti, Tanzania. Two villages were involved namely Nyamburi and Bonchugu. Data were collected by using household questionnaire, focus group discussion and archive information. Data were analyzed by use of SPSS (Version 18) software.Field results indicates that; Chilling method is effective (83%)in mitigating human elephant conflicts. However, statistics for crop damaged by elephants before and after introduction of the method shows that the crops damage decreased* ***by*** *25****%.*** *The most observable strengths of the method were; it control HEC without harm people and elephants, it is easy to apply (55%), it does not consume time and use appropriate technology. Despite the effectiveness of the method, major weaknesses observed to face the method were; insufficient used oil and pepper (61%), elephants observed to be a clever animal as sometimes they inter into the farms backwards and also during rain seasons, chill method observed to be ineffective as it can be washed/removed easily. However, the respondents recommended that; the challenges can be solved by local community to cultivate pepper and other stakeholders such as district, different NGO and companies to support farmers the provision of used oil, chill should be applied regularly once washed out by rain and for the effectiveness of the method community should be more trained on how to use the method.*

Keywords— Human-elephant conflict (HEC), Chilling method, Crop raids.

I. INTRODUCTION

1.1 Background information

Protected areas (PAs) are important in the conservation of biodiversity, economic value of resources and their potential to contribute to sustainable development (URT, 2005). Despite this development, local people living adjacent to the PAs live in abject poverty and human wildlife conflict (Kaswamila*et al*, 2007). Human elephant conflict (HEC) is a growing global problem which is common to all areas where elephants and human population coexist as well as share resources (Distefano, 2005). HEC occurs wherever people and elephants coincide, and poses a serious challenge to wildlife managers, local communities and elephants alike.

Human elephant conflicts are one of the major threats to conservation in Africa (Holmern& Anne, 2004). HEC occurs throughout the elephant range, and has been reported in most of the 37 elephant range states of the African continent in both savanna and forest situations (Parker *et al.*, 2007). Hence, knowledge about human-elephant conflict in and around protected areas is crucial in wildlife management (Holmern& Anne, 2004; Kideghesho, 2006).

In several parts of East Africa Conflict between elephant and local communities are wide spread and are major concern for both elephant conservation and rural development (Distefano, 2005).HEC in East Africa is increasingly becoming significant as human populations and agricultureexpand into elephant habitat (*ibid)*. Elephants continue to threaten farmers' income and food security despite considerable research and resources that has been devoted to resolving this problem (Woodroffe *et al.*, 2005). In Tanzania encroachment of protected areas by local communities has resulted into tremendous human wildlife conflict (Severre, 2000).

To mitigate HEC, farmers use both lethal and non-lethal measures such as fencing, scares, chilling, barriers, translocation and use of guard animals (Breitenmoser*et al*., 2005). In order to be conservation-effective, non lethal methods must be acceptable (in accordance with local traditions) and applicable on a large scale(cheap and easy to use) (Woodroffe *et al*., 2005). Furthermore, the implementation and application of non-lethal techniques must be considered in the context of the conservation goals and all other management action *(ibid)*1.2

1.2 Statement of the Problem

Conflict between humans and African elephants occur wherever they coexist, especially in the interface between the elephants' range and agricultural land. Most HEC incidents involve crop-raiding animals that consume or destroy food crops and injure or kill those people trying to protect their fields (Distefano, 2005).

The Serengeti ecosystem in Tanzania embodies a variety of human elephant conflicts as evidenced both within and around the protected areas of the ecosystem (Homewood *et al*., 2001).). The root cause of human-elephant conflict is the exploding human population growth and resultant pressure on elephant habitat (Parker *et al*, 2007).According to Kaswamila (2009), HEC is more alarming in Serengeti district. Crop destruction by elephant impacted on both household food security and cash income. The annual crop loss is estimated to amounts to 390 tones from annual crop yields of 129,670 tones of various crops .

In trying to address the problem, the Tanzania Wildlife Research Institute in collaboration with Serengeti District Council intervened by introducing the use of chilli (pepper) in mitigating destruction of crops by elephants. However since its introduction in 2007, no study has been carried out to assess its effectiveness. This study is an attempt to that end.

1.3 Research Objectives

1.3.1 General Objective

The main objective of this study was to assess the effectiveness of chilling method in mitigating HEC in Western Serengeti.

1.3.2 Specific Objectives

Specifically this study intended to:

(i) Assess effectiveness of chilling method
(ii) Identify strengths and weakness of the method
(iii) Suggest measures for improvement

1.4 Conceptual Framework

For effective control of Human Elephant Conflict, good application of chilling method will be very much needed. In order for the method to be applicable, there must be a positive community attitude towards control of elephants and community should be well trained on chill application. Furthermore, the control will be effective if the elephants will not be habituated and there is conducive environment for chill application such as absence of rain as chill is affected by rain.

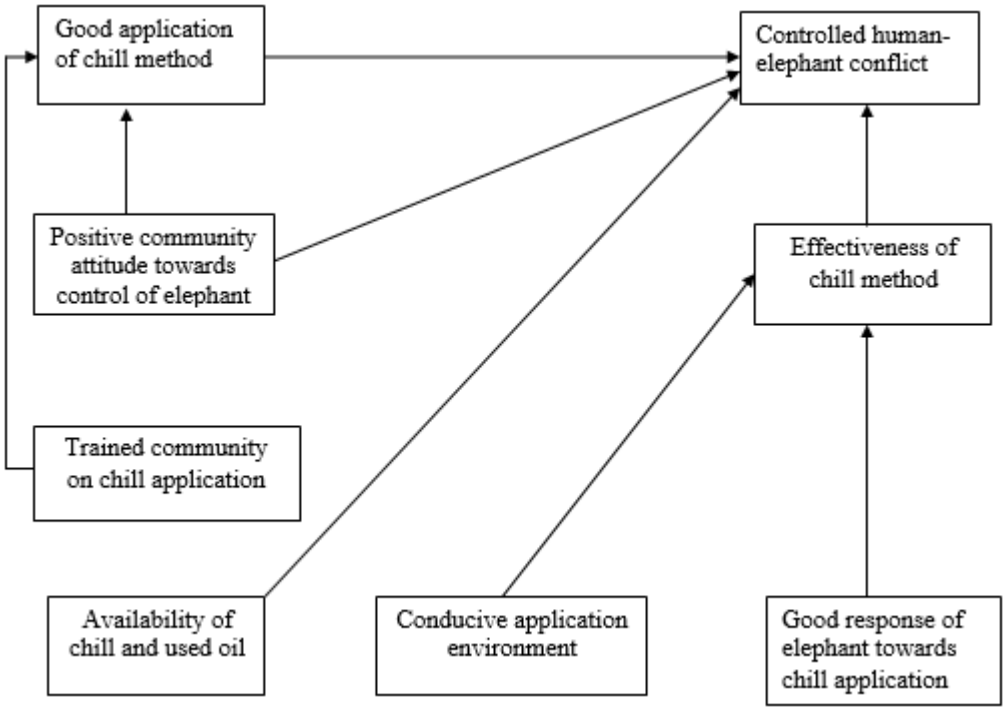


Fig.1: Conceptual Frame work

1.5 The Study area

1.5.1. Location

Nyamburi and Bonchugu are among villages in Serengeti District. (See figure 1). Nyamburi villagelies between 34° 40" E and 1° 47" S while Bonchugu village lies between 34º 45" E and 1º 50" S with an average altitude of 1480 m (SDC, 2011). Nyamburi has a total population of 3865 people and 787 households while Bonchugu has total population of 6114 and 579 households.

http://dx.doi.org/10.22161/ijeab/2.1.60 *ISSN: 2456-1878*

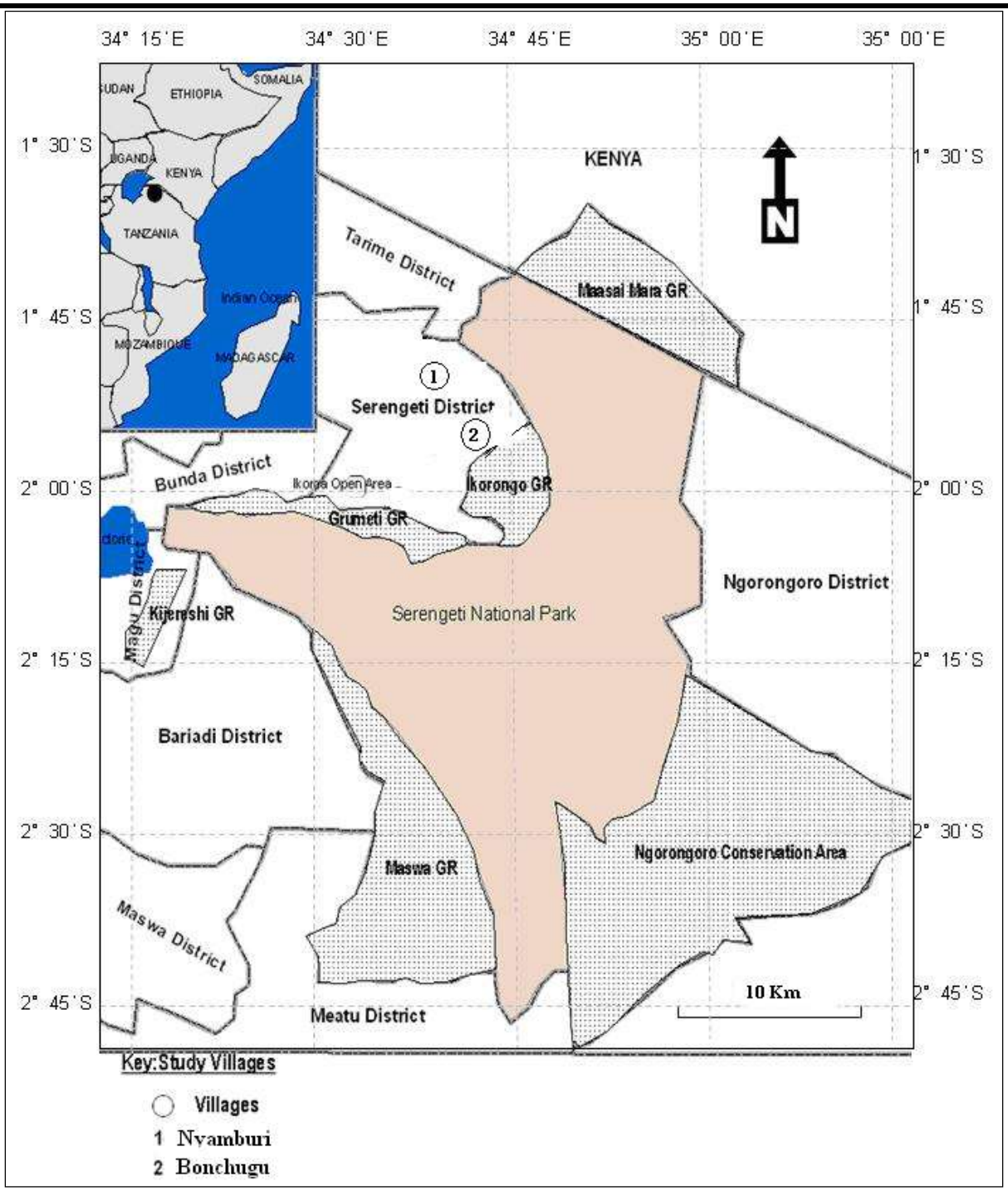


Fig.2: Map of the study area

1.5.2 Climate and topography

The study villages are part of the high interior plateau of East Africa. It slopes to its highest part (1850 m) on the eastern plains near the Gol Mountains towards Speke Gulf (920m) along Lake Victoria. The temperature shows a relatively constant mean monthly maximum of 27^0-28^0 C. The minimum temperature varies from 16^0 C in the hot month of October-March to 13^0C during May-August (SDC, 2011). Rain typically falls in a bimodal pattern with the long rains during March-May and the short rains during November-December. Rainfall varies from 1200 mm in the north to 600 mm on the south-eastern plains and the Rift Valley (*ibid*).

1.5.3 Economic activities

About three quarters of the population in the study villages are mainly small-scale farmers who, to a varying degree complement with livestock keeping (Holmern, *et al*., 2004). Livestock (cattle, sheep and goats) which is kept by 61% of the population is seen both as a source of income and a

http://dx.doi.org/10.22161/ijeab/2.1.60 *ISSN: 2456-1878*

source of meat for consumption and some 73% earn income from the sale of animals or meat (*ibid*). Wildlife-induced damage to crops and domestic animals is a major problem in the area (SDC, 2011).

II. RESEARCH METHODOLOGY

2.1 The Study Area Selection

The study was conducted in Bonchugu and Nyamburi villages adjacent to Western Serengeti. These villages were selected because the problem of HEC is more alarming due to human elephant interaction and proximity to Serengeti National Park.

2.2 Sample size and Sampling technique

A totalof 82 respondents were picked. Sample size composition included (68) households respondents, (2) Village chairperson, (2) VEO,(1) DGO, (1) DALDO, (2)SENAPA Ecologist, (2) Elders,(2) Youth and (2) women.In determining the sample size two factors were considered: required level of precision in the results and the available budget.

According to Henn*et al*., 2006, it is worth mentioning that, it is the absolute size of the sample, which is important in determining the sample size, not its size relative to the population. Sixty eighty households from each Village were sampled from the Village register list using a simple random sampling method. Where a candidate happened to come from the same household, one was dropped. The age from 18 and above was picked from each household using a table of numbers following the procedures described in Bouma (2000). Simple randomly sampling is considered to be simpler and more cost effective system (Henn *et al*., 2006). Key informants and group discussion members were selected purposively to meet the objectives of the study.

2.3 Data collection methods

2.3.1 Questionnaire survey

Face-to-face semi-structured questionnaires were administered to the sampled households. Semi-structured questionnaire survey was preferred to structured because it normally yields better quality data than the latter. According to Gillham (2005), Semi-structured questionnaire allows the interviewer greater flexibility at the expense of possibly incurring greater bias as the same questions may be asked in the same order but supplementary questions (probes) may be allowed to clarify the responses. The household questionnaire contained aspects such as: socio-economic characteristics, crop destruction, and assessment of chilling method, strengths and weaknesses of the method and suggestions for improvement.

2.3.1.1 Questionnairepre-testing

Questionnaire pre-testing aimed to test questionnaire wording, sequencing and layout; to train fieldworkers; and to estimate response rates and time. Pre-testing also assessed whether the questions are clear, specific, answerable, interconnected and substantially relevant. The exercise helped to fine-tune the questionnaire. Some ambiguous questions were removed and others were re-phrased. After revision, the questionnaires were duplicated ready for use in the social survey.

2.3.1.2 Questionnaire administration

Face to face household semi-structured questionnaire surveys were administered by the researcher and research assistants to interview sample local residents in Villages.Research team visited the selected household sample at their residential areas. The questionnaire consisted both open and closed end questions. The open-ended questions were intended to give respondents an opportunity to express their views more freely and to increase the level of interaction between the two subjects.

2.3.2 Focus group discussion

Focus group discussion (FGD), help researcher tap many different forms of communication that people use in day to day interactions, including jokes, anecdotes, teasing and arguments (Morgan,1998). FGD needs to comprise 5 to 10 people so as to have effective and participatory group discussion (Krueger *et al.*,2004). In this study FGD comprised 6 people. A checklist was used to obtain information's from villages elders, women and youths (group members). During discussion the researcher acted as a facilitator to make sure that every one participates effectively.

2.3.3 Archive information

Documented information in Village, ward and District offices related to average crops destroyed by elephants, Introduction of chilling method to the district, location and population of the study villages. Similar information was also obtained from Village experts (agriculture and wildlife officials). This information supplemented data collected from interviewed households.

2.3.4 Direct field Observation

Field observation was made for the purpose of observing farms located adjacent to protectedareas to compare the incidence of destruction as compared to distant villages. Also field observation was made to observe the way chilling method was applied by the farmer. Using physical visit as a tool for data collection ,the observer goes to the field and makes the study of the phenomena and once observes things in a scientific manner, he or she thoughtfully studies the fact (Rwegoshora,2006).

2.4 Data analyses

Data collection using questionnaire survey, group discussion and archive information were mainly qualitative in nature. As pointed out by several social science researchers, qualitative data analysis has no one right way to proceed with analysis (Hesse-Biber&Leavy, 2004) and this necessitated use of coding and memoing for narrative information and/or secondary data. Coding is the reading

the text line by line and carefully coding each line, sentence and paragraphs thereby describing themes/ideas (Punch, 2000). Memoing (memo writing) on the other hand is the theorising write up of the ideas about codes, which assist researchers to illuminate ideas and relationships in the data (*ibid*.).

Before the detailed data analysis, questionnaires were thoroughly examined, variables coded and then imported into SPSS version 18 software package. This examination process will be done to all questionnaires used in the survey. The data analysis then followed the two main stages of reduction and display (Coffey & Atkinson, 1996). Data reduction involved editing and summarizing of data through coding. With data already entered into SPPS and secondary data from government offices and group discussion, data analysis to answer research questions were carried.

III. RESULTS AND DISCUSSION

3.1 Social economic characteristics of respondents

The socio-economic characteristics of the study area are presented in Table 1 below. Overall, in the two villages combined, the majority of the respondents were males (60%; N=68). As for age most of the respondents (68%) were between 18 and 54 years. This shows that the majority of the populations at study villages are still economically productive. Regarding social economic activities of the study villages, about 78% of the population depends on crop-based agriculture.

Table.1: Social economic characteristics of respondents

Village	**N** =68	**Sex (%)**		**Age (%)**			**Education (%)**			**Socio-economic activities (%)**		Household size%		
		M	F	18 - 34	35 - 54	>54	NF	P	S &A	A	L	1-5p	6-10p	>10p
Nyamburi	34	61	39	30	36	34	41	55	4	76	24	10	53	25
Bonchugu	34	58	42	32	38	30	38	57	5	80	20	12	50	50
Average	34	50	40.5	31	37	32	40	56	4.5	78	22	11	51.5	37.5

Keys: N =sample size M=Male F=female >=Above NF=Non Formal P=Primary S & A =Secondary and AboveA= AgricultureL=Livestock p=person

The literacy level in these two villages is low as only (4.5%) have attained secondary education. This implies that, the illiteracy level in terms of formal education is high. Education is a necessary condition for social economic and technological development in any society(Author, pers. Obs.).With education one can easily learn new technological advancement, adapt to change environmental conditions and learn new skills.

Regarding household size, findings reveals that, the average size of household is 8 people. The higher number of family size could probably be due to polygamy culture of the people in the area. InMara region,particularly Serengeti and Tarime districts the culture of marrying many wives is rampant (Author, pers. Obs.).Having many wives increases the probability of having many children when compared to monogamy families and hence increased poverty level. This is in agreement by Kaswamila (2007) where he observed that income in the study area ranged between TZS784,000 and 930,000.

3.2 Human Wildlife Conflict (HWC) status

Local communities were asked to assess the current status of HWCs in their areas. Answers were limited to Yes or No. In both villages the findings reveals that, HWC is a problem (Figure 3). In Nyamburi all respondents perceived HWC a problem whereas in Bonchugu the proportion was 97%. The most destructive game being elephants (*Lexodanta Africana*), other problem animals includedwild pigs (*Potamochoerusporcus*), porcupine (*Potamochoerusporcus*), vervet monkeys (*Cercopithecus aethiops*), wildebeest (*Connochaetestaurinus*), warthog (*Phacochoerusaethiopicus*) and gazelle (*Gazella grant*). The most affected crops were maize, sorghum and finger millet which are basically the main staple food in the study area. The reasons for favouring these crops could not be established. However, probably thereasons couldbe the nature of the crops and elephants prefer succulent crops.Results from Focus Group Discussion (FGDs) revealed that,crop damage by elephants not only affect farmer's ability to feed his or her family, but also reduces cash income and has repercussions for health, nutrition, education and ultimately, development. As farmers depends on crops for selling to obtain cash for school fees

http://dx.doi.org/10.22161/ijeab/2.1.60 *ISSN: 2456-1878*

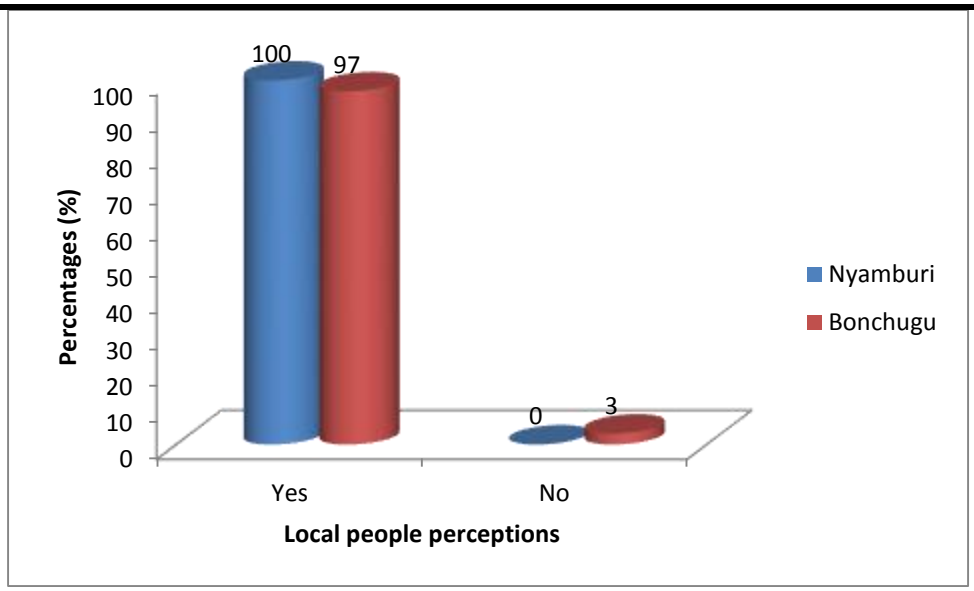


Fig.1: Local community perception on HWC status

3.3 Assessment of effectiveness of chilling

The effectiveness of the method was assessed through local people perception and through the status of crop destruction by elephants before the introduction of chill and after the use of the method.

Table.2: Households perception on effectiveness of chilling method

Village	Sample size (N)=68	Perceptions %	
		Chilling is effective	Chilling is not effective
Nyamburi	34	77	23
Bonchugu	34	90	10
Average	34	83.5	16.5

They argued that, the incidence of destruction by elephants has gone down. For example one respondent had this to say: "We thank the government and Tanzania Wildlife Research Institute (TAWIRI) for introducing this method of deterring elephants, we were not happy with the situation." Jones & Elliott (2006), in their study in Namibia found that; chilling method is effective because it worked as olfactory deterrent for elephant.

Focus group discussant's view on the effectiveness of the method was that,the method is effective because it has improved food security through reducing crop raids. Interview with District Game Officer (DGO) on the matter revealed that chilling has been instrumental in mitigating HEC.When asked to give reasons for,he argued that; elephants have a highly sensitive olfactory system and chilies therefore cause them pain. Thisargument is supported by Hoare (2001), who argues that chillsare effective in deterring elephants due to its irritant properties.

3.3.1 Local Communities perception

Local communities were asked to assess if the method is effective or not. Results indicate that more than 80% were of the opinion that chilling method was effective in mitigating HEC (Table 2).

3.4 Status of destruction by elephants before and after introducing chilling method

The status of destruction of crops (crop raids) by elephants before and after the introduction of chill was assessed. Data were obtained from District Game Officer (DGO).

3.4.1 Status before introduction of chilling method in Nyamburi village

In Nyamburi village, crop destruction over years (2003-2006) fluctuated (Figire 4). The average destruction was about 367ha/annum. This seems to be extremely high. Taking into the account, the total arable land of the area which is 2450ha; thedestruction is about15% of the total arable land.Assuming the destruction was for maize which is the most preferred crop by elephants and which is also a staple food in the area. This situation has two implications; that is, in food security and cash income. In the study area the crop is of multipurpose nature. That means is used as cash crop as well as food crop. For example assessing maize yield/ha in the area which is 5bags/ha;this implies that the loss of 367 ha/annum is equivalent to 182 tones of maize/annum which could feed a large number of families.According to Kaswamila (2007) one family consume 0.72tone/year therefore the loss of 182 tones means food shortagefor 2000people.

http://dx.doi.org/10.22161/ijeab/2.1.60 *ISSN: 2456-1878*

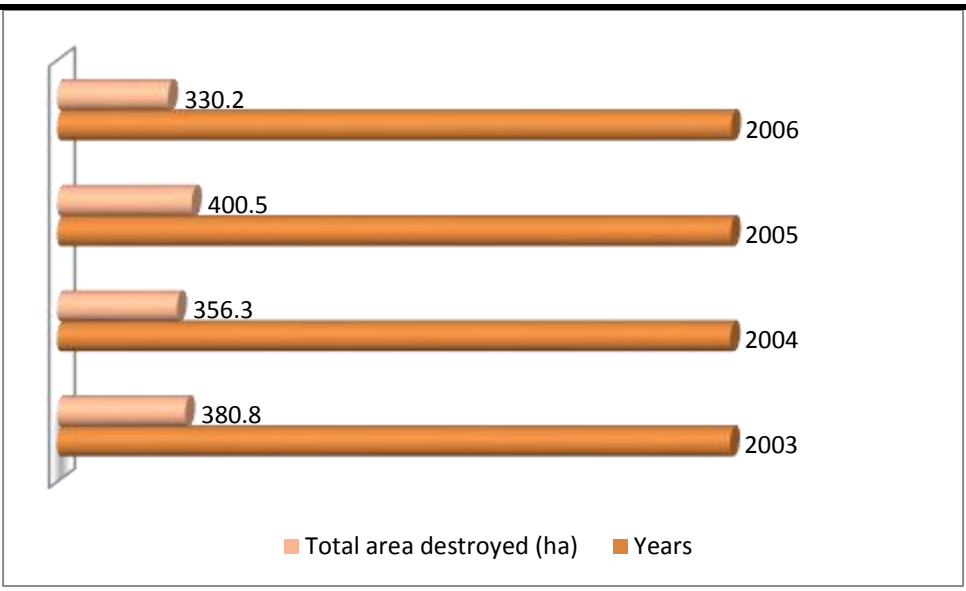


Fig.2: Extent of elephant destruction at Nyamburi village before introduction of Chilling method (2003 - 2006)

3.4.2 Status after introduction of chilling method in Nyamburi village

The situation of elephant's crop destruction status at Nyamburivillage after the introduction of chill was also assessed (Figure 5).Results indicate that the trend of crop destruction in general decreased. This could be argued that,among other things, probably the method has been instrumental in deterring elephants. On average only 231ha was destroyed between 2007and 2010 which was 25% lower compared to the situation before chill introduction. Therefore the method is effective in mitigating HEC.

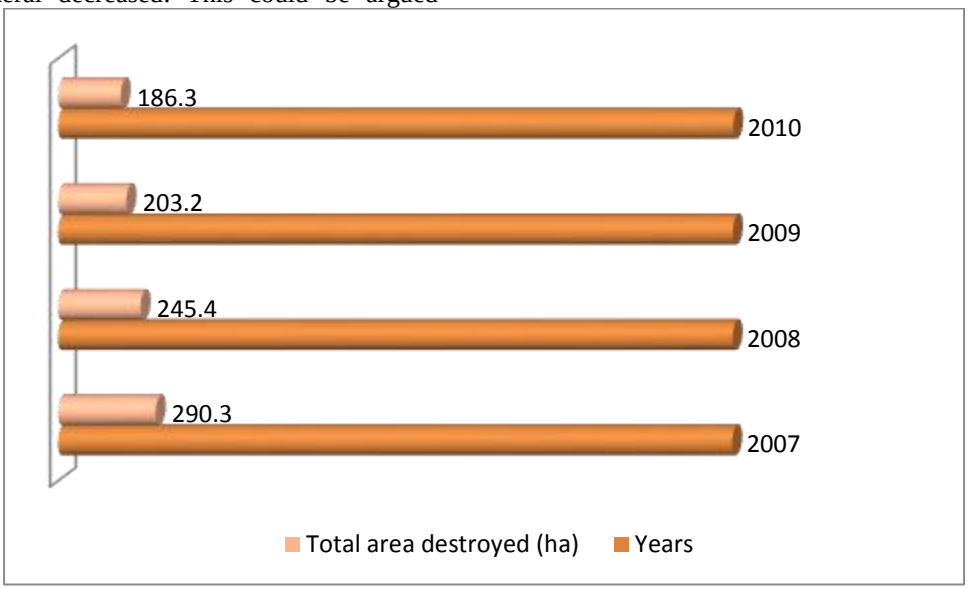


Fig.3: Extent of elephant destruction at Nyamburi village after introduction of Chilling method (2007 – 2010)

3.4.3 Status before introduction of chilling method in Bonchugu village

In Bonchugu results show that, crop destruction over years (2003-2006) also fluctuated (Figure 6). However, by all standards the average destruction of 401ha/annum.This was relatively higher compared to Nyamburi. Taking into the account, the total arable land of the area which is 1273.8 Ha; destruction was about 32% of the total arable land.

http://dx.doi.org/10.22161/ijeab/2.1.60 *ISSN: 2456-1878*

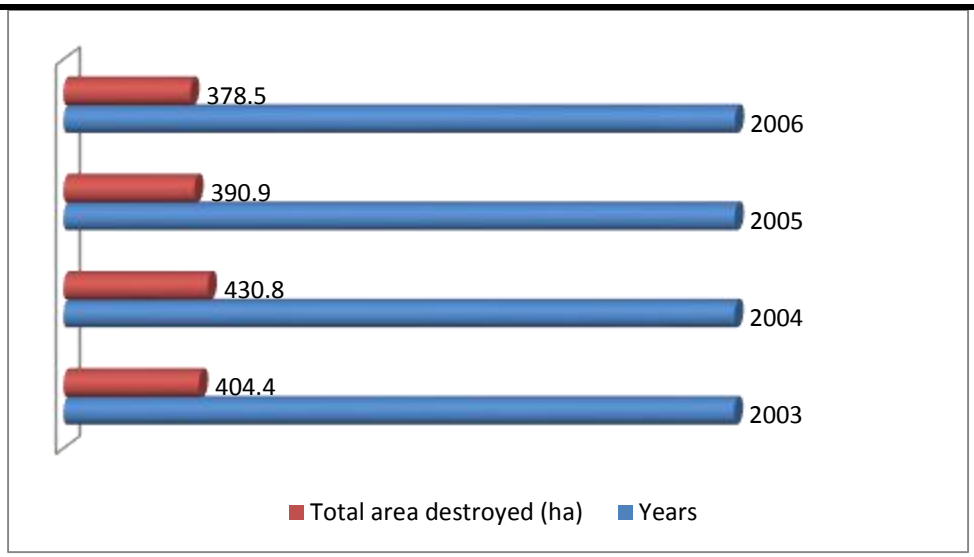


Fig.4: Extent of elephant destruction at Bonchugu village before introduction of Chilling method (2003 - 2006)

3.4.4 Status after introduction of chilling method in Bonchugu village

The situation of elephant's crop destruction status atBonchuguvillage after the introduction of chill was also assessed (Figure 7).Results indicates that the trend of crop destruction was decreasing at a decreasing rate. The decline trend could probably due to the effective of the method. On average only 291ha was destroyed between 2007and 2010 which was 25 % lower compared to the situation before chill introduction. This could be argued that, among other things, the method is instrumental in deterring elephants.

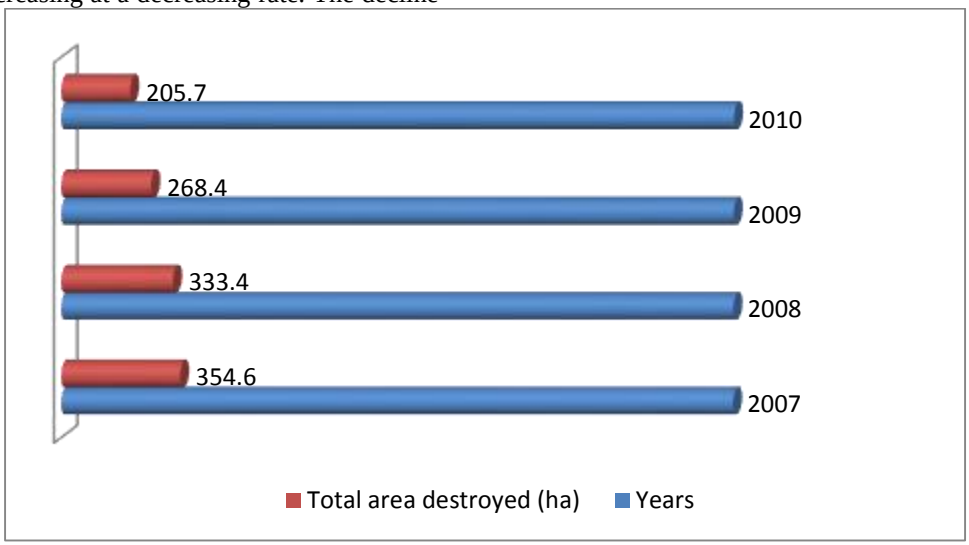


Fig.5: Extent of elephant destruction at Bonchugu village after introduction of Chilling method (2007 - 2010)

A study made by Jackson *et al*. (2008) shows that, chill method definitely works as crop raids from elephants every year in Zambia witnessed to decrease after farmers received training from Zambian trainers on how to use chili pepper to stop elephants raiding farmers field. According to Parker and Osborn (2006), it is estimated that in 2001, farms close to the eastern wing of Kakum National park (Ghana) where elephant activities were highest, recorded between 0.5bags of maize/ha during the main season depending on the number of wildlife damage the farm had. In 2003, such farms recorded up to 7 bags/ha after chilling crop raiding deterrents were put in place to scare off elephant.

3.5 Strengths of chilling method

Perception of local communities on the strengths of chilling was sought through questionnaire survey and group discussions (Table 3). Questionnaire results show several strengths. In order of importance the strengths viewed by households in both villages were easiness to use in field.Other strengths were cost effective of the method and itis user friendly.During FGDs the most observed strengths were for the chill to be harmless to both human and elephants and that it is simple to use.. InZambia Jackson *et al*. (2008), found that, when the crops supply with chillies, as an olfactory deterrent for elephants, it was sufficient,without harm both human and elephant.

Table.3: Strengths of chilling method

Villages	N=68	Views		
		Households	%	FGD
Nyamburi	34	Easiness in use	58.6	Control HEC without harming people and elephant
		Cost effective	22.3	
		User friendly	19.1	
Bonchugu	34	Easiness in use	51.2	Its use is simple and use appropriate technology
		User friendly	31.4	
		It use simple technology	17.4	

3.6 Weaknesses of Chilling method

Research findings from households revealed several weaknesses (Figure 8). The most notable one was the tendency of elephant to inter in the farm backwards after recognition that, chill deter them by generating unpleasant smell. Other weakness observed by households was the effect of heavy rainfall. They revealed that, insufficient used oil and chill. During FGDs they revealed that during rain seasons the pepper can be removed easily hence the method becomesineffective. Results from FGD do not differ with that of households. Theyargued that, elephantsare clever animals, they soon learn that, they pose no real threat and then ignore them, with time they entering in the farms/field backwards.Muruthi, (2005), argument on weaknesses of chilling method were similar with FGDs.He pointed out that, chilling method like other modern method face the same problem of elephants to overcome their fear by becoming habituated and less effective overtime.DGO argued that, the availability of pepper and used oil does not match with the high demands. In northern Mozambique for instance, in a region where chili-pepper has been tried, villagers very rapidly lost confidence in the method, due to difficult in maintaining the deterrent (FAO, 2005).

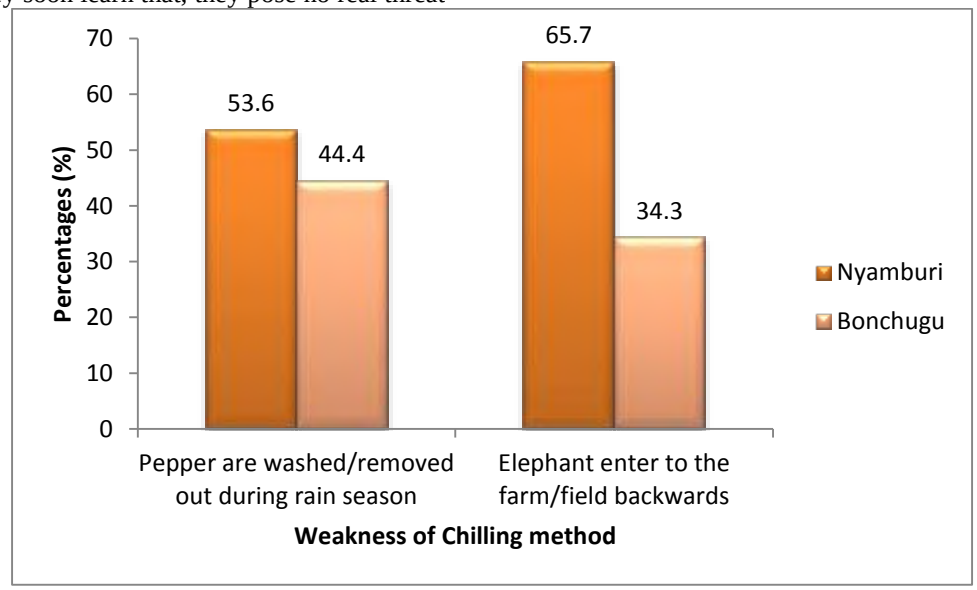


Fig.6: Weaknesses of Chilling method

3.7 Suggested measures for Improving Chilling Method.

Suggestions for local communities on the improvement of the method weresought through Questionnaire survey, group discussions and government officials (Table 4).

http://dx.doi.org/10.22161/ijeab/2.1.60 *ISSN: 2456-1878*

Table.4: Suggestions for improving chilling method

Villages	Suggestions		
	Households	FGDs	Officials
Nyamburi	Chill pepper cultivation by community and provision of used oil	Provision of used oil from different organizations and more training of community on chill application	Chilli pepper cultivation by community and provisions of used oil from surrounding companies and institutions
Bonchugu	Chill pepper cultivation and provision of used oil	Regular application of chill once washed out by rain and Provision of used oil from different organization	Positive community attitude towards the control of the elephant

The most suggested measures byhousehold's respondents and FGDs were about the farmer to cultivate chill pepper and availability of used oil from surrounded companies and different institution. However the study made by Kioko*etal*.,(2006), show that cultivation of chill will depend on farmer investment, climate and soil suitability, as well as the ability to market such crops. The benefits of having elephants living close to communities must exceed the cost of daily or constant exposure to people and their arable land (*ibid*).

It was also suggested that, chill should be applied regularlyonce washed out by rain and for the effectiveness of the method community should be well trained on how to use the method.

DGO suggested that,farmers should cultivate peppers, and he has already involved Districts authorities andBarick Company Limited as the supplier of used oil to the farmer to improve the method. He also suggested that for the method to be more effective, community should have positive attitude towards the use of the method. FAO, (2005), suggestions on improvement of chilling method does not differ with that of households' perceptions and DGO. They suggested that, government or NGO support is required to maintain the deterrents over most of the more remote areas where human-elephant conflict occurs.

IV. CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

The results of the study revealed that; chilling method is effective in mitigating HEC.The most observable strengths of the method was that; it can deter elephants without harming people and elephants, it is easy to apply, it does not consume time and use appropriate technology.Despite the effectiveness of the method, major challenges which observed to face the method were; insufficient used oil and pepper, elephants observed to be a clever animal as sometimes they inter into the farms/field backwards and alsoduring rain seasons,chill method observed to be ineffective as it can be washed/removed easily.However, the respondents recommended that; the challenges can be solved by local community to cultivate pepper and other stakeholders such as district, different NGO and companies to support farmers the provision of used oil,chill should be applied regularly once washed out by rain and for the effectiveness of the method community should be more trained on how to use the method.

4.2 Recommendations

- ✓ Community should cultivate more peppers to simplify the exercise of chilling method.
- ✓ Government officialssuch as VEO or WEO should have to report immediately to game officers once elephants destruct crops.
- ✓ Districts Authorities should have to collaborate with other companies outside the district such as Barick Company Limited to support the provision of used oil to the farmer.
- ✓ Capacity building of local wildlife managers to deal with HEC
- ✓ The government should have to develop substantial benefits for local communities living adjacent to the protected areas to increase local tolerance of HEC.
- ✓ Frequency application of the method should be intensified particularly during rain season.
- ✓ Capacity building on how to use the method particularly on the ratio required.

REFERENCES

[1] Bouma, G.D. (2000). The research Process(4thed).Oxford University Press, London.

[2] Breitenmoser, U.R.S., Angst, C., Landry, J., Breitenmoser-Wursten, C., Linnell, J. & Webber, J. (2005).Non-lethal Techniques for Reducing Depredation. In R. Woodroffe; S. Thirgood& A. Rabinowitz (Eds.), *People and wildlife: conflict or existence*? (pg 49-71). Cambridge, UK: Cambridge University Press.

[3] Coffey, A.& Atkinson, P. (1996).*Making Sense of Qualitative Data:* Complementary Research Strategies. Thousand Oaks, CA: Sage.

[4] Distefano, E. (2005). Human- Wildlife Conflicts Worldwide:A*Collection of Case Studies, Analysis of Management Strategies and Good Practices.* SARD Initiative Report, FAO, Rome.

[5] FAO,**(**2005).*Strategies to mitigate Human-Wildlife Conflict in Mozambique*.Anderson, J. &Pariela, F. Report for the National Directorate of Forests & Wildlife. Pg 68 .

[6] Gillham, B. (2005). *Research Interviewing*: The Range of Techniques: London: Open University.

[7] Henn, M., Weinstein, M., Foard, N. (2006).*A Short Introduction to Social Research*.Sage Publications, London.

[8] Hesse-Biber, S., &Leavy, P. (2004). *Approaches to Qualitative Research*: A Reader on Theory and Practice. Oxford University Press.

[9] Hoare, R.E. (2001a). *A decision support system for managing human-elephant conflict situations in Africa*.Pages 1-110.IUCN African Elephant Specialist Group Report.

[10] Hoare, R.E. (2001b). *Management implications of new research on problem elephants*.*Pachyderm*, 30: 44-49.

[11] Holmern, T &Anne B. (2004), "Human –wildlife conflicts and hunting in the Western Serengeti Tanzania."NINA Project Report, No 26.

[12] Homewood, K., Lambin, E.F., Coast, E(2001).Long – term changes in Serengeti-Mara Wildebeest and Land Cover pastoralism, population or policies? *Proceedings of the National Academy of Sciences of the USA.*

[13] Jackson, T.P., Mosojane, S., Ferreira, S.M. & van Aarde, R.J. (2008). Solutions forelephant *Loxodontaafricana*crop raiding in northern Botswana: moving away fromsymptomatic approaches. *Oryx* 42**,** 83-91

[14] Jones, B. T. B. & Elliott, W. J. (2006). Human Wildlife Conflict in Namibia: Experiences from a Portfolio of Practical Solutions. *Nature &Faune*21**,** 20-25.

[15] Kaswamila, A.L., Shaun, R., &McGibbon, M. (2007).Impacts of Wildlife on Household Food Security and Income in Northeastern Tanzania.*J. Human Wildlife Dimension,***12**: 391-404.

[16] Kaswamila, A. (2009). *Impacts of Game on Household Food Security: A Case of Serengeti District,* University ofDodoma –Department of Geography and Environment.

[17] Kideghesho, J.R. (2006).*Constraints in Implementing the Benefit-Based approaches as a Strategy for Conserving WildlifeIn Western Serengeti, Tanzania,* A paper presented at the 11th Biennial Conference of the International Association for the Study of Common Property in Bali, Indonesia June 19-23, 2006.

[18] Kideghesho, J.R.Nyahongo,J. W,Hassan, S.N, Tarimo,T.C, &Mbije, N.E. (2006),*Factors and Ecological Impacts of Wildlife Habitat Destruction in Serengeti Ecosystem in Northern Tanzania.*Sokoine University of Agriculture,Department of Wildlife, Morogoro,Tanzania.

[19] Krueger, A., Kahneman, D., Schkade, D., Schwarz, N. and Stone, A. (2004), *Measuring the Subjective Well-Being of Nations: National Accounts of Time Use and Well-Being*, National Bureau of Economic Research, University of Chicago Press, 79 pp.

[20] Morgan, D.L.(1998).Focus Groups as Qualitative Research, London; Sage.

[21] Muruthi, P. **(**2005). Human Wildlife conflicts: Lessons learned from AWD's African heratlands. *AWF Working Papers*, July 2005, 12 p.

[22] Parker, G.E. & Osborn, F.V**. (**2006).Investigating the potential for chilli Capsicum annuum to reduce human-wildlife conflict in Zimbabwe. Oryx 40(3):1-4.

[23] Parker G.E., Osborn, F.V., Hoare R.E. &Niskanen, L.S. (eds.) (2007): *Human-Elephant Conflict Mitigation: A Training Course for Community-Based Approaches in Africa.* Participant's Manual. Elephant Pepper Development Trust, Livingstone, Zambia and IUCN/SSC AfESG, Nairobi, Kenya.

[24] Punch, K. (2000). *Developing Effective Research Proposals*. Sage Publications, London, 125pp

[25] Rwegoshora, H.M.M. (2006). *A Guide to Social Science Research*, Dar es Salaam, Tanzania MkukinaNyota Publishers.

[26] Severre, E.L.M.(2000).*Conservation of wildlife outside core areas in the new millennium*. Dar-re-salaam printers

[27] URT. (2005).*Assessment of Game Controlled Area Category as Wildlife Conservation Areas.*Ministry of Natural Resources and Tourism, Wildlife Division, Dar es Salaam: Government Printer.

[28] Woodroffe, R., Thirgood, S & Rabinowitz, A. (2005). The Future of Coexistence: Resolving Human-Wildlife Conflicts in Changing World. In R. Woodroffe; S. Thirgood& A. Rabinowitz (Eds.), *People and wildlife: conflict or existence*? (Pg 388-405). Cambridge, UK: Cambridge University Press.

[29] Woodroffe,R., Thirgood,S&Rabinowitz,A. (2005),*People and Wildlife Conflict orCoexistence*? Cambrige University Press.

http://dx.doi.org/10.22161/ijeab/2.1.61 *ISSN: 2456-1878*

Germination and Seedling Growth of a Set of Rapeseed (*Brassica napus*) Varieties under Drought Stress Conditions

Souhail Channaoui[1,2], Rahal El Kahkahi[2], Jamal Charafi[1], Hamid Mazouz[2], Mohamed El Fechtali[1], Abdelghani Nabloussi[1*]

[1]Research Unit of Plant Breeding and Plant Genetic Resources Conservation, National Institute of Agricultural Research, Regional Agricultural Research Center of Meknes, PO. Box 578, Meknes 50000, Morocco.
[2]Laboratory of Plant Biotechnology and Molecular Biology, Department of Biology, Faculty of Science, University Moulay Ismail, PO. Box 11201, Zitoune, Meknes 50000, Morocco.

***Abstract*—** *Drought stress is one of the major abiotic factors affecting seed germination and plant growth especially in arid and semi-arid regions. In this study, we investigated the effects of drought stress on seed germination and seedling growth of five varieties of rapeseed. Seven drought stress levels of zero (control), -3, -5, -7, -9, -11 and -13 bars were performed using polyethylene glycol-6000 (PEG-6000). A completely randomized design with three replications was used for this experiment. Germination percentage (GP), germination rate (GR), mean germination time (MGT), root length (RL) and shoot length (SL) were measured to evaluate the varieties response to PEG-induced drought stress. Drought stress, variety and the interaction drought × variety had a significant effect on all studied parameters. GP and GR decreased with the increase in stress level, while MGT increased. There were no seeds germinated for all varieties at -11 bars and -13 bars. Shoot length decreased with increasing drought stress but different varieties show different performance under stress environment. Root length decreased with increasing level of severe drought stress. However, the presence of moderate drought stress could even improve the root growth of the investigated varieties. The varieties 'INRA-CZH2' and 'INRA-CZH3' exhibited the highest germination percentage and the best early seedling growth. Thus, they could be recommended for environments with early cropping cycle drought.*

***Keywords*— Drought stress, Germination, Rapeseed, Seedling growth.**

I. INTRODUCTION

Rapeseed (*Brassica napus* L.), belonging to the family of *Brassicaceae*, is one of the most important sources of vegetable oils and protein-rich meals worldwide. Rapeseed is the world's third most important source of vegetable oil after palm and soybean [1]. Also, rapeseed oil is one of the most interesting edible oils in the world. Its nutritive value is excellent due to the abundant unsaturated fatty acids. Rapeseed meal (remains after oil extraction) is used for livestock feed industry. Its amino acid content is ideal and it has a high content of fiber, several minerals and vitamins [2, 3].

Drought was always present in Morocco's history, but during this last decade, it has become more frequent, with a net reduction in precipitation and an increasing temperature trend. Climatic data during the period 1961-2004 showed an increase in drought frequency, severity and spatial distribution [4]. In Morocco with an arid and semi-arid climate, rapeseed is more often planted in late autumn and harvested in early summer. Accordingly, this stress is also considered as an essential limiting factor for rapeseed growth and production due to poorly distributed rainfalls over the crop growing season. Although drought can occur at any time during the growing season, two main periods of drought are more likely, the early one that coincides with seed germination and seedling emergence and the terminal drought that is more frequent and affects grains set and growth [5].

Drought can be most simply defined as a period of below normal precipitation that limits plant productivity in a natural or agricultural system [6, 7]. In the field, drought is a severe limitation of plant growth, development and productivity, particularly in arid and semi-arid regions [8], where the rainfall varies from year to year. However, depending upon plant species, certain stages such as germination, seedling or flowering could be the most critical stages for drought stress. Seed germination is first critical and at the same time the most sensitive stage in the life cycle of plants [9] and the seeds exposed to unfavorable environmental conditions like drought stress may have to compromise the seedlings establishment [10]. Soil water supply is an important environmental factor controlling seed germination [11]. If the osmotic

potential is reduced, seed germination will be delayed or prevented, depending on the extent of its reduction [12]. One technique for studying the effect of drought stress on germination is to simulate stress conditions using artificial solutions to provide variable osmotic potentials [13, 14]. Polyethylene glycol (PEG) causes osmotic stress and could be used as a drought simulator [15, 16, 17, 18] as an inert osmoticum in germination tests [19] and a non-penetrating solute [20]. This results in osmotic stress that inhibits seed germination through the prevention of water uptake.

The present research was carried out to study the effect of water stress (PEG) on rapeseed germination and early seedling growth, evaluating various levels of PEG and different rapeseed varieties.

II. MATERIALS AND METHODS

The plant material used in this study consisted of five varieties of rapeseed (*Brassica napus* L.) from the collection of the National Institute for Agricultural Research (INRA) of Morocco (Regional Center of Meknes). These are 'INRA-CZH2', 'INRA-CZH3', 'INRA-CZSyn1', 'Moufida' and 'Narjisse'.

The experiment was conducted in a double factorial completely randomized design, with three replications. The first factor was the variety, with five levels, and the second was the stress solution of PEG, with seven levels.

Drought stress was induced by polyethylene glycol (PEG-6000) solutions. Application of six levels of drought stress, with osmotic potentials of -3, -5, -7, -9,-11 and -13 bars, was prepared as described by [21]. Distilled water was used as control. For each treatment, 100 seeds were sterilized in a solution of 5% sodium hypochlorite for 1 min and then carefully rinsed with distilled water to remove any traces of sterilizing agent, and were allowed to germinate in a Petri dish double lined with filter paper moistened with 15 ml of the appropriate level of PEG-6000 at 25 ± 1 °C for 8 days. Germination parameters were counted after 2, 4, 6 and 8 days following seeds placement in Petri dishes for germination. Seeds were considered germinated when the radicle was at least 3 mm long. For germination percentage, the number of seeds germinated on day 8 was considered. The germination rate index was determined by GR = ∑ (Ni/Di) as described by Carlton et al. 1968 [22], where Ni is the number of seeds germinated between two counts and Di represents the day of counting. Mean Germination Time (MGT) expressed in d, is the inverse of GR (MGT = 1/GR). Root and shoot length were measured on the eighth day after germination (end of experiment). Shoot length was measured from the cotyledons to the collar, and the root length was measured from the collar to the root tip.

Statistical analysis was conducted with the software package SPSS for Windows (Version 22). Data were subjected to an analysis of variance (ANOVA) to determine statistically significant differences among varieties, drought levels and their interaction levels. Duncan's new multiple range test (DMRT) was applied to compare treatment means.

III. RESULTS

3.1 Drought stress effects on germination

Results of analysis of variance indicated that drought stress affected significantly rapeseed seed germination percentage (GP), germination rate index (GR) and mean germination time (MGT). There was also a significant effect of variety and its interaction with drought on these parameters (Table 1). GP and GR decreased, while MGT increased with the increase in drought level (Table 2). The highest GP (97.40 %) was observed in absence of drought stress (control), and the most significant decline was recorded at -9 bars (64.20%). Below this osmotic potential, i.e. at -11 and -13 bars, no germination was recorded. The germination percentage at the -5 and -7 bars was 90.33 and 85.00 %, respectively.

In absence of stress (control treatment), significant differences among varieties were observed for GP. 'INRA-CZH2', 'INRA-CZH3' and 'Moufida' were the most interesting, having a GP of 100, 99 and 98.70%, respectively, while 'INRA-CZSyn1' and 'Narjisse', had 94% and 95%, respectively (Fig. 1). Under moderate stress (-3 bars), 'INRA-CZH2' had the highest GP (100%), whilst 'INRA-CZSyn1' had the lowest one (87%). Under severe-intermediate stress (-9 bars), again, 'INRA-CZH2' maintained the highest GP (93%), while 'Narjisse' showed the most drastic reduction in GP (40%) (Fig. 1). 'INRA-CZH3' exhibited quite high GP (80%), whilst 'Moufida' and 'INRA-CZSyn1' had too much lower GP, with an average of 58% and 50%, respectively (Fig. 1). This indicated that, in terms of germination, 'INRA-CZH2' and 'INRA-CZH3' were the most tolerant to such stress, and 'Narjisse' was the most sensitive. The other varieties were intermediate.

Mean germination time (MGT) of all experimented varieties was significantly delayed by increasing the drought level (Fig. 2). 'INRA-CZH2' and 'INRA-CZH3' were the least affected as they showed the lowest MGT values for all drought levels (Fig. 2). By increasing drought stress, germination rate (GR) decreased significantly in all varieties (Fig. 3). Again, 'INRA-CZH2' and 'INRA-CZH3' confirmed their highest tolerance to various drought levels, maintaining the highest GR values, compared to the other varieties. Overall, they had a GR of about 30 and 28.6%, respectively, which was significantly higher than 23.3,

18.2 and 17.8%, observed in 'Moufida', 'INRA-CZSyn1' and 'Narjisse', respectively.

3.2 Drought stress effects on seedling growth

There were significant differences among varieties and between drought stress levels for shoot length (SL) and root length (RL) (Table 1). Also, the effect of drought stress × variety interaction on both parameters was significant, indicating that varieties reacted differently to the drought levels.

Shoot length decreased with increase in drought level. The highest average shoot length, 4.77 cm, was observed in absence of drought (control). At the osmotic potential of -3 bars, corresponding to the moderate drought stress, the average shoot length was 2.42 cm. The first drastic reduction was recorded at -7 bars (0.67 cm) and the lowest shoot length ever observed was 0.28 cm, recorded at -9 bars.

Overall, the varieties 'Narjisse' and 'INRA-CZSyn1' showed the highest average shoot length (1.62 cm) while the variety 'INRA-CZH2' showed the lowest one (1.04 cm). The other varieties were intermediate (Fig. 4). In absence of stress, 'Narjisse' and 'INRA-CZSyn1' had the highest average shoot length (5.70 cm). At moderate stress (-3 bars), all the varieties were comparable, with an average of about 2.40 cm. 'Narjisse' maintained the highest SL (2.55 cm) at -5 bars followed by 'INRA-CZH3', 'INRA-CZSyn1', 'Moufida' and 'INRA-CZH2', having an average SL of 1.99, 2.03, 1.80 and 1.46 cm, respectively. However, under intermediate-severe stress (-9 bars), 'INRA-CZSyn1' and 'Narjisse' had the lowest average SL (0.21 cm) while 'INRA-CZH3' exhibited the highest one (0.41 cm). 'INRA-CZH2' and 'Moufida' were comparable, with a mean value of 0.27 cm (Fig. 4).

On the other hand, and for all drought stress levels combined, the variety 'INRA-CZH3' developed the longest root, with an overall average of 4.23 cm, whilst the variety 'Narjisse' had the lowest average root length, i.e. 3.58 cm. Unlike shoot length, root length (RL) increased with moderate and moderate-intermediate drought stress (-3 and -5 bars, respectively). The highest average RL, 7.70 cm, was observed for moderate-intermediate stress (-5 bars). The varieties 'INRA-CZH2' and 'Narjisse' exhibited the highest mean RL, i.e. 8.35 cm. At the osmotic potential of -3 bars, corresponding to the moderate drought stress, the average RL was 6.70 cm. 'INRA-CZH3' was the most interesting variety, with RL of 7.95 cm. In absence of drought (control), average RL was 5.10 cm, and 'INRA-CZSyn1' developed the longest root (about 6 cm). This indicates that moderate up to intermediate drought stress did not affect the root growth, but could even improve it. Under intermediate drought stress (-7 bars), RL was unchanged for the varieties 'INRA-CZH3' and 'Moufida', decreased for 'Narjisse' and increased for 'INRA-CZH2'. Finally under severe-intermediate stress (-9 bars), a decrease of this parameter was observed for all varieties (Fig. 5). However, the variety 'INRA-CZH2' was the least affected, maintaining a RL of about 3.50 cm, compared to 3.80 cm recorded in absence of drought stress (control).

IV. DISCUSSION

Seed germination and early seedling growth are critical stages for plant establishment [23, 24], and plants are more sensitive to drought stress during these stages. Drought stress greatly affects seed germination, but the response intensity and harmful effects of such stress depend on the species. In the present study, all the seed germination parameters measured were affected by drought stress. Particularly, GP and GR decreased with the increase in stress levels. This is in agreement with findings of previous studies in *Brassica napus* [18] and in *Brassica juncea* [15]. MGT increased with the increase in stress levels.

Table 1. Analysis of variance (mean squares) for seed germination and seedling growth related traits of five rapeseed varieties evaluated under different drought stress levels.

Source of variation	Degree of freedom	Germination percentage	Germination rate	Mean germination time	Root length	Shoot length
Variety (V)	4	1003.32***	681.11***	0.0018***	3.45*	20.10***
Drought (D)	6	28360.17***	5142.93***	0.0103***	383.33***	748.11***
V × D	24	200.20***	62.16***	0.0005***	3.35***	9.26***
*, **, *** Significant at 0.05; 0.01 and 0.001 probability levels, respectively.						

http://dx.doi.org/10.22161/ijeab/2.1.61 *ISSN: 2456-1878*

Table.2: Overall averages of seed germination and seedling growth related traits for each PEG applied osmotic potential.

	Germination percentage	Germination rate	Mean germination time	Root length (cm)	Shoot Length (cm)
0	97.40 a	42.74 a	0.026 d	5.09 c	4.77 a
-3	94.67 b	42.51 a	0.024 d	6.72 b	2.42 b
-5	90.33 c	36.05 b	0.030 c	7.70 a	2.00 c
-7	85.00 d	27.28 c	0.040 b	4.71 c	0.67 d
-9	64.20 e	16.49 d	0.077 a	2.66 d	0.28 e
-11	0.00 f	0.00 e	0.00 e	0.00 e	0.00 f
-13	0.00 f	0.00 e	0.00 e	0.00 e	0.00 f
Mean values, in each column, followed by the same letter are not significantly different.					

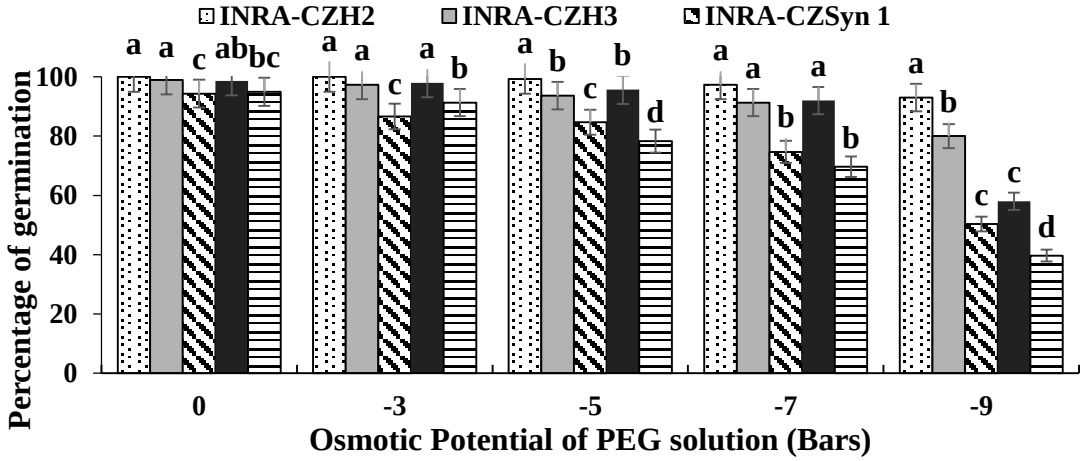


Fig. 1: Effect of PEG induced drought stress on germination percentage of seeds from five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

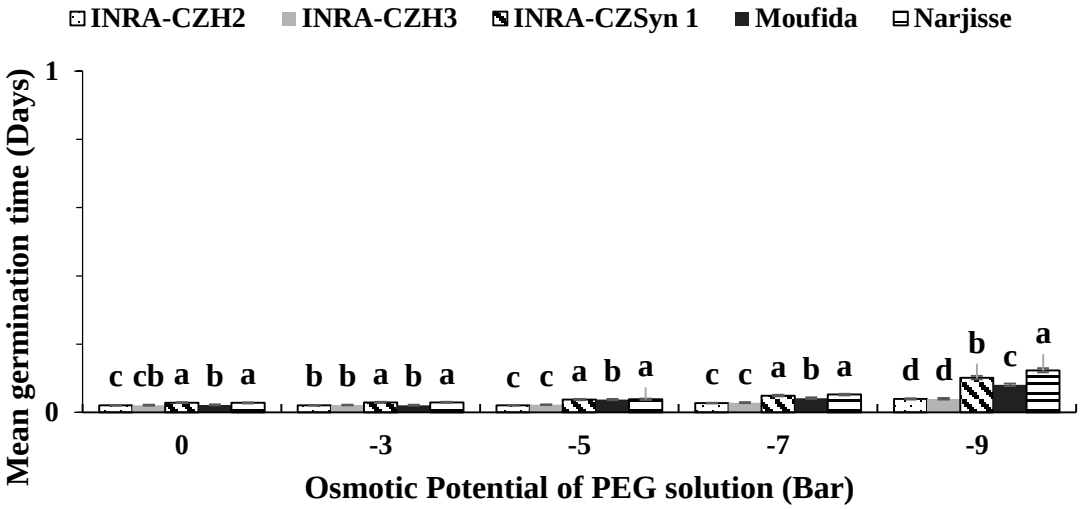


Fig. 2. Effect of PEG induced drought stress on mean germination time (MGT) of seeds from five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

http://dx.doi.org/10.22161/ijeab/2.1.61 *ISSN: 2456-1878*

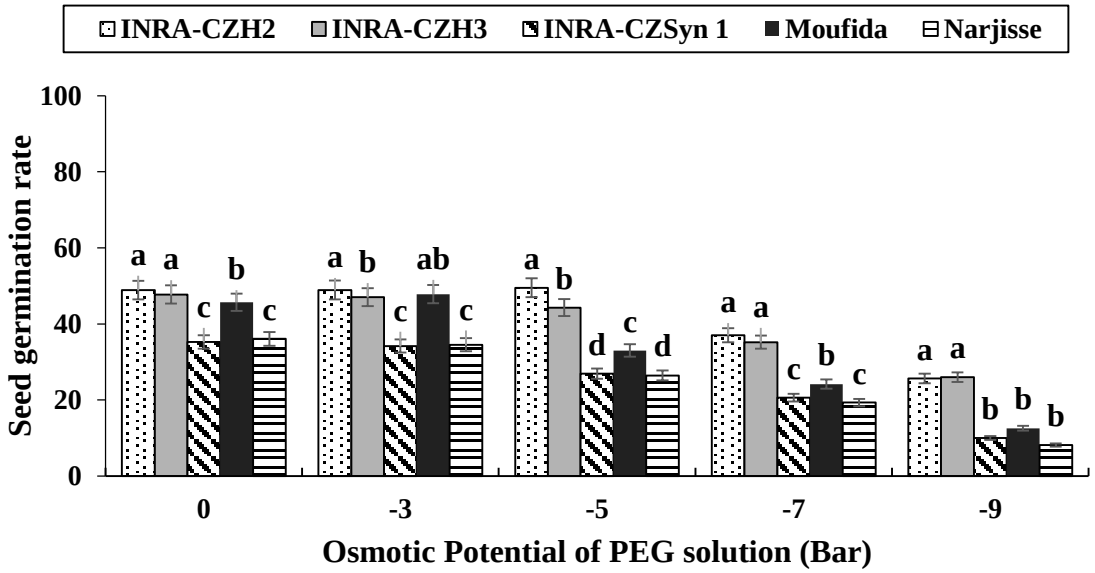


Fig. 3. Effect of PEG induced drought stress on germination rate of seeds from five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

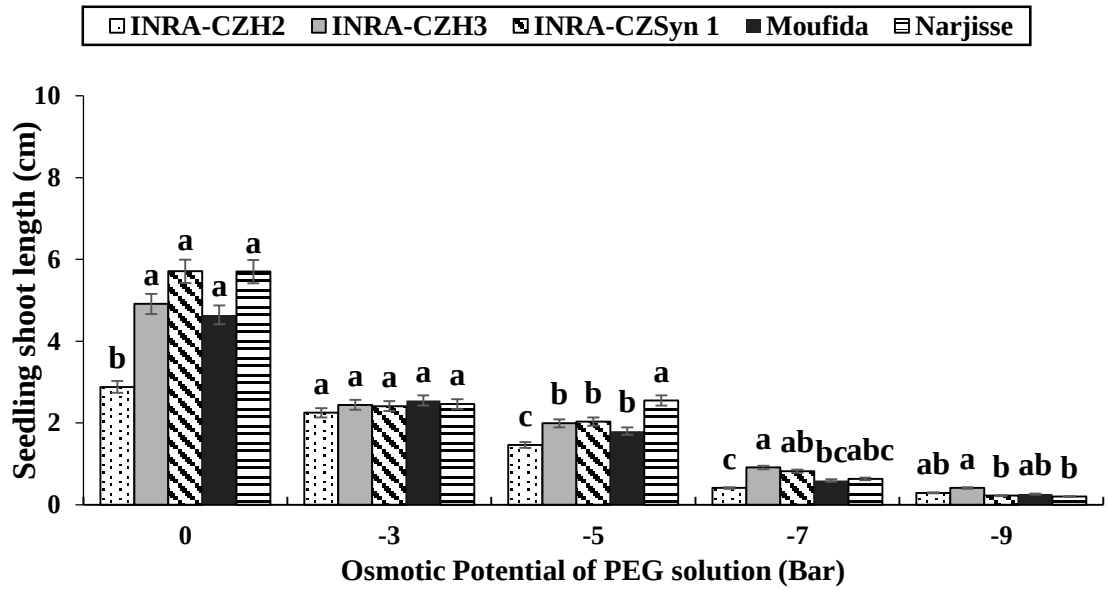


Fig. 4. Effect of PEG induced drought stress on seedling shoot length of five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

The same result had been found in safflower [25] and in sesame [26]. The germination of 'INRA-CZH2' and 'INRA-CZH3' was less affected than that of 'INRA-CZSyn1', 'Moufida' and 'Narjisse', indicating that, during germination, 'INRA-CZH2' and 'INRA-CZH3' were more tolerant to drought stress than the other varieties. To germinate, all varieties of rapeseed could tolerate until -9 bars, and from -11 bars, no germination was recorded. Toosi et al. [15] reported that -10 and -12 bars completely inhibited seed germination in *Brassica juncea* Var. Ensabi. Kaya et al [27] found that none of the sunflower seeds could germinate at -12 bars, and in a recent study on sesame, it was also shown that seeds ceased to germinate from -12 bars [26]. However, Zraibi et al. [25] reported that safflower seeds ceased to germinate already from -2.5 bars. These findings indicate that rapeseed is less tolerant than sunflower and sesame and more tolerant than safflower to drought stress during germination stage.

For all varieties, drought stress affected the germination and early seedling growth of rapeseed. This is may be due to alteration of enzymes and hormones found in the seed [28] or to the metabolic disorders induced by stress and generation of Reactive Oxygen Species [29].

http://dx.doi.org/10.22161/ijeab/2.1.61 *ISSN: 2456-1878*

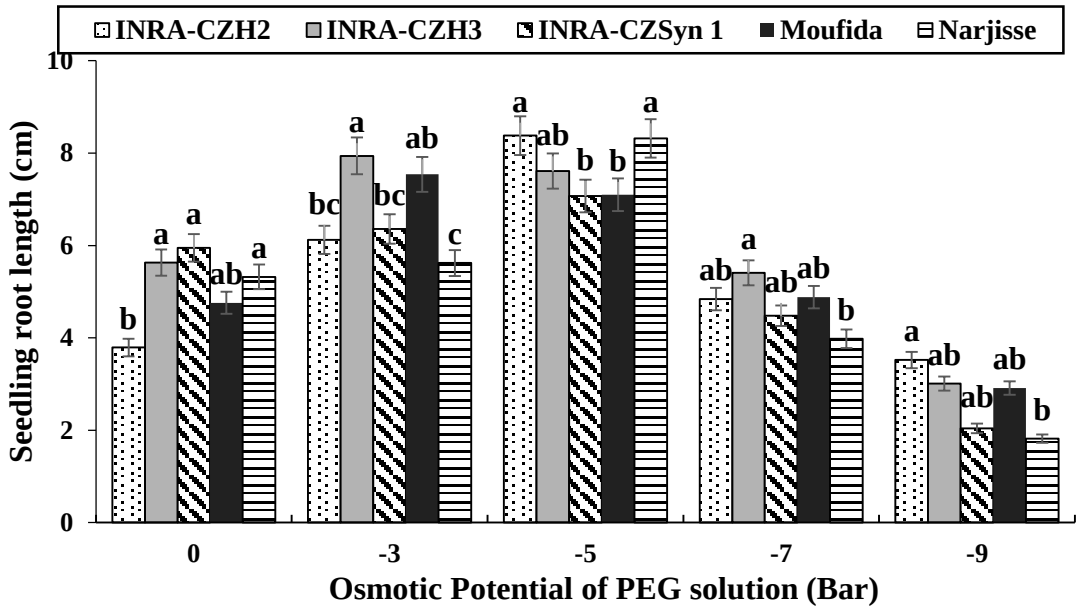


Fig. 5. Effect of PEG induced drought stress on seedling root length of five rapeseed varieties. (Values with different alphabetical superscripts are significantly different ($p \leq 0.05$) according to DMRT).

It could also be a deficit of hydration of the seeds due to high osmotic potential causing inhibition of the mechanisms leading to the output of the radicle out of the coat and therefore a seed germination delay [30].

In our study, shoot length generally decreased with increased drought levels. There are many reports on various crops that are in accordance with this finding [15, 25, 26]. However, we found that the varieties investigated showed different performances under this stress. In absence of stress, 'Narjisse' and 'INRA-CZSyn1' had the highest average shoot length, whilst under elevated drought stress, 'INRA-CZH3' was the most tolerant, having exhibited the highest shoot growth. Similar results were previously shown in other crops, including *Brassica juncea* Var. Ensabi [15], safflower [25] and sesame [26]. Also, root length decreased with the increased drought levels. However, the presence of moderate drought stress could even improve the root growth of the varieties investigated. These results agree with those reported in *Brassica juncea* Var. Ensabi [15], in cowpea [31], in pearl millet [32] and in triticale [33]. Our investigation revealed that, in absence of any stress, the varieties 'Narjisse', 'INRA-CZSyn1' and 'INRA-CZH3' developed the longest root. However, for all drought stress levels combined, the variety 'INRA-CZH3' was the most tolerant, exhibiting the longest average root,

The variation in rapeseed varieties performance determined by some seedling growth parameters such as shoot length and root length indicated that seedling growth is a reliable and efficient stage for the study of rapeseed genotypes reaction to moisture stress. The varieties having genetic potential to maintain higher seedling growth under moisture stress conditions are drought tolerant in this particular stage, and their tolerance should be confirmed at adult plant stages.

V. CONCLUSION

Based on the results of this study, the varieties 'INRA-CZH2' and 'INRA-CZH3' germinated better than the other varieties under drought conditions. The observed variation among varieties is a reliable indicator of genotypic differential for drought tolerance in rapeseed. This suggests that the choice of the rapeseed variety to be planted in a given environment should depend upon the presence and the degree of the stress observed in such environment. In a non-stressed environment, the varieties, 'INRA-CZSyn1' and 'Narjisse', even with an intermediate germination percentage, should be planted due to their best seedling growth (root and shoot length). In drought stressed environments, the varieties 'INRA-CZH2' and 'INRA-CZH3', exhibiting the highest germination percentage, should be recommended.

REFERENCES

[1] FAO, FAOSTAT, 2014. Available at: http://www.fao.org/faostat/en/#data/. (Accessed 12 February 2017).

[2] M. Naczk, R. Amarowicz, A. Sullivan, and F. Shahidi, "Current research developments on polyphenolics of rapeseed/canola: a review," Food Chem, 1998, 62: 489-502.

[3] R.K. Downey and J.M. Bell, "New developments in canola research. In: Canola and rapeseed. Production, chemistry, nutrition and processing technology.

Shahidi, F. (toim.)," Van Nostrand Reinhold, United States of America, 1990, pp. 37-45.

[4] L. Stour, and A. Agoumi, "Climatic drought in Morocco during the last decades. Hydroécol," Appl, 2008, 16:215–232.

[5] D.G. Watts and M. El Mourid, "Rainfall Patterns and probabilities in the semi-arid cereal production region of Morocco," USAID project No, 1988, 608-0136.

[6] J.S. Boyer. "Plant productivity and environment," Science, 1982, 218, 443–448.

[7] P.J. Kramer and J.S. Boyer, "Water Relations of Plants and Soils," San Diego: Academic Press, 1995.

[8] A. Galle, P. Haldimann and U. Feller, "Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery," New Phytol, 2007, 174:799-810.

[9] M. Ashraf and S. Mehmood, "Response of four Brassica species to drought stress," Environ. Expt. Bot, 1990, 30: 93-100.

[10] F.M.C. de Albuquerque and N.M. de Carvalho, "Effect of type of environmental stress on the emergence of sunflower (*Helianthus annuus* L.), soyabean (*Glycine max* (L.) Merril) and maize (*Zea mays* L.) seeds with different levels of vigor," Seed Sci. Technol, 2003, 31: 465-467.

[11] P.J. Kramer and T.T. Kozlowski, "Physiology of woody plants," Academic Press, New York, 1979, pp 811.

[12] T.W. Hegarty, "The physiology of seed hydration and dehydration, and the relation between water stress and control of germination: a review," Plant Cell Environ, 1978, 1: 101–109.

[13] M.M. Larson, and G.N. Shubert, "Effect of osmotic water stress on germination and initial development of Ponderosa pine seedlings," Forest Sci, 1969, 15: 30–36.

[14] M. Falusi, R. Calamassi and A. Tocci, "Sensitivity of seed germination and seedling root growth to moisture stress in four provenances of *Pinus halepensis* Mill," Silvae Genetica, 1983, 32: 4–9.

[15] A.F. Toosi, B. B. Bakar and M. Azizi, "Effect of drought stress by using peg 6000 on germination and early seedling growth of *Brassica juncea* Var. Ensabi," Scientific Papers. Series A. Agronomy, 2014, Vol. LVII: 360-363.

[16] B. Torabi, F. G. Ardestani, "Effect of salt and drought stresses on germination components in canola (*Brassica napus* L.)," IJACS, 2013, 5-15:1642-1647.

[17] V. Jajarmi, R. Abazarian and K. Khosroyar, "Effects of drought stress and salt stress on components factors germination of oilseed rape cultivars," Indian J.Sci.Res, 2014, 7 (1): 1042-1044.

[18] G.R. Mohammadi and F. Amiri, "The Effect of Priming on Seed Performance of Canola (*Brassica napus* L.) Under Drought Stress," American-Eurasian J. Agric. & Environ. Sci., 2010, 9 (2): 202-207.

[19] G.L. Dodd, L.A. Donovan, "Water potential and ionic effects on germination and seedling growth of two cold desert shrubs," Am J Bot, 1999, 86: 1146-1153.

[20] M. Almansouri, J.M. Kinet and S. Lutts, "Effect of salt and osmotic stresses on germination in durum wheat (*Triticum Durum* Desf.)," Plant Soil, 2001, 231: 243-254.

[21] B.E. Michel and K. Kaufmann, "The osmotic potential of polyethylene glycol 6000," Plant Physiol, 1973, 51: 914–916.

[22] A.E. Carlton, C.S. Cooper and L.E. Wiesner, "Effect of seedpod and temperature on speed of germination and seedling elongation of sainfoin (*Onobrychis viciaefolia* Scop)," Agron. J, 1968, 60: 81-84

[23] S. Ahmad, R. Ahmad, M.Y. Ashraf, M. Ashraf and E.A. Waraich, "Sunflower (*Helianthus Annuus* L.) response to drought stress at germination and seedling growth stages," Pak J Bot, 2009, 41:647-654.

[24] F.L. Li, W.K. Bao and N. Wu, "Morphological, anatomical and physiological responses of *Campylotropis polyantha* (French.) Schindl. seedlings to progressive water stress," Sci Hortic-Amsterdam, 2011, 127:436-43.

[25] L. Zraibi, A. Nabloussi, M. Kajeiou, A. El Amrani, A. Khalid and H. Serghini Caid, "Comparative germination and seedling growth response to drought and salt stresses in a set of safflower (*Carthamus tinctorius*) varieties," Seed Technol, 2011, 33: 39–52.

[26] M. El Harfi, H. Hanine, H. Rizki, H. Latrache and A. Nabloussi, "Effect of Drought and Salt Stresses on Germination and Early SeedlingGrowth of Different Color-seeds Sesame (*Sesamum indicum* L.)," Int. J. Agric. Biol, 2015, Vol. 00, No. 0, 201x.

[27] M.D. Kaya, A. Ipek and A. Ozturk. "Effects of different soil salinity levels on germination and seedling growth of safflower (*Carthamus tinctorius* L.)," Turk. J. Agric, 2003, 27:221–227.

[28] P. Botia, M. Carvajal, A. Cerda and V. Martinez, "Response of eight Cucumis melo cultivars to salinity during germination and early vegetative growth," Agronomy, 1998, 18: 503–513.

[29] D.E. Almas, S. Bagherikia and K.M. Mashaki, "Effects of salt and water stresses on germination and seedling growth of Artemisia vulgaris L. Int. J. Agric," Crop Sci, 2013, 56: 762–765.

[30] P.K. Gill, A.D. Sharma, P. Singh and S.S. Bhullar, "Changes in germination, growth and soluble sugar contents of *Sorghum bicolor* (L.) Moench seeds under various abiotic stresses," Plant Growth Regul, 2003, 40: 157–162.

[31] B. Murillo-Amador, R. Lopez-Aguilar, C. Kaya, J. Larrinaga-Mayoral, and A. Flores-Hernandez, "Comparative effects of NaCl and polyethylene glycol on Germination, emergence and Seedling growth of Cowpea," Journal of Agronomy and Crop Science, 2002, vol. 188, no. 4, pp. 235–247.

[32] L. Radhouane, "Response of Tunisian autochthonous pearl millet (*Pennisetum glaucum* L.) to drought stress induced by polyethylene glycol (PEG) 6000," African Journal of Biotechnology, 2007, 6:1102-1105.

[33] M. Yagmur, D. Kaydan, "Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments," African Journal of Biotechnology, 2008, 7:2156-2162.

http://dx.doi.org/10.22161/ijeab/2.1.62 *ISSN: 2456-1878*

Seeds Potentialities of Medicks in Sub-Humid Area to be used in Steppe Zone

Alane F[2], R.Chabaca[1], Abdelguerfi-Laouar.M[3], Abdelguerfi A[1]

[1,3]National Superior School of Agronomy El Harrach, Algeria
[2] National Agronomy Research Institute of Nari Baraki, Algeria

Abstract— *New pulse crops varieties more productive as medics should be made available to breeders located in semi-arid region of Algeria. So, and through two years of testing, pods yield and seeds production of twenty populations belonging to species* ***M.intertexta , M.ciliaris, M.polymorpha , M.Truncatula*** *and an introduced population* ***M.muricoleptis*** *is an Australian cultivar (Jemalong) are appreciated.Pods' yields of year 2013 vary between 78,66g/m² with* $poly_{27}$ *and 3637,33g/m² with* I_{107}*. Values of the second essay (2015) are different, they vary between 40,89g /m² with* $Poly_{236}$ *and 464,36 g/m² with* I_{11}***.****The cultivar Jemalong offers a yield of 172,4 g/m². The corresponding seed yields also show a low production in year 2015. The ratio leaf / stem varies between 1,03 and 5. The average of yields in dry matter of 27 populations in 2013 was of 457,79g/m² against 127,41 g/m² in 18 populations in 2015. Jemalong cultivar records a yield of 12,8g/m². For the same dose of seed, number of plants by square meter varies between 44 and 112. Number of stems by square meter varies between 136 in* C_{204} *and 420 in* I_{52}*..The average width of population's ramifications varies between 13cm in* Tr_{334} *and 44 cm in* I_{52}*, The Jemalong cultivar offers an average of 17cm. So, production results of medicks depend of weather conditions in littoral zone of lower altitude than 600-700m. In steppe zones, we recommend to make tests in situ with these same populations.*

Keywords— Medicago, medicKs, populations, pods, yield in seed.

I. INTRODUCTION

Husbandry of ruminants is mainly located in arid regions of Algeria, in about 1/5 of the total area. This important availability of agricultural land requires fodder production In fact, fodder grown is one of the solutions considered, pass through intensification of fodder production, per unit of area, not only in quantitative terms (kg of fodder produced by m^2) but also in qualitative terms with a net improvement of nutritional quality. New pulse crops varieties more productive and with better nutritional value should be available to the breeders. Much appreciated by livestock, as well in green as in dry, pulse crops are richer in nutrients mainly in digestible nitrogenous matter, in vitamins and in mineral (2,5 kg of alfalfa hay represents 1 UF) (**Bouaboub -Mousab., 2012).** *M. truncatula* is a potential grazing cultivation that products high fodder level of good quality. A study carried out in South-East of Wyoming during 3 years, on three cultivars of ***M truncatula*** which are ***Calife, Mogul et Paraggio,*** revealed that average yield in dry matter was respectively of 1**.6, 3.1 et 4.0 t /ha (Babita., 2008).**

The first researchers' objectives have been to study kinds of ***Medicago, Trifolium, Scorpiurus, Hedysarum and Onobrychis*** **(Abdelguerfi., 2000),** They showed a positive correlation between bio geographical parameters, particularly altitutde and rainfall, and biological aspects linked to the growth and reproduction – flowering – inflorescences, pods and seeds. The 2/3 of Medicago's genus species (they are gathered under the term of medicKs). Originating of Mediterranean zone, are particularly adapted to drought conditions: Australia, Chile, California…**Piano et Talamucci, 1996).** Thanks to accumulation of dry grains, MedicKs' meadow regenerates between two cereal crops even after several years. This system is applied in various dry cereal areas: Maghreb, Middle East, Spain… (SAREP. 2013).

Diversification of steppe pastures by a combination of medicKs 'species allows increasing a chance to succeed in their plantation. If one of the cultures dies due to the drought or to the pests, another culture can allow saving the pasture. This allows to put in place pastures more sustainable and increasing soil security. In a well drained soil, sprigs of medicKs can contribute to increase soil organic matter. So, by improving soil health, pulses also help to biodiversity of micro-organisms present into the soil. They can biologically fix more than 350 kg of nitrogen by hectare and by year (FAO., 2015).

The production of pulses is a water-saving, notably if we compare it to other protein sources, such as for instance: Indian dal (split peas, lentils) which necessitates 50 water liters by kg. Conversely, it is needed 4.325 water's liters to product 1 kg of poultry, 5.520 water's liters to product 1kg of mutton and 13.000 water liters to product 1 kg of beef. The low water footprint of pulses is an intelligent cultivation choice in areas and in arid regions, subject to drought (**FAO. 2015**). Thanks to their hard grains and

http://dx.doi.org/10.22161/ijeab/2.1.62 *ISSN: 2456-1878*

drought tolerant, roots can enter 5 feet (1.524m) in depth to maintain soil in place and value drops of water (SAREP, 2013). Much constraint prevents annual alfalfa extension, local or introduced in Algeria.

However, we have been interested through two testing years in pods yield and to seeds production of about 20 populations belonging to species of *M.intertexta M.ciliaris, M.polymorpha , M.Truncatula* and a population introduced belonging to *M.muricoleptis* and so an Australian cultivar (Jemalong).At the same time, we have tried to approach determining parameters of this production which is: (i) green and dry matter (ii) width of ramifications (iii) weight of 50 pods and their seed (iiii) and number of stems by square meter. These data are thus, very interesting because during dry period, from June to October, pods constitute an available food for animals.

II. MATERIAL AND METHODS

The first trial is set on 13/12/2012 and the second on 24/12/2014 at the experimental farm of National Superior School of Agronomy, located in Algiers City.This station is at 30° 8' of longitude and latitude of 36°43' north and altitude of 48m.The previous cultural element of the first essay was corn, and for the second one was a fallow. The essay is a full random block with three repetitions. In each block, we have 20 populations for the first essay and 22 populations for the second one, represented each one by four lines of 1 meter with interval of 60cm with 80 grains by population and by line. Every block is spaced from other of 1.50m for the first essay and of 60cm for the second one.

The chemical-physics analysis of the soil was carried out on samples harvested on the plot before seeding, at depths of 20cm and 40cm. At the first essay the texture is silty (soils texture triangle), the soil is rich in calcium and low in nitrogen and organic matters, potassium and sodium. As for the second essay, texture is silty-clayey, low in nitrogen and in organic matter, a pH>7,23 (analysis carried out in Department of Soil Science of ENSA in 2013 and 2015.

Climatic conditions of the experiment period of the first essay 2013 gave a monthly temperature, the highest in May, with an average of 17.8°c with maximal of 23°c. The coldest month was February with an average of 11.5°c. The coldest day recorded 5.9°c. The highest rainfall amount recorded was of 137cm in three days. As for year 2015, the most rainy month was January with 98,9 mm and the hottest month was August with an average of 38.4°c. The maximum and minimum of temperature was also recorded in August with 39°c and 1.2°c (The agro-meteorology of ENSA, 2013 and 2015).The plant material used which comes from collection of the school is shown in table 1.

Table.1: Code and origins of the populations studied and the average weight of 1000 seeds

Species	Populations' code	Weight of 1000 grains	Origins
M.truncatula	Tr_{201},Tr_{407} Tr_{238} Tr $_{334}$	3,58g	Algeria (2004)
M .intertexta	I_{756} I_{107} I_{4O7} I_{11} I_{31} I_{52}	15,77g	Algeria (2004)
M.ciliaris	S_3 C_2 C_{204} S_5 S_{15} S_7 C_{11} C_{58}	13,21g	Algeria (2004)
M .polymorpha	poly $_{27}$ Poly $_{205}$	3,24g	Algeria (2004)
M.muricoleptis	Aus_{106}	6,26g	Turkey (2004)
M.truncatula CV Jemalong	CV	3,9 g.	Algeria (-)

Softwares used for statistical analysis are: Stat View and Statistica.

III. RESULTS AND DISCUSSION

Yields in dry matter of population vary between 1132,1g/m² in I_{52} and 38,85 g/m² in Tr_{221} en 2013 while in 2015 they vary between 453,33g/m² in I_{31} and 12,56g/m² in S_{15}. The average of 20 populations in 2013 is of 457,79 g/m² against 127,41g/m² in 18 populations in2015. Jemalong cultivar records a yield of 12,8g/m^2. In region of Tunisia yields in gram by square meter of dry matter (DM) of *M.polymorpha* vary from 84 to 530, *M.truncatula* varies from 31 to 450 while Jemalong CV witness varies from 0 to 340. The average of six species of annual alfalfa varies from 102 g/m² to 483g/m² (**Seklani et Hassen., 1990 in Seklani et *al*., 1996.).**

En Alaska, yields of 339 to 384 g/m² of DM have been obtained in average on two doses of fertilizer N, 0 and 90 kg /ha, on a neutral ground **(Panciera et Sparrow., 1995).** The average of ramifications width of populations varies between 13cm in Tr $_{334}$ and 44cm in I_{52} Jemalong cultivar gives an average of 17cm. Number of stems by square meter varies between 136 chez C_{204} and 420 chez I_{52}. Number of plants by square meter varies between 112 to 44; cultivar Jemalong gives a number of 48 (Tab. 2).

Generally, it is well known that leaves-stems ratio is a good indicator of the forage quality and offers a potential for cultivars selection, this last one varies at the beginning of flowering in 2015 between 5 in population of S_5 and 1.03 in population of Tr_{334}. In *M.truncatula,* it varies of 1.03 in Tr_{334} and 2,81 in Tr_{238} while in Jemalong cultivar species, it gives a ratio close of the latter (2,8) (Tab.2) while in 2013, it varies between 0,5 in $Poly_{205}$ and 2,91 in Tr_{407}. **Porquiddu (2001)** records lower values in *M.polymorpha cv***.** Cercle Valley that *M truncatula*. cv. Chypre et *M. Tornata* cv. Dornafield with respectively 0.97, 0.80 and 0,76. A decrease of this ratio is observed during pods and grains formation probably linked to their high demand for products of photosynthesis with presence of species interaction x age **Derkaoui et *al*., 1990).** The percentage of viable plants at early flowering stage varies in 2013 between 37,08% in Tr_{334} and 19,16% in Tr_{55}. Average between species varies between 24.06% in *M.polymorpha* and between 29.32% in *M.intertexta*. In 2015 this same parameter (Tab.2) varies between 0% in *M.polymorpha* and most of *M.truncatula* reaches 35% in *M.intertexta*, Jemalong cultivar offers a viability of 15%. We can say that both species (*M.truncatula , M.intertexta)* are more resistant to abiotic stress than other both species *(M.plymorpha, M.truncatula)* probably due to the grains size.

The Fisher's test offers homogeneous groups for biometrical parameters of forage production (Tab.2). By contrast, for production of pods, no group is formed. The growing cycle parameter of the plan offers seven groups which overlap, except for populations Tr_{27} and I_{52} where there is not overlapping. As for matter, green, dry and width of ramifications, population I_{52} is individualized from other groups. The LS ratio in dry, forms with population S_5 the homogeneous group *a* and population I_{52} and Tr_{334} form another group d which did not overlap with other.

Table.2: Average of biometrical parameters studied for production of dry matter in MedicKs populations studied.

Populations	Cycle in days	Number of plants by m²	Number stems by m²	Width in cm	MV g/m²	MS g/m²	F/T Sec	Perc of viability
S_5	110fg	70,67abc	209,33 abc	18ef	160efg	53,79 def	4,5a	22,08
S_{15}	110g	66bc	154 c	13,5ef	91,2efg	12,56 def	2,43bcd	20,625
C_2	114efg	100bc	244 bc	19,5ef	129,613	27,253 def	4,02bcd	31,25
S_7	115efg	64bc	260 abc	22,25ef	205,46 efg	37,06 def	3,16bcd	20
S_3	116def	44bc	156 bc	20ef	80efg	320def	5b	13,75
C_{11}	116def	64abc	276 bc	18ef	244,4efg	47,68 def	3,21bc	20
C_{204}	116def	56abc	136 bc	23ef	158,04 efg	33def	3,64bcd	17,5
C_{58}	120,33cd	54,67bc	189,33 bc	20,33def	252,87 Defg	91,567 cde	2,33bcd	18,125
CV	118de	48c	116 c	17ef	78,96	12,8 def	2,8cd	15
Tr_{238}	117de	76ac	312 abc	18,5ef	256,54 efg	74,06 def	2,81bcd	23,75
Tr_{334}	128 ,33ab	70,67bc	209,33 bc	13f	80fg	51,72f	1,03d	18,125
Tr_{27}	130a	28bc	172 c	19ef	140efg	36bef	1,28cd	18,500
I_{253}	124bc	112a-	420 a	38,33abc	1072abc	196ab	2,07bcd	35
I_{756}	124,67bc	94,67ab-	353,33 ab	36abcd	749,33 abcd	141,33 abc	1,94bcd	23,88
I_{107}	124,33	60bc	314,67 abc	40,67	942,67	174,67	1,66cd	25,79

	bc			ab	ab	Ab		
I_{11}	126ab	86,67ab-	332 ab	27cde	41,5cdef	166	2,53bcd	27,083
I_{58}	126,67ab	61,33bc	248 abc	29,67bcde	530,67 bcde	117,33	1,55cd	19,167
I_{31}	126,67 ab	74abc	290bc	28,33 abc	113,33 abc	453,33 ab	1,32cd	25
I_{52}	130a	86,67ab-	420a	44a	1277,06a	260a	1,11d	27,08

Our yields pods in littoral region in 2013 vary between 79.88g/m^2 in poly$_{27}$ and 3637.33g/m^2 in I_{107} (Alane et *al*., 2014)**.** Values of the second essay (2015) are different, they vary between 40,89g /m² in Poly$_{236\ and}$ 464,36 g/m² in I_{11}. The cultivar Jemalong offers a yield of 172.4g/m2, if we compare it to local species of *M.truncatula*, population $Tr_{238,}$ it gives a higher yield of 247,51 g/m² (Tableau 3) while yield of pods obtained by l'ITGC in 1977in sub-littoral zone was of 10g/m^2. The results comparison of both experiment years show a different yield: for instance year 2013, yield of both quoted populations (Aus_{106} 944g/m², I_{11} 2428, 444g/m²) is superior to that of year 2015 (AUS $_{106}$13g/m², I_{11}464, 36 g/m²). Furthermore, some populations as Poly$_{27}$ have not germinated. This result may be explained by difference of weather conditions between both years of experiments. Soil of the first essay is well fertilized having experienced several essays on medicKs, thus well provided in rhizobium, unlike to soil of the second essay which was a fallow during several years. In addition, climatic data of the first essay record an abundant rainfall compared to that of the second test.

Yields in seeds corresponding to performances in pods produced (g/m²), also show for the same reasons, a low production in 2015 compared to 2013. Since they vary in 2013 between 21,533 g/m² in Poly$_{27}$ and 900,375g/m² in I_{107} while introduced population Aus_{106} gives a yield of 359,077 g/m² (Alane et *al*., 2014). In 2015 the essay gives in seeds yield which varies between 425, 67g/m² in S_7 and of 5,71g/m² in I_{52} (Table.3). According to **Laouar et *al* (2000)** *M .ciliaris* is earlier and products more plant/pods but with a low number of pod/grains, contrary to *M intertexta.*

However, according to results of our essays, we confirm precociousness of *M.ciliaris's* populations (Fig.1), but for production of seed, yield of *M.intertexta* depends of annual weather conditions since in 2013, average yield of M.intertexa exceeds that of *M.ciliaris* (S :354,602g/m², I :821,997g/m²) while in 2015 the contrary occurred (S: 106,20575g/m², I : 55,215 g/m²).Jemalong cultivar gives a yield of 40,936g/m². This also has been observed in clover by Clark (2014) who noticed that cycle of clover varies from 100 to 126 days after seeding depending of the place, and of timing of sowing, and so of cultivars used. The introduced population in our essay Aus_{106} gives the lowest yield 4,51g/m² (Table 3).

For information purpose, populations with early flowering have trend to belong to drier habitats and the hottest, and numerous authors found similar results in several other annual pulses (**Graziano et *al,* 2010).**

Furthermore, population's origin influences directly on weight of 1000 grains where itself influences on yield. In effect, populations which come from areas with more annual rainfalls had more small pods and grains at upper sizes than of dry environments **(Graziano et *al*., 2010).** These results are confirmed by **Del Pozo et *al* (2002)** who affirm that sizes of grains and pods have an adaptive value. Nevertheless, the grains performance may be reduced through over-grazing, freeze, and drought. (**Muir et *al*., 2005).** Since 70% of feedings have been insured by pods of MedicKs, proportion decreased rapidly to zero when green material becomes available after rain **(Porqueddu., 2001).** Much of leguminous fodder species are depending to permanent grassland **(Duc et *al*., 2010).** Weight of grains constitutes a compensation mechanism to support grain yield under impact of the drought (**Yousfi et *al*., 2012).**

Table.3: Average of biometrical parameters studied for production of pods and seed in medics' populations studied.

Populations	**Weight of 50 pods**	**Weight of seed of 50 pods**	**Yield in pods g/m²**	**Yield of seed g/m²**
S_5	12,1 3	2,98	254,8	63,72
S_{15}	9,64	2,89	142,54	42,27
C_2	13,64	3,38	345,8	87,01
S_7	15,62	3,84	425,67	425,67
S_3	13,28	3,58	245,9	66,29
C_{11}	12,97	3,42	241,2	60,72

C_{204}	17,08	4,85	163,47	46,25
C_{58}	9,71	2,74	392,93	57,75
CV Tr	1,99	0,47	172,4	40,94
Tr_{407}	3,32	0,74	66,567	16,18
Tr_{201}	6,43	1,56	170,8	41,42
Tr_{238}	5,87	1,524	247,51	65,25
Tr_{334}	4,99	1,40	113,3	31,96
I_{755}	13,46	3,19	86,133	20,73
I_{756}	17,76	4,79	320,8	86,17
I_{107}	18,27	4,24	283,53	71,58
I_{11}	17,82	3,70	464,36	97,07
I_{31}	16,53	4,023	197,3	50,03
I_{52}	21,53	36,80	175,45	5,71
$Poly_{205}$	4,76	1,16	75,6	36,80
$Poly_{236}$	1,18	0,29	40,89	9,97
AUS $_{106}$	3,353	1,16	13	4,51

Variance analysis of dry matter production parameters, shows great significance **(p<0, 0001)** (Table 4). The correlation matrix of these parameters for a ddI of 48 is reported in table (5). Parameters' variance analysis of pods yield and populations 'seed studied also show a highly significant difference (p**<0,0001)** (**Table 6).** Correlation matrix of these parameters for ddI of 68 is reported in table (7).

Table.4: Univariate analysis of dry matter production parameters

Parameters	Variance	DDL	Chi2	P	95%inf	95%sup
Number of plant	47,930	56	2684,097	<0,0001	36,043	67,437
Number of stems	896,770	54	48425,602	<0,0001	671,150	1270,472
Width (cm)	123,931	55	6816,214	<0,0001	92,976	174,963
L/S (green)	49,077	56	2748,304	<0,0001	36,906	69,051
L/S(dry)	13,896	56	778,173	<0,0001	10,450	19,551
GMg/m²	177410,971	56	9935014,349	<0,0001	133412,622	249615,463
DMg/m²	6283,627	55	345599,462	<0,0001	4714,124	8871,072

Table.5: Correlation matix of dry matter production parameters

	Number of plant	**Number of stems**	**Width of ramifications**	**Rapport L/S(dry)**	**GM g/m²**	**DM g /m²**
Number of plant	1					
Number of stems	0,685***	1				
Width of ramifications	0,188	0,481***	1			
Rapport L/S(vert)	0,256	-0,139	-0, 314*			
Rapport L/S(sec)	0,127	-0,167	-0,291*	1		
GM g/m²	0,447***	0,772***	0,862***	-0,218	1	
DM g /m²	0,401***	0,687***	0,788***	-0,177	0,934***	1

Table.6: Univariate analysis of pods yield parameters

Parameters	**Variance**	**DDL**	**Chi2**	**p**	**95%inf**	**95%sup**
Weight of 50 pods	680,096	69	46926,641	<0,0001	524,958	922,214
Seed of 50 pods	7,303	69	503,884	<0,0001	5,637	9,904
Weight of collected Pods	10029,572	69	692040,458	<0,0001	7741,706	13601,626

Seed of collected pods	3778,920	69	260745,462	<0,0001	2916,903	5124,790
Yield of pods in g/m²	27789,048	69	191744,294	<0,0001	21450,032	37686,179
Yield of seed in g/m²	1754,650	69	121070,833	<0,0001	1354,393	2379,572

Table.7: Correlation matrix of dry matter production parameters

	Weight of 50 pods	**Seed of 50 pods**	**Weight of collected pods**	**Seed of collected pods**	**Yield of pods g/m²**	**Yield of seed g/m²**
Weight of 50 pods	1					
Seed of 50 pods	0,925***	1				
Weight of collected pods	-0,091	0,085	1			
Seed of collected pods	0,043	0,187	0,584***	1		
Yield of pods g/m²	0,203	0, 346**	0,749***	0,535***	1	
Yield of seed g/m²	0,203	0,368***	0,690***	0,534***	0,973***	1

Number of homogeneous groups given by Fisher's test for green matter production parameters at early flowering is shown in table (2). No homogeneous group is given for pods production parameters.

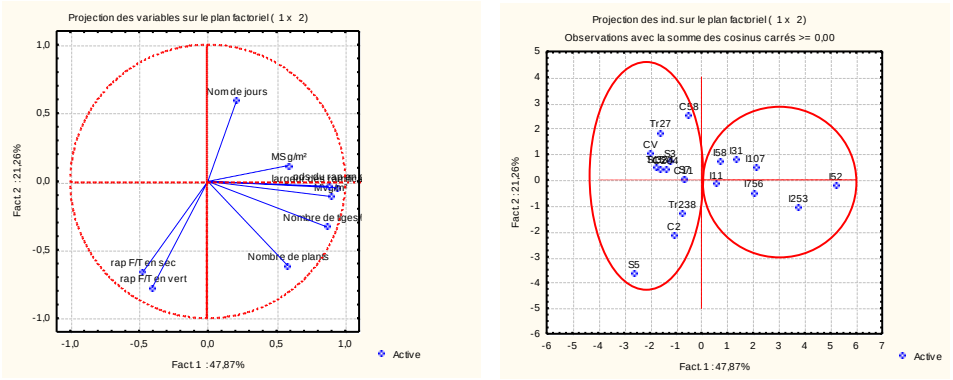


Fig.2: Principal Components Analysis (PCA) of dry production parameters

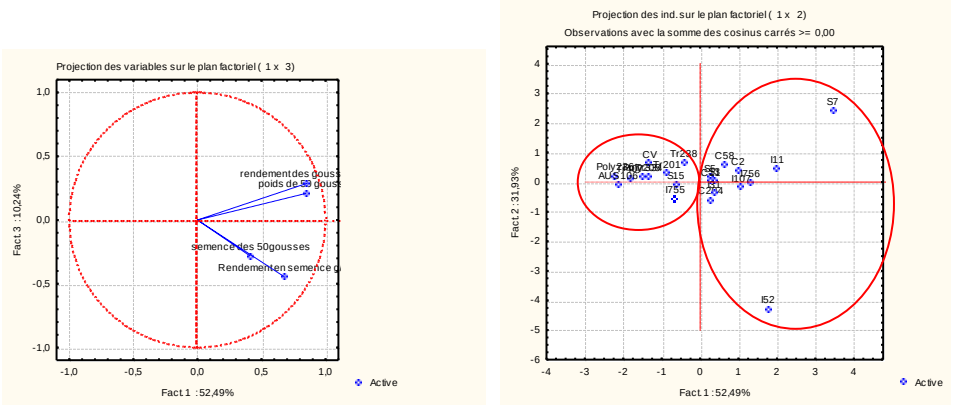


Fig.3: Principal Components Analysis (PCA) of pods production parameters.

Principal Components Analysis (PCA) led us to the study of Kaiser Criterion, this one offers us for dry matter production parameters, three designs (1,2), (1,3), 2,3) and in elbow criterion, we observe an important fall from the first axis (of 47 ,87% to 8,96% of inertia). We retained the first factorial design (1 and 2) (Figure 2) which gives information of 69.13% (47,87 + 21,26 = 69,13%) parameters that are close to circle are GM(g/m^2), width of ramifications, weight which served to the ratio determination, number of stems, number of plants and

ratio in green and dry, thus they are effectively well correlated with the two factors used for this design (F1 and F2), by contrast, dry matter (DM/m^2) and cycle of the plant in day number are less near to the circle.

The first information axis, whereby is preserved by projection of maximum initial dispersion of points of the cloud. All variables occupy a fairly narrow zone inside of the correlations circle. Maximum angle between two variables is below 90°. This suggests that all variables are positively correlated between them. So, two large populations groups are formed, the first one based on parameters : GM, DM, width of ramifications, weight served to ratio determination, number of stems containing *M.intertexta* (I_{31},I_{11},I_{107},I_{58},I_{756},I_{253},I_{52}) the second group containing *M.ciliaris* and *M.truncatula* (*S_3,S_5,cv,C_{204}, C_{11},Tr_{27}, Tr_{334}, Tr_{238}, C_2,C_{58})* are determined by parameters ratio leaves/stems in green and in

For pods production, the Kaiser Criterion, led us to retain two axes and in elbow criterion, we observe an important fall from the first axis (de 52,49% to 10,24% of inertia). In the first design, all parameters are close of the circle, thus, they are effectively well correlated with both factors constituting this design (F1 and F2). The first factorial axis gives (52,49%+ 31,93%=84,42%), axis that is preserved , by projection of maximum initial dispersion of cloud points. All variables occupy a rather limited area inside of the correlations circle Maximum angle between two variables is below 90°. This suggests that all variables are positively correlated between. And so, two populations groups are formed. The first group determined by all parameters studied includes only both species : *M.ciliaris* and *M.intertexta* (S_7,S_5,I_{52}, C_{58},S_3,I_{756},C_{11},C_2,C_{204},I_{107},I_{11}) while the second group is a mixture of species examined *M.ciliaris* , *M.intertexta*, *M.truncatula* ,*M.polymorpha*, *M.granadensis* ($poly_{236}$, Aus_{106}, Tr_{106}, Tr_{407}, CV ,$poly_{205}$, Tr_{334}, Tr_{201}, Tr_{238},S_{15},I_{755}).

IV. CONCLUSION

Evaluation of genetic material of medics based on agronomic aspects should take into account of appropriate variability for some characteristics linked to fodder and to production of pods. The results of comparison of both years of experimentation show a different yield: that of 2013 is higher than of 2015. Some populations had not germinated in the last essay. Weather conditions strongly influence on quantity of dry matter produced and also on yield of pods and seed. Both species (*M.ciliaris, M.intertexta)* are more resistant to abiotic stress than other both species *M.plymorpha, M.truncatula)* probably due to the grain's size. *M.ciliaris* are earlier than other local species but for seed production, yield of *M.intertexta* depends of annual weather conditions since in 2013 average yield of *M.intertexta* exceeds that of *M.ciliaris* (S :354,602g/m², I :821,997g/m²) while in 2015 the opposite happened (S: 106,20575g/m², I : 55,215 g/m²). Moreover, this is the last ones which lose their leaves and their greenery until half of June and have a broader land use than other species.

Jemalong cultivar is earlier than all populations studied, but it records a low yield in dry matter and in pods. Variance analysis of dry matter production parameters shows a very high significance (p<0, 0001). Principal components analysis (PCA) of dry matter production parameters forms two large populations groups: the first based on parameters: green matter (GM), dry matter (DM), width of ramifications, weight served to the ratio determination, number of stems containing only in *M.intertexta* (I_{31},I_{11},I_{107},I_{58},I_{756},I_{253},I_{52}), the second group containing *M.ciliaris* and *M.truncatula* (S_3,S_5,cv,C_{204}, C_{11},Tr_{27}, Tr_{334}, Tr_{238}, C_2,C_{58}*)* are determined by ratio parameters leaves/stems in green and in dry. Therefore, parameters of pods production form two other populations groups. The first group determined by all parameters examined comprises only both species *M.ciliaris* and *M.intertexta* (S_7,S_5,I_{52}, C_{58}, S_3,I_{756}, C_{11},C_2, C_{204}, I_{107},I_{11}) while the second group is a mixture of species examined *M.ciliaris* , *M.intertexta*, *M.truncatula* ,*M.polymorpha*, *M.granadensis* ($poly_{236}$, Aus_{106}, Tr_{106}, Tr_{407}, CV ,$poly_{205}$, Tr_{334}, Tr_{201}, Tr_{238},S_{15},I_{755}).

Results of medics seed production depend on weather conditions in littoral area, of altitude lower than 600/700m. In steppe regions, it is necessary to make trials in situ with these known populations. Mixture of population seeding with small and large grains: will give a better ecological balance and in the same time a high nutritional value. Finally, to control seed population of annual alfalfa (Medics) some number of significant barriers must be overcome: seed bed, maintenance, operation, cultivation through grazing, are determinant on results of pods harvesting. This is particularly delicate and requires an appropriate material.

REFERENCES

[1] Abdeguerfi.A . (2002). ressources génétiques d'interêt pastoral et /ou fourrager : Distribution et variabilité chez le légumineuses spontanées (Medicago, Trifolum, Scorpiurus, Hedysarum et Onobrychis) en Algérie :thése doctorat INA .433p.

[2] Alane.F., Chabaca.R., Abdelgherfi-Laouar and M,Abdelgherfi A. (2015) . Seed Potentialities Of Medics In Subhumid ZoneWith A View To Use Them In Steppe Zone.International Journal of Agriculture and Crop Sciences. Available online at www.ijagcs.com IJACS/2015/8-2/181-186 ISSN 2227-670X ©2015 IJACS Journal

[3] Babita Thapa .(2008).Understanding cold acclimation in Medicago truncatula". Graduate Theses and Dissertations. Paper 119 40. http://lib.dr.iastate.edu/cgi/viewcontent.cgi?article=2970&context=etd

[4] Bouaboub- Moussab .K.(2001). Comportement des variétés et des populations de la luzerne pérenne *Medicago sativa* L. dans la région de d'Adrar. thèse de magister INA El Harrach. 152p.

[5] Clark, Shawnna. (2014). Plant guide for bur clover (*Medicago polymorpha* L.) USDA-Natural Resources Conservation Service, Big Flats Plant Materials Center, corning New York..

[6] Del Pozo .A., Ovalle.C., Aronson.J., Avendaño.J. (2002) . Ecotypic differentiation in **MEDICAGO POLYMORPHA** L. along an environmental gradient in central Chile. II. Winter growth as related to phenology and temperature regime .Volume 160, Issue 1, pp 53-59

[7] Derkaoui.M ., Caddel .J.L. and Stroup, W.W.(1990). Biomass partitioning and root development in annual *Medicago* spp. *Agr. Med.*, 120: 407-416.

[8] Duc. G., Mignolet . C., Carrouée .B., Huyghe.C. (2010) . Importance économique passée et présente des légumineuses : Rôle historique dans les assolements et facteurs d'évolution. Innovations Agronomiques 11 (2010), 1-24

[9] FAO. (2015). 2016 Année internationale des légumineuses: des graines pour nourrir l'avenir. Brochure

[10] Graziano. D., G. DiGiorgio, P. Ruisi ., G. Amato and D. Gianbalvo (2010). Variation in pheno-morphological and agronomic traits among burr medic (*Medicago polymorpha* L.) populations collected in Sicily, Italy. Crop and Pasture Science. 61:59-69.

[11] ITGC (Institut de développement des grandes cultures). (1976). Résultats et perspectives d'extension de la luzerne annuelle(Medicago).Revue céréaliculture n °1 .28-31

[12] James .P. Muir , William.R., Ocumpaugh and Twain J. Butler .(2005). Trade-Offs in Forage and Seed Parameters of Annual *Medicago* and *Trifolium* Species in North-Central Texas as Affected by Harvest Intensity . American Society of Agronomy. American Society of Agronomy https://www.agronomy.org/

[13] Laouar . M..,Abdelguerfi .A. and Kouchi K. (2000) . Etude du complexe d'espèce *Medicago clliaris-M.inlertexta* variabilité morphologie et phénologique. Annales de f1nstitut National Agronomique - EI-HamJch - Vol .21, N" 1 et 2, 2000 5 1

[14] Nasreddine.Yousfi.,Ines. Slama.,Chedly.,Abdelly.(2012) . Phenology, leaf gas exchange, growth, and seed yield in contrasting *Medicago truncatula* and *Medicago laciniata* populations during prolonged water deficit and recovery . Botanique, 90(2): 79-91, 10.1139/b11-093

[15] Panciera . Sparrow., Panciera. MT., Sparrow .SD. (1995) . Effects of nitrogen fertilizer on dry matter and nitrogen yields of herbaceous legumes in interior. Alaska. Can J Plant Sci 1995

[16] Piano . E. and Talamucci,. P. (l996). Annual self-regenerating legumes in Mediterranean areas. *Grassland Science in Europe*, I, 895-909. ERSA.

[17] Porqueddu .C. (2001) .Screening germplasm and varieties for forage quality: Cons traints and potentials in annual medics *in* Delgado I. (ed.) , Lloveras J. (ed.) Quality in lucerne and medics for animal production .Zaragoza : CIHEAM Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 45 pages 89- 98

[18] SAREP. (2013). SAREP Cover Crops. 2013. Burr Médic. http://www.sarep.ucdavis.edu/database/covercrops (accessed 3 Feb 2013)

[19] Seklani.H ., Hassen .H (1990). Contribution à l'étude des espèces spontanées du genre *Medicago* en Tunisie. Ann. INRAT **63**: 3-15 . In Seklani H., Zoghlami A., Mezni

[20] M .., Hassen .(1996.) Synthèse des travaux de recherche réalisés sur les Medicago à l'Institut National de la Recherche Agronomique de Tunisie. in Genier G. (ed.),Prosperi J.M. (ed.). The Genus Medicago in the Mediterranean region: Current situation and prospects in research Zaragoza: CIHEAM Cahiers Options Méditerranéennes; n. 18 1 . pages 31-37

http://dx.doi.org/10.22161/ijeab/2.1.63 *ISSN: 2456-1878*

Assessment of Drumstick Tree (M. deifera) Accessions for Genetic Diversity in the Southern guinea Region of Nigeria

Vange T.[2], Jibung G.G.[1], N.I.Odiaka[3]

[1]Plateau State College of Agriculture, Garkawa, Plateau State, Nigreia.
[2]Department of Plant Breeding and Seed Science
[3]Department of Crop Production
Federal University of Agriculture Makurdi, Benue State, Nigeria.

Abstract— *An experiment was conducted to analyze the genetic diversity among 9 drumstick tree (Moringaoleifera) accessions in the Teaching and Research Farm of the University of Agriculture Makurdi. The experiment was laid out in a Randomized Complete Block Design (RCBD) replicated three times. Data were recorded on growth and yield characteristics before and after pruning. The result obtained showed that at 18 weeks after transplanting, accession UAM-NI had the tallest plants (3.63m) while UAM-BE had the shortest mean plant height (2.84m) under no pruning. Other parameters that showed significant differences were number of leaves per tree and stem diameter. Although accession UAM-OY recorded highest fresh (220.22g), dry (113.42g) and leaf powder (82.60g) weights, it was not significantly different from other accessions. However, at 18 weeks after pruning, there was a significant difference among the accessions with regard to leaf length. Although accession UAM-NA recorded highest fresh leaf weight (286.60g), dry leaf weight (90.67g) and leaf powder weight (85.60g), it was not statistically different from other accessions. For the pruned accessions, significant differences were recorded in leaf length, number of flowers/tree, days to podding and fifty percent podding, pod length, pod girth, pod weight, number of seeds/pod, number of seeds/tree and 100seed weight. The result also indicated that the pruned accessions recorded higher leaf yield than the unpruned. The result of the cluster analysis grouped the accessions into two clusters and an outlier both for the pruned and unpruned accessions irrespective of area of collection.*

Keywords— Drumstick, accession, cluster, pruned, unpruned, outlier.

I. INTRODUCTION

Drumstick (*Moringaoleifera*L.) is believed to have originated from Northern India and has been distributed world wide in the tropics and sub-tropics (Olson, 2002). It is one of the thirteen species belonging to the family moringaceae with only one genus, *moringa*. In West Africa, the family is represented by 10 species while in Nigeria, the plant is represented by the only species of *Moringaoleifera* (Keay, 1989). Drumstick (*Moringaoleifera*) is commonly named horse-radish tree, drumstick tree, mothers best friend, Indian ben among others. It is a fast growing drought resistant tree that is widely adaptable to the tropics and subtropics (Olson, 2002). Its fast growing attributes coupled with its ability to grow on marginal soils has particularly made drumstick an invaluable vegetable and fodder crop especially during the dry season (Price, 2007; Rajangam*et al.,* 2001; Foidl*et al.,* 2001). *Moringaoleifera*is considered one of the world's most useful trees as almost every part of the plant is useful in one way or the other. It is a multipurpose plant with a tremendous variety of potential uses and recently attracted the attention of several authors (Abubakar*et al.,* 2011).Thus *Moringaoleifera*could be useful in alley farming, animal forage, vegetable, biogas, dye, medicinal, water purification, edible oil (Foidl*et al.,* 2001, Rajangam*et al.,* 2001). The leaf of this plant is known to be rich in micro-nutrients and vitamins such as zinc, iron and vitamin A and thus, has been use in the treatment of malnutrition in children and in the improvement of the diets of lactating mothers in some African countries. The oil extracted from the seeds is used in lubricating delicate machines, cosmetics, perfume and pharmaceutical industries. Foidl*et al.,* (2001) has reported on the possibility of using the ben oil as a biofuel considering the high cost of crude oil in the international market. However, in the aspect of water purification, Aho and Agumba (2010), Foidl*et al.* (2001) had

reported that up to 99% of colloids can be removed from dirty (turbid) water and works more effectively than the imported alum (aluminiumsulphate). It has been documented that the drumstick plant have a lot of pharmaceutical properties for the control of terminal ailments such as high blood pressure, diabetes, typhoid, rheumatism and asthma due to the presence of antioxidants (Rajangam*et al.*, 2001, Foidl*et al.*, 2001).

However, despite the numerous economic significance of this crop, there is no evidence that it has benefited from adequate research and agronomic management attention that would promote its cultivation among the local farmers for increase yield. At present, its production in Nigeria is still in the hands of peasant farmers who cultivate it in home gardens and the few accessions under cultivation in Nigeria have not been fully characterized. Therefore, to wean its production from hands of the resource-poor farmers and integrate it into the commercial scale agriculture, knowledge of the extent of genetic variability in the population and the magnitude of genetic diversity in the accessions is extremely important. Similarly, Christopher (2010) had earlier reported that *moringa* trees should be trimmed in order to promote branching, increase yield and facilitates harvesting. If left to grow without pruning, the plants will grow straight and tall producing leaves and pods only on the primary stem. According to Palada and Chang (2003), pinching of drumstick trees at 1.0 – 2.0m height enhances production, controlled tree height and takes only three (3) weeks for the tree to be ready for a leaf harvest after the pruning. Thus, the present study was aimed at assessing the yield (leaf) performance of the moringa accessions under pruned and unpruned in order to ascertain the degree of similarity among the different accessions so as to establish their breeding potentials.

II. MATERIALS AND METHODS

The experiment was conducted at the Teaching and Research Farm of the Federal University of Agriculture Makurdi, Benue State. Makurdi is located on latitude 07^{o} 41'N, Longitude 08^{o} 37'E and altitude of 106.4 m above sea level. The experiment consisted of 9 accessions of drumstick seeds collected 9 different states of the country including Nasarawa, Benue, Kogi, Oyo, Kebbi, Niger, Adamawa, Abuja and Akwa-Ibom States. For the sake of identification, acronyms were used (Table 1).

The treatments were laid out in a Randomize Complete Block Design(RCBD) with three replicates in a 3m x 3m spacing as described by Patricio *et al*.(2011).Six weeks old seedlings were transplanted to the the field according to Palada and Chang(2003). Data were taken from two plants randomly selected and tagged in each plot on a weekly basis as follows.

Plant height (cm):Heights of two plants from each plot randomly selected were measured with a measuring tape from the base of the plant at soil level to the apical tip.

Number of branches/plant*:* These were taken by counting the number of branches in the selected plants on weekly bases.

Stem diameter (cm):The stems of the selected plants were measured at soil level using verniercalliper on weekly bases.

Table.1: Locations of M. oleifera accessions collected for yield performance

S/No	Accession Code	State	Location	Latitude	Longitude
1	UAM-NA	Nasarawa	Kolo	8^0 48´N	7^0 33´E
2	UAM-BE	Benue	Makurdi	7^0 41´N	8^0 37´E
3	UAM-KO	Kogi	Ankpa	7^0 30´N	6^0 42´E
4	UAM-OY	Oyo	Idere	7^0 23´N	3^0 55´E
5	UAM-NI	Niger	Kontagora	10^0 24´N	5^0 28´E
6	UAM-KE	Kebbi	Zuru	11^0 26´N	5^0 13´E
7	UAM-AD	Adamawa	Yola	9^0 14´N	12^0 18´E
8	UAM-AB	Abuja	Kuje	9^0 28´N	7^0 25´E
9	UAM-AK	AkwaIbom	Uyo	5^0 05´N	7^0 39´E

Number of leaves/plant*:* The numbers of leaves of selected plants were counted on weekly bases.

Leaf Length (cm):Lengths of the leaves selected on each plot were measured and the means were determined in centimeter on weekly bases.

Forty percent(40%) of total number of leaves on each selected plant were harvested at 18 weeks after transplanting and pruning respectively as described by Freer,(2006) and the fresh, dried and leaf powder weights were recorded. Pods were equally harvested from the selected plants as they dried and turned brown.

After trimming the trees at 2m height ,data were equally collected on number of branches/tree, stem diameter, number of leaves/ tree, leaf length, number of flowers/tree, number of pods/tree, pod length, pod girth, pod weight,

number of seeds/pod, number of seeds/tree and 100-seed weight.

The data collected were subjected to Analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to separate between significant treatments means (Singh and Chaudhary, 1979). Test of significance was at 5% probability level. Cluster Analysis was also computed according to Singh and Chaudhary (1979) using SAS 2000.

III. RESULTS AND DISCUSSION

Choice of parents for developing base population is a crucial step in plant breeding since it largely predetermines the outcome of subsequent selection steps in breeding programmes. The analysis of genetic diversity and the relationships among germplasm therefore, facilitates the selection of parents with diverse genetic background (Subramanian and Subbaraman, 2010). The result of this study showed that significant variations exists among the accessions in many of the characters studied. For the unprune, accessions, UAM-NI produced the tallest (3.63m) plants while UAM-BE(2.84m) produced the least. Accession UAM-OY recorded the highest (96) number of leaves/tree as UAM-NA had the least (37). On number of branches/tree, Accession UAM-OY recorded the highest (12.33) as the least (4) was recorded by UAM-NA. Other parameters which equally exhibited significant differences were stem diameter, days to flowering and days to fifty percent flowering (Table2). The result of the scatter plot analysis for the unpruned drumstick accessions therefore, classified the nine accessions into two broad groups, cluster I and cluster II and an outlier with distinct genetic potentials (Figure 1). The result further revealed that cluster I comprised of two accessions, UAM-NI and UAM-KE while cluster II consisted of six accessions including UAM-AK, UAM-AD, UAM-AB, UAM-KO, UAM-BE and UAM-NA (Figure 1) while the outlier, UAM-OY alienated from the other accessions because of its genetic dissimilarity and uniqueness. The accessions in cluster I are characterized by tall plants and highly profused branching habit which also translated into higher number of leaves per tree (71) as indicated by cluster mean (Table 3). However, cluster II comprised of accessions that had intermediate performances while the outlier was quite unique, indicating its distinct identity. It is comparatively short, and also gave the least number of branches and leaves. Consequently, it recorded the least fresh, dry and leaf powder weights.

Eighteen weeks (18weeks) after pruning, the result of the study showed that accession UAM-BE (66.07cm) recorded the longest leaf lengths while UAM-AB recorded the least(41.13cm).On number of flowers/ tree, accession UAM-OY had the highest(350) UAM-KO had the least(91).Similarly ,accession UAM-OY recorded the least number of days(189days and 206days) to podding and fifty percent podding respectively. Other parameters which exhibited significant differences were pod length, pod girth, pod weight, number of seeds/pod, number of seeds/tree and 100-seed (Table4). The result also revealed that the pattern of grouping of the accessions were similar to that observed in the unpruned data. The cluster analysis revealed that the 9 accessions were classified into two clusters, I and II and an outlier with the same members both in number and in kind. The cluster mean (Table 4) showed that cluster I was characterized by least number of leaves and branches, least values for fresh, dry and leaf powder weights were also obtained in this cluster. Cluster II showed intermediate performances in the characters studied while the outlier (UAM-OY) produced the highest number of leaves, highly branched and consequently produced the highest fresh, dry and leaf powder weights contrary to when it was unpruned. On the general note, the accessions exhibited increased in fresh, dry and leaf powder weights pruned (Table 4) than unpruned (Table 2). This finding supports the report of Palada and Chang (2003) who reported that trimming of drumstick trees promotes branching and increases leaf yield. However, the data for the unpruned and the pruned drumstick accessions were pooled together, but the clustering pattern was still consistent for the unpruned, pruned and when these results were pooled together. In all these situations, accession UAM-OY (outlier) from Oyo State maintained its distinctiveness and uniqueness. However, the clustering of two accessions UAM-NI from Niger State (guinea savanna) and UAM-KE from Kebbi State (sahel agro-ecological zone) into cluster I is indicative of the fact that regional boundary was not a criteria for genotype differentiation (Figure 1). Similarly, the accessions comprised in cluster II were also collected from across different agro-climatic areas. For instance, UAM-AK from Akwa-Ibom State in the rainforest zone, UAM-AB from Federal Capital Territory Abuja, UAM-KO from Kogi State, UAM-BE from Benue State and UAM-NA from Nasarawa State in the guinea savanna zone, UAM-AD from Adamawa State in the sudan savanna zone. Thus, it is evident that clustering of accessions was based on similarity irrespective of their place of collection;

Hence, regional boundary was not a criterion for genotype differentiation. This result agrees with the report of Thul*et al.* (2009) who reported four clusters and two outliers from

a collection of Capsicum species studied irrespective of area of collection

Abubakar*et al.* (2011) also reported six clusters from the 21 accessions of *Moringaoleifera*studied irrespective of where the accessions were collected. This appears to mean that factors other than regional boundaries are responsible for divergence in drumstick trees studied. Thus, the growth and vegetative characters differentiated the accessions into different groups from which superior hybrids can be derived. Based on the level of divergence among the clusters as revealed by the scatter plot analysis and mean cluster distance, cluster II and the outlier are highly dissimilar, suggesting that accessions from these two groups could be evaluated for their combining ability for possible utilization as parents in the heterosis breeding programme in drumstick as suggested in maize (Betran*et al.*, 2003) and Brassica (Mahmuda*et al.*, 2008) crops.

Table.2: *Performance of some vegetative and phenological characters of unprunedMoringa accessions evaluated in Makurdi. Means within column followed by same letter are not significantly different by Duncan at 5%.*

TRAITS	UAM-NA	UAM-BE	UAM-KO	UAM-OY	UAM-NI	UAM-KE	UAM-AD	UAM-AB	UAM-AK	MEAN
Plant height (cm)	3.11abc	2.84c	3.19abc	3.60a	3.63a	3.54ab	3.30abc	3.02bc	3.15abc	3.26
Number of leaves	37c	44c	46bc	96a	60bc	81ba	50bc	56bc	57bc	58.55
Number of branches	4c	6.33bc	7.17bc	12.33a	8.33abc	10.00ab	6.00bc	7.66bc	8.00bc	7.76
Leaf length (cm)	74.10	76.27	74.60	76.73	70.07	72.67	67.67	72.50	62.57	71.91
Stem diameter(cm)	21.17ab	19.07bc	17.60bc	23.00a	18.50bc	18.50bc	17.30c	17.93bc	18.50bc	19.06
Fresh leaf weight (g)	118.10	121.60	134.85	220.22	157.17	162.90	133.37	133.48	135.25	146.33
Dry leaf weight (g)	52.25	53.78	56.53	113.42	74.70	77.02	59.70	66.42	62.97	68.53
Leaf powder weight (g)	40.22	41.70	45.70	82.60	53.25	54.25	43.03	44.28	46.12	50.13
Days to flowering	152c	176a	179a	151c	153c	147c	159bc	154c	168ab	159.89
Days to 50% flowering	157bc	177a	180a	152c	155bc	148c	161bc	157bc	169ab	161.78

Table.3: Cluster means for unprunedM. oleifera accessions evaluated in Makurdi in 2012.

Trait	Cluster I	Cluster II	Outlier
Plant height (cm)	3.59	3.17cm	3.11
Number of leaves per plant	71.0	55.0	37.0
Number of branches per plant	9.0	7.0	4.0
Leaf length (cm)	71.37	72.06	74.0
Stem diameter (cm)	18.50	19.22	21.17
Fresh leaf weight (g)	160.04	142.41	118.10
Dry leaf weight (g)	75.86	66.44	52.25
Leaf powder weight (g)	53.75	49.09	40.22
Days to flowering	150.0	163.0	152.0
Days to 50% flowering	152.0	165.0	157.0

Table.4. Performance of some vegetative and phenological characters of pruned Moringa accessions evaluated in Makurdi

http://dx.doi.org/10.22161/ijeab/2.1.63 *ISSN: 2456-1878*

	NOB	NOL	STD	LLGT	FLW/T	DLW/T	LPW/T	NFL/T	DPD	D50%P	NPD/T	PDL	PDG	PDW	NS/PD	NS/T	100-SW
UAM-NA	24	183	36.87	45.23 bc	286.60	90.67	85.60	279a bc	250 ab	269a	57	28.22 bc	4.62d	6.57d	8.0bc	429.0	22.95 bc
UAM-BE	21	136	34.47	66.07 a	253.11	82.93	78.33	197a bcd	274 a	285a	47	24.48 c	4.80bc	6.21d	6.0c	309	21.66 cd
UAM-KO	17	136	32.97	52.50 b	177.60	67.63	50.07	91d	282 a	290a	38	34.50 ba	5.22bc	10.67 abc	11.0 ab	420	27.27 a
UAM-OY	27	200	39.10	52.07 b	216.27	70.80	65.13	350a	189 c	206b	88	34.80 a	5.56b	11.78 ab	13.0 a	1329	23.09 bc
UAM-NI	16	113	32.37	44.00 bc	114.27	36.73	25.17	197a bcd	206 c	215b	72	35.02 a	6.22a	13.40 a	14.0 a	1070	25.78 a
UAM-KE	20	135	34.20	44.57 bc	115.10	42.67	39.37	316a b	193 c	206b	73	32.05 ab	5.52b	11.48 ab	11.0 ab	778	25.52 ab
UAM-AD	19	160	31.07	42.30 bc	184.63	64.53	53.13	191a bcd	225 bc	234b	60	28.91 bc	5.14bcd	8.50cd	13.0 a	753	19.65 d
UAM-AB	22	152	32.77	41.13 c	222.43	73.10	68.43	160b cd	203 c	218b	43	29.04 bc	6.06a	8.74bcd	9.0bc	372	26.56 a
UAM-AK	21	150	33.27	47.17 bc	188.00	68.32	54.67	120cd	283 a	290a	49	30.62 ab	5.13bcd	9.05bcd	13.0 a	588	20.56 cd
Mean	20.78	151.67	34.12	48.34	195.33	66.38	57.77	211.22	233.89	245.89	58.56	30.85	5.36	8.32	10.89	672.0	23.67

NOB=Number of branches/tree, NOL=Number of leaves/tree, STD=Stem diameter (cm), LLGT=leaf length (cm), FLW/T=Fresh leaf weight/tree (g), DLW/T=Dry leaf weight/tree (g), LPW/T=Leaf powder weight/tree (g), NFL/T=Number of flowers/tree, DPD=Days to podding, D50%P=Days to 50% podding, NPD/T=Number of pods/tree, PDL=Pod length (cm), PDG=Pod girth (cm), PDW=Pod weight (g), NS/PD=Number of seeds/pod, NS/T=Number of seeds/tree, 100-SD=100-seed weight (g).

Table.4: Performance of some vegetative and phenological characters of pruned Moringa accessions evaluated in Makurdi

Parameters	**UAM-NA**	**UAM-BE**	**UAM-KO**	**UAM-OY**	**UAM-NI**	**UAM-KE**	**UAM-AD**	**UAM-AB**	**UAM-AK**	**Mean**
Number of branches/tree	24	21	17	27	16	20	19	22	21	20.78
Number of leaves/ tree	183	136	136	200	113	135	160	152	150	151.67
Stem diameter (cm)	36.87	34.47.	32.97	39.10	32.37	34.20	31.07	32.77	33.27	34.12
leaf length (cm)	45.23bc	66.07a	52.50b	52.07b	44.00bc	44.57bc	42.30bc	41.13c	47.17bc	48.34
Fresh leaf weight/tree (g)	286.60	253.11	177.60	216.27	114.27	115.10	184.63	222.43	188.00	195.33
Dry leaf weigh/tree (g)	90.67	82.93	67.63	70.80	36.73	42.67	64.53	73.10	68.32	66.38
Leaf powder weight (g)	85.60	78.33	50.07	65.13	25.17	39.37	53.13	68.43	54.6	57.77
Number of flowers/tree,	279abc	197abcd	91d	350a	197abcd	316ab	191abcd	160bcd	120cd	211.22
Days to podding	250ab	274a	282a	189c	206c	193c	225bc	203c	283a	233.89

http://dx.doi.org/10.22161/ijeab/2.1.63 *ISSN: 2456-1878*

Days to 50% podding	269a	285a	290a	206b	215b	206b	234b	218b	290a	245.89
Number of pods/ tree	57	47	38	88	72	73	60	43	49	58.56
Pod length (cm),	28.22bc	24.48c	34.50ba	34.80a	35.02a	32.05ab	28.91bc	29.04bc	30.62ab	30.85
Pod girth (cm),	4.62d	4.80bc	5.22bc	5.56b	6.22a	5.52b	5.14bcd	6.06a	5.13bcd	5.36
Pod weight (g),	6.57d	6.21d	10.67abc	11.78ab	13.40a	11.48abc	8.50cd	8.74bcd	9.05bcd	8.32
Number of seeds/pod	8.0bc	6.0c	11.0ab	13.0a	14.0a	11.0ab	13.0a	9.0bc	13.0a	10.89
Number of seeds/tree	429	309	420	1329	1070	778	753	372	588	672.0
100-seed weight (g).	22.95bc	21.66cd	27.27a	23.09bc	25.78a	25.52ab	1965d	26.56a	20.56cd	23.67

Means within row followed by same letter are not significantly different by Duncan at 5%.

Table.5: Cluster means of pruned Nine Moringaoleifera accessions evaluated in Makurdi in 2013.

Trait	**Cluster I**	**Cluster II**	**Outlier**
Number of branches per tree	18.0	22.0	27.0
Number of leaves per tree	124.0	160.0	200.0
Stem diameter (cm)	33.29	34.36	39.10
Leaf length (cm)	44.29	49.50	52.07
Fresh leaf weight (g)	114.69	189.80	216.27
Dry leaf weight (g)	39.70	74.0	70.80
Leaf powder weight (g)	32.27	65.05	65.13
Number of flowers per tree	257.0	198.0	350.0
Days to first podding	200.0	244.0	189.0
Days to 50% podding	211.0	256.0	206.0
Number of pods per tree	73.0	55.0	88.0
Pod length (cm)	33.54	30.84	34.80
Pod girth (cm)	5.87	5.22	3.56
Pod weight (g)	12.44	8.79	11.78
Number of seeds per pod	13.0	10.0	13.0
Number of seeds per tree	924.0	600.0	1329
100-seed weight (g)	25.65	23.11	23.09

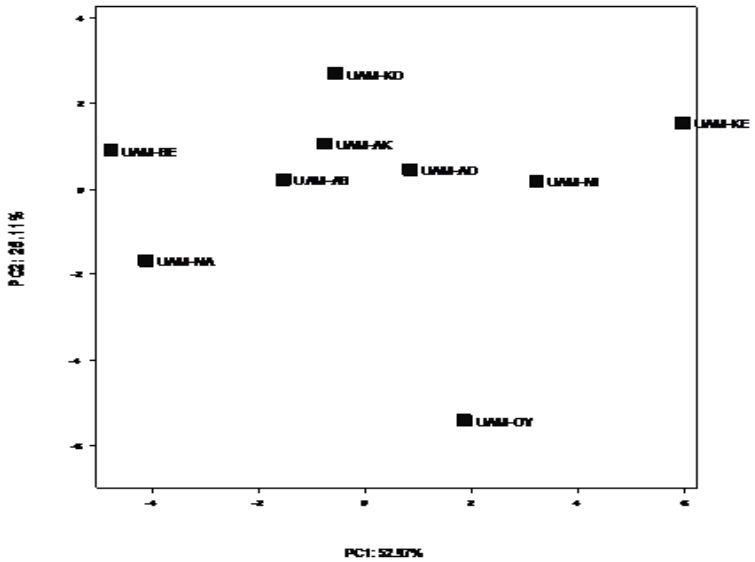


Fig.1: Scatter plot analysis for pruned Moringaoleifera accessions evaluated in Makurdi.

http://dx.doi.org/10.22161/ijeab/2.1.63 *ISSN: 2456-1878*

IV. CONCLUSION

This study indicated that there is sufficient variability in the studied population to warrant the commencement of genetic improvement of drumstick through selection as revealed by the diversity analysis. It was also observed that the pruned accessions recorded higher fresh, dry and leaf powder weights than unpruned, with accession UAM-OY (outlier) expressing superiority over other accessions with regards to the fresh, dry and leaf powder weights and in most of the phenological characters studied.

REFERENCES

[1] Abubakar, B.Y. Muazu, S. Khan, A.U. and Adamu, A.K. (2011). Morpho – Anatomical variation in some accessions of *Moringaoliefera* from Northern Nigeria. African *Journal of Plant Science*, 5 (12): 742 – 748.

[2] Aho, M.I. and Agunwamba, J.C. (2002).Comparism between water extract of *Moringaoliefera* seeds and moringa seed powder in the treatment of water. Paper presented at National submit on *Moringa* Development, organized by the Raw Material Research Development Council (R.M.R.D.C.). Headquarter–Abuja 16th–18th November, (2002).

[3] Betran F.J., Rabaut J.M, Becker D. and Gonzalez L.D. (2003).Genetic diversity, specific combining ability and heterosis in tropical maize under stress and non-stress environments.*Crop Science*, 43:797-806.

[4] Foidl, N. Makkar, H.PS and Becker, K. (2001).The Potentials of *Moringaoleifera* for Agricultural and Industrial uses. What development of moringa products – Oct. 20th– Nov. 2nd 2001. DarEssalam. Pp. 10-12.

[5] Keay, R.W.J. (1989).Trees of Nigeria, Clarendon Press Oxford. Pp. 24-27.

[6] Mahmuda, M.D., Golam, R. and Abdur, M.D. (2008).Genetic diversity analysis in some advance lines of *Brassicanapus*. *Science Asia*, 34:432-434.

[7] Olson, M.E. (2002). Combing data from D.N.A. sequences morphology for a phylogeny of moringaceae (Brassicales). *Systematic Bot*., 27 (i).55-73.

[8] Palada, M.C. and Chang, I.C. (2003).Suggested Cultural Practices for Moringa.International Cooperators Guide. Pp. 3 – 4.

[9] Price, M.L. (2007).The Moringa tree.An Echo Technical note.p.19.www.echonet.org.

[10] Rajangam, J. Azahakia, M.R.S. Thangarai, T. Vikjayakumar, A. and Muthukrishan, N. (2001).Status of production and utilization of moringa in southern India. Development potential for *Moringa* products – Oct. 29th - Nov. 2nd 2001, DaresSalaam, Tanzania. Pp. 3 – 4.

[11] Subramanian, A. and Subbraranan, N. (2010).Hierarchical cluster analysis of genetic diversity in maize germplasm. *Electronic Journal of Plant Breeding*, 1(4): 431 – 436.

[12] Thul, S.T. Lai, R.K. Shasany, A.K. Darokar, M.P. Gupta, A.K. Gupta, M.M. Verma, R.K. and Khanuja, S.P.S. (2009). Estimation of phenotypic divergence in a collection of *Capsicum* species for yield related traits. Euphytica. 168:189.doi:10.1007/s10681-009-9882-y.

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

Evaluation of Returns and Risks in the Forms of Garlic Market: Seed Versus in Natura

Dr. Luciano Bendlin[1], Katia Adrieli Vichinheski[2], Nazareth Pires Martins Spautz[3],Viviane Maciel Scherer[4]

[1]Professor, Contestado of University, Brazil
[2] Profa. Master student, Contestado of University, Brazil
[3,4]Bel., Contestado of University, Brazil

Abstract**—** *The garlic plant hortense Liliaceae, very used as spice, giving flavor to food and can be used in medicinal treatment. Its cultivation is an annual crop with all its processes since the preparation of the earth to their harvest. The context of this work was to evaluate their production, their use, their risks and seed and its consumption in natura. Their samplings were selected in Três Barras Town, Santa Catarina in 2014 harvest period. Among the seed (bulb) and in natura even with the risk of losses with fungal or bacterial their culture is considered a good harvest, because the climate is very favorable to its cultivation, detailing its costs of production and the difference between the crop seed and in natura. This study has used the Multi-index methodology to the analyzes and calculations for the comprehensive, such as: analysis of the 8.25% ROIA for garlic in natura and 7.44% in garlic seed on the initial investment. The cash flow based on monthly statements, involving expenditure on machinery, preparation of the land, among others. An initial investment of R$ 39.756,64 for both harvests. Getting a VPL R$63.188,55 for garlic in natura and R$54.303,99 for garlic seed, its IBC considering the values of 2.59% for garlic in natura and 2.37 for garlic seed. The information about TMA/TRI were well satisfactory for the return of the harvest, as all cultures have risks, this harvest is approximately 50% for both types being in the seed and in natura. The data was passed by the Crystal Ball for attesting and validation of calculations where is approved investment in agribusiness.*

Keywords**— Risk and Return. Garlic. Multi-index methodology.***

I. INTRODUCTION

Agribusiness in Brazil has presented an evolution over the years, becoming a prosperous, safe and profitable activity, favorable for the diversified climate, being today the main locomotive of the Brazilian economy. It is more representative in the production of grains such as soy, corn, and in the sale of beef, pork and poultry. But the market has been expanding in other cultures, such as onions and garlic.

The production of garlic in Santa Catarina has been showing evolution and growth in the national production, being favored by relief, climate and land and social structure, making garlic a product of great socioeconomic importance for the state, making small and medium producers viable. ALHO, 2014).

However, the small farmer has no control of his costs in relation to the direct labor, with maintenance of the cultivation of his production and mainly with depreciation occurring in his machinery, where he indirectly affects his real cost in relation to the Production, and therefore the importance of obtaining a more targeted view of these issues, giving greater emphasis and obtaining the necessary data for risk assessment and feedback that helps decision making in order to minimize their losses and wastes.

This work gains importance due to the fact that it has a greater focus on the northern plateau of Santa Catarina, being a great help for the decision making mainly of the small farmers, where they can evaluate the best way of producing garlic in the region with a focus on profitability and Associated with the product.

The objective of this study is to analyze the production costs and expectations of return and risks associated with garlic agribusiness in the region of Planalto Norte Catarinense. Evaluate the risks and demonstrate the return of garlic production, applying the indicators Net Present Value (NPV), Annualized Net Present Value (NPV), Benefit / Cost Index (IBC), Return on Investment Added (ROIA), Internal Rate (TIR), Garlic Agribusiness Playback Period, using the multi-index methodology to obtain a better perception of the risks and the return of garlic production in this region, the data were confirmed through the Crystal software Ball.

This article is divided in five sections, beginning with introduction, followed by the theoretical reference where it deals with agribusiness, the garlic market, garlic cultivation, rural accounting and multi-index indicators. The third section deals with the methodological

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

procedures in the elaboration of the research, section four is exposed the calculations and analyzes, and in the fifth section the final considerations.

II. THEORETICAL FOUNDATION

For the development of the theoretical reference of this article, it became necessary the bibliographical research, with definition of the concepts related to the study, such as agribusiness, garlic culture, rural accounting, multi-index methodology and its indicators and Monte Carlo simulation - Crystal Ball.

2.1 AGRIBUSINESS

Agribusiness in Brazil shows great credibility and growth after the consolidation of Brazil in the International scenario. Soil, climate, water and Brazilian relief are characteristics that favor agribusiness in the country. New technologies and processes have significantly changed the industry over the years, involving small and large companies.

Although these segments had their own functions, they have a very important link in the chain of production, becoming responsible for the link between the rural property and the final consumer, creating an integration of agriculture with commerce, industry, service providers and producer.

With this new scenario, the concept of primary agriculture no longer makes sense, and a new conception is necessary, which arises in the United States in the year 1957 through university professors the term agribusiness, defined as:

> [...] the set of all operations and transactions involved from the production of agricultural inputs, from production operations in agricultural units to processing and distribution and consumption of 'in natura' or industrialized agricultural products (RUFINO, 1999 apud ARAÚJO, 2007, p.16).

The term spread throughout the world, being widespread in Brazil in the 80's and still being in English, the nomenclature Agribusiness began to be accepted in Brazil in the mid-90's, being used in books and newspapers.

The agribusiness presents some particularities that affect the production as:

a) Seasonality of Production - causes variations in the value of the commodity, between the harvesting periods between harvest, where it is necessary to improve the infrastructure for storage and conservation, with greater use of inputs;
b) Influences of biological factors - diseases and pests, which affect the cost of production, requiring adequate products, machinery and appropriate equipment to better preserve the quality of the product offered to the consumer;
c) Rapid perishability - after-harvest products have a fast cycle, which affects their useful life, so specific care is needed to extend the product life cycle.

The agribusiness productive chain acts in the analysis of the structure and functionality of the economic sectors of the activity. However, it must be emphasized that the productive chain is not limited to the process between the producer and the market, this affects other segments necessary for the development of agribusiness. The process can be classified into three segments, according to:

Classified into three segments, according to:

. Prior to Porteira: this segment is composed of suppliers of inputs and services necessary for agricultural production, such as machinery, implements, pesticides, fertilizers, correctives, seeds, water and energy. The services in this segment involve research and development institutions, financial institutions, and government agencies that operate in the sector. It should be noted that the components may vary according to the activity developed. In some cases, the number of suppliers may be reduced due to the specific characteristics of the activity.

. Within the gate: a segment constituted by the agricultural activity from the beginning of the activity of preparation of the inputs (soil, seeds, fertilizers, plantation) until obtaining the agricultural product ready for the industrialization process. In some activities, the process of industrialization still occurs within the gate, with the transformation of the agricultural product into industrialized product still in the agricultural property, this is the case of some dairy products, sugar, alcohol, sweets, among others. Although this industrial process is relatively common within the gate, in most agricultural activities the final product is traded in the primary form, without any type of value added.

. Porter: after harvest, agricultural products can be destined directly to the final consumer or to the processing industry. Products destined for final consumption are sold in bulk, or only a simple package, as in the case of some fruits, roots, grains and various vegetables, marketed in fairs or supermarkets that they buy directly from farmers. The products destined for processing receive the most diverse types of processing, in order to add value to the product offered to the final consumer. In addition to processing, it is the responsibility of the

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

post-consumer segment to develop the logistics chain necessary for products to reach the consumer markets (SANTOS, SILVA, 2014).

2.2 GARLIC

Garlic, scientific name Allium sativum, a plant of Asian origin, belonging to the family Aliacea, can reach a height of 50 cm to 120 cm, has an operational cycle around one hundred and thirty five days for cultivars precoce and one hundred and sixty Five days for late cultivars, and may present changes depending on the planting season, the planted variety and the production region.

The consumption is made by bulbs (head), composed of bulbilhos (teeth), and can be consumed raw, cooked or roasted, being used as seasonings, condiments and for medicinal purposes.

It can be verified that in the last twelve years it obtained a reduction in the planted area in 74% but its production increased in 34%. In 1990 the area cultivated in Brazil was 17,535 hectares with production of 71 thousand tons and in 2012 was 10,064 hectares with 107 thousand tons (Revista Nosso Alho, 2014).

It is noticed that the production obtained increase due to the capacity of the producers to evaluate the factors that affect the productivity being the one of greater impact the quality of the garlic seed.

The production of garlic in Brazil, does not meet the consumption of the country, and with this needs to import. In the 2013/2014 harvest, Brazil's production accounted for 40% of garlic consumed by Brazilians, with the main garlic producing states of Minas Gerais, Goiás, Rio Grande do Sul, Santa Catarina and Bahia.

Garlic production in the country shows a productivity difference (tons per hectare), in 2012 the states with the highest production were Goiás (33%), Santa Catarina (18%), Minas Gerais (17%) and Rio Grande do Sul %).

The garlic has been used in addition to the kitchens, due to the benefits it produces, which has higher consumption. Garlic contains alliin and allicin components responsible for its odor and taste and its biological properties. The benefits are obtained by breaking the cell, by chewing or cooking, but frying decreases its properties.

It was found that Brazilian garlic is more beneficial than Chinese garlic, even though both have the same properties, but the Brazilian garlic conserves for a longer time the qualities of the existing nutrients. In addition, the Brazilian product has antioxidant in 70%, contributing to prevent aging (Revista Nosso Alho, 2008).

The production of garlic goes through a critical period, because its profitability is low and its high cost, which does not allow the investment in the production, since the price practiced in the imports, mainly by China, is low. However, in order to obtain greater investments in the crop, it is necessary that the influential factors be optimized, aiming at the greater profitability of the national production and thus reducing the dependence on imports.

a) Cultivation of Garlic

The garlic culture in Brazil began in the states of Minas Gerais and Goiás, where they produced white common garlic. Over the years the producers improved and began to grow purple garlic. The state of Santa Catarina was the pioneer in the cultivation of the noble purple garlic, specifically in the region of Curitibanos (Nosso Alho Magazine, 2008).

The Brazilian production presents variation over the years, with periods of growth and in others of reduction, nevertheless, it has presented a stability in the last years. This variation occurs due to the supply from the outside, which affects the value of the national merchandise, which ends up at certain moments discouraging the producers.

With the opening of the import market, garlic consumption in the country increased, but it destabilized some producers who ended up abandoning the crop and migrating to other crops. This occurred mainly in the region of Curitibanos and the Gaúcha Reaching the southern region of the country, which suffers from the competitiveness of Argentine garlic.

The alternative for the southern producer to remain in the market will be to invest in the cultivation of late varieties with high productive potential, with good quality seed and corrected soil.

b) Climate

The production of garlic in Brazil can be made in several states, except for hot or rainy regions. It is necessary to adapt to the variety and planting season respecting the climate of each region. The cultivation of garlic requires at the beginning or in the intermediary phase of temperatures between 0ºC and 15ºC (Portal Hortas), thus stimulating the formation of the bulbs.

Temperatures influence the development of the plant, being ideal temperatures in the period of formation of the bulb, and warmer in maturation, with periods of solar luminosity that will vary the time according to the cultivated variety.

c) Alone

Regarding the soil the garlic cultivation is not so demanding, being ideal soils light, drained and rich in organic material. However, if crop rotation is necessary, soil preparation varies according to the previous crop and should occur in advance of 35 to 45 days (Portal Hortas).

Irrigation has its contribution, being necessary at the outset.

Table.1: Production stages of Garlic

Planting	To obtain a good result it is necessary to make a good planting in an appropriate and cautious way. The finest part of the bulb should be facing upwards, spacing between 25 and 30 cm between rows and 10 cm between the plants (Portal Hortas). The spacing between lines and plants contributes to the productivity of garlic.
Harvest	Harvest varies from sixteen to thirty-six weeks after planting depending on the variety planted, planting season and region. The determination of the harvest period is done through visual methods such as amount of green leaves, bulb formation. Harvesting can be done in two ways, either manually or mechanically.
Storage	When manual harvesting takes place, it is necessary to pre-cure the production, where the plant loses its excess water through sun exposure, which occurs between two to four days depending on the climate, after it is collected and stored in a dry place and With good ventilation, care must be taken at this stage so that the garlic is not beaten and the bulbs are not exposed to the sun. Mechanical harvesting does not occur during the pre-cure period, where the machine is harvested and lashed after production is stored in a cool, dry place with good ventilation, without the shape of braids.
Preparation of the Garlic for commercialization:	After the harvest and cure of the production the garlic goes through four phases to be ready for commercialization, which are: Cut where the garlic is cut, conditioned, and occurs the first selection of industrial garlic. Garlic should be exposed to the sun or fans after cutting. Classification garlic is classified by size by mechanical means, being careful not to affect the quality of the bulbs. Cleaning consists of removing the dirty outer tunics, and selecting by quality, removing the bulbs with defects. Packaging varies according to specification, garlic for consumption is commercially available in bulk and in bulk in sacks.

Source: Adapted Portal Hortas (2016).

2.3 RURAL ACCOUNTING

Accounting can be applied generally or privately, while encompassing all branches is defined as general or financial accounting, in particular this is retained in the branch to be studied or applied to proper accounting. In this way, one has as defined Marion (2014).

1. Agricultural accounting applied to agricultural enterprises;
2. Rural accounting applied to rural enterprises;
3. Zootechnical Accounting applied to companies that operate Zootechnics;
4. Livestock Accounting applied to livestock enterprises;
5. "Agricultural Accounting" applied to agricultural enterprises;
6. Agribusiness Accounting applied to agroindustrial companies.

a) Agricultural activity

The economic development of agricultural activity is influenced by market conditions and material resources, so the producer must have knowledge and thus make the decisions of which crop to invest, how much and how to produce, control the initial activity, analyze the results and compare with the Originally planned.

Agricultural activity is influenced by external factors such as climate, price of products, credit and financing policy, transportation and availability of labor and internal factors crop yields and combinations of productive activities, labor efficiency and Equipment and the administrator's personal conditions.

Revenue from the activity is normally concentrated during or shortly after harvest, being seasonal, with closure of the agricultural year soon after harvesting. In conducting the calculation of the result after harvest and commercialization, a better evaluation of the performance of the agricultural harvest is obtained, thus contributing to the decision-making and programming of the next agricultural year.

In case the company opts for several crops the agricultural year follows the cycle of the crop of greater economic representativeness, being carried out the evaluation of the harvest and commercialization of the main crop and evaluation of the other crops in formation.

With Law no. 7,450 / 85, making the Income Tax mandatory for all companies, making the fiscal year of the agricultural enterprise coincide with a calendar year, affecting rural accounting, as this does not normally coincide with the agricultural year. However, the

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

agricultural year can be used for managerial purposes, where it will contribute to the evaluation of the activity.

b) Rural activity in the new Civil Code

With the amendment of the Civil Code that was in force in 2003, one can define the term entrepreneur as:

> [...] who carries out professionally organized economic activity for the production or circulation of goods and services. Thus, the rural producer will be called a rural entrepreneur depending on the definition above, as long as he joins the trade board. Not signing up for the trade board, he will be an autonomous rural producer (MARION, 2014, p.7).
>
> And if you consider the company:
>
> [...] when people enter into a contract and reciprocally undertake to contribute goods and services to the exercise of economic activity and the sharing of results between them. Thus, the term business society replaces the previous term (commercial company). In this way, rural society (when there are two or more people together) is now seen as an entrepreneurial society (MARION, 2014 p.7).

With this, the entrepreneur whose rural activity is his main profession can exercise in the following legal forms: autonomous, individual entrepreneur, business society (MARION, 2014).

> A) Temporary crops
>
> In temporary culture, replanting after harvesting is required. These are short-lived crops, also known as annual crops, that is, every year it will be necessary to plant such crops as soybeans, corn, potatoes, onions, garlic.
>
> The accounting is carried out in Current Assets in the same way as in the industry under the Inventory in Progress account. The costs will be accumulated in the culture sub-account in specific training of the temporary culture.
>
> When only one crop is produced, the costs are direct, and when there is more than one crop it will be necessary to apportion indirect costs, proportional to each crop. The costs are seeds, fertilizers, seedlings, demarcations, labor, charges, electricity, social charges, fuel, insurance, professional services, insecticides, depreciation of tractors and other assets in the crop.

The agricultural activity has expenses and costs in the period, which has its differentiation where according to Marion (2014 p.15), it is considered:

> Crop cost All expenses directly or indirectly identifiable with the crop (or product), such as seeds, fertilizers, labor (directly or indirectly), fuel, depreciation of machines and equipment used in the crop, agronomic and topographical services etc. .
>
> Expenditures for the period are all expenditures that are not identifiable with the crop and are therefore not accumulated in the stock (temporary crops), but are appropriated as expenses for the period. Sales expenses (advertising, salesmen's commission ...), administrative expenses (directors' fees, office staff ...) and financial expenses (interest, bank fees ...).

The costs arising from the harvest will be recorded in the Culture account, after which the finished products will be downloaded and transferred to the new crop account, such as soybean, corn, potatoes, garlic and onion (MARION, 2014). Production costs can be classified as direct or indirect.

Expenses are expenses not identified with the crop, so it is not accumulated in the inventory and is appropriated with Expenses of the Period, they are expenses with sales, advertisements, administrative expenses, and financial expenses. These expenses are classified as Selling Expenses in the Operating Expenses group and not in the Cost of the Product, in the event that the agricultural product may be in stock, some prefer to count as accumulated cost of storage.

Depreciation, there are some difficulties to make the exact calculations of the equipment used in the crop, this item has gained space in recent times due to its use with its goal to improve productivity.

2.4 MULTI-INDEX METHODOLOGY

The multi-index methodology used for the decision-making process of a project with respect to its acceptance or rejection uses in addition to the indicators already mentioned above the Degree of Revenue Commitment (GCR), Risk Management and Business Risk, thus obtaining a better perception Of the risk offered by the project.

The essence of the Multi-index Methodology according to Souza and Clemente (2008: 124), consists of:

1. not incorporate the risk premium as a spread over the TMA;
2. to express the ROIA's profitability as an additional return, in addition to what would be earned by the application of capital in low-risk securities;
3. use the environmental analysis to deepen the assessment of the risks involved;
4. compare the expected gains with the perception of the risks of each project.

The degree of revenue impairment (GCR) is the proximity of the Operational Equilibrium (PEO) to the maximum capacity, where the operational break-even point is the

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

minimum quantity to be sold in order to obtain settlement of production costs but without profit. The maximum capacity refers to the market's ability to sell.

The Management Risk associates with the degree of knowledge and competence of similar projects, being necessary the knowledge and experience accumulated on the productive process, commercialization process, distribution channels and in the condition of negotiations, aiding in periods of turbulence and unfavorable. It is necessary for the Management Risk assessment to make an assessment of the company's areas (SOUZA; CLEMENTE, 2008).

Business risk is associated with short-term and uncontrolled factors that end up affecting the project environment, being the degree of competition, barriers to entry and exit, trends in the economy and the sector of activity. It is possible to adopt evaluation adjustments, in relation to the factors and the techniques of analysis, being more effective in relation to the perception of the risks involved (SOUZA; CLEMENTE, 2008).

The investment is the disbursement aimed at generating a flow of future benefits, with prospects of receiving them.

The indicators generated help for the investment decision process through analysis. By analyzing the generated indicators, it becomes possible to follow the decision-making process of the investment. It is possible to ascertain viable alternatives and which are financially attractive, thus making it possible to decide between investing or refusing the project.

According to Souza and Clemente (2008, page 67) the indicators of analysis of investment projects can be subdivided into two groups:

a) Indicators of return on investment

The first index of profitability to be verified is the Net Present Value (NPV) that according to Souza and Clemente (2008, p.74),

> The Net Present Value, as its name implies, is nothing more than the concentration of all expected values of a cash flow at date zero. To do so, the Company's Minimum Rate of Attraction (TMA) is used as the discount rate.

However, the NPV index is not enough for decision making, it is necessary to apply the others, initially no number is good or bad, but it should be compared with another reference, and the primary rule for NPV is as follows: NPV> 0 □ indicates that the project deserves further analysis (SOUZA; CLEMENTE, 2008).

For projects with long planning horizon, it is necessary to have a more approximate value for each period. With this, we can use average NPV, making it easier to reach a rational decision maker in terms of gain for the period than the accumulated gain in the long term.

Souza and Clemente (2008, page 77), describes the VPLa as:

> The Annualized Net Present Value (VPLa), also known as Equivalent Uniform Annual Value (VAUE), is a variation of the Net Present Value Method. While the NPV concentrates all cash flow values at date zero, in the VPLa the cash flow representative of the investment project is transformed into a uniform series

Another index of profitability is the Benefit / Cost Index (IBC) that according to Souza and Clemente (2008, p. 78).

> [...] is a measure of how much is expected to be earned per unit of invested capital. The hypothesis implicit in the IBC calculation is that resources released over the life of the project are reinvested at the rate of least attractiveness.

The benefit / cost ratio is the ratio of the expected benefit flow of a project and the expected flow of investments required to carry out the project (SOUZA & CLEMENTE, 2008).

It can be calculated with the following formula:

$$IBC = \frac{\text{Present value of the benefit stream}}{Present\ value\ of\ the\ investment\ flow}$$

The Additional Return on Investment (ROIA) is the best profitability alternative, and the wealth generated by the project derives from the IBC equivalent rate of each project period.

a) Investment Risk Indicators

After verifying the profitability indexes of the project, it is time to look for risk indicators. The Internal Rate of Return (IRR) can be defined as: "rate that makes the Net Present Value (NPV) of a cash flow equal to zero" (SOUZA; CLEMENTE, 2008, p.81). It can be used to analyze the return dimension or the risk dimension.

By size return can be considered as upper limit for profitability of the project invested. The IRR can be considered representative of the project if the IRR and TMA values are the same. Regarding the risk dimension, the IRR is more relevant, being the upper limit for the variability of the TMA, due to the fact that the NPV is decreasing as the TMA approaches the IRR, where with the TMA equal to the IRR the project gain Is equal to zero (SOUZA; CLEMENTE, 2008).

The Payback Period is the "number of periods necessary for the flow of benefits to exceed the capital invested" (SOUZA; CLEMENTE, 2008, p.38).

As an indicator of importance in the investment decision process, it presents continuous and marked trends within the economy, since the invested capital must present a rapid return, to avoid the exclusion of investments in future projects. (SOUZA; CLEMENTE, 2008).

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

These indicators have a better perception of the expected behavior between risk and return, where greater risks lead to an increase in the desired return (SOUZA; CLEMENTE, 2008).

The project must present a financial attractiveness, which results from the expected cash flow less the amount invested, in order to obtain the expected flow it will be necessary for the current value to be converted to future value. For this, it is necessary to have the value of the rate, known as the Minimum Attraction Rate.

The minimum attractiveness rate is the best low-risk rate available for the capital investment. If it is necessary for decision-making at least two alternatives, invest in the project or invest in the minimum rate of attractiveness.

> The basis for establishing an MRA is the market interest rate. The interest rates that most impact the TMA are: Basic Financial Rate (TBF); Reference Rate (TR); Long-Term Interest Rate (TJLP) and Special System of Settlement and Custody Rate (SELIC) (SOUZA; CLEMENTE, 2008, page 71).

What is difficult to establish the TMA are the oscillations during the investment period of the rates that serve as floor and the ceiling for the TMA, being it possible that the rate of capture is greater than the rate of application.

However, even with the development of all the indicators, a very relevant analysis is necessary, as these can present different results, which makes it difficult to obtain an immediate and effective solution. It is necessary at the end with the indicators in hand to ascertain and identify the performance presented for the investment in question, and the impact on the profitability of the company (Bendlin, 2016).

2.4.1 Monte Carlo - Crystal Ball

The Monte Carlo method is a simulator whose central idea is the use of random samples with the objective of allowing the observation of the performance of a variable in a system or process, being an effective method in the solution of problems encountered in the development of the project In several sectors.

Because it is a method associated with risk management, it is possible to obtain an estimate for decision making by constructing an activity template model, which includes the development of appropriate formulas and equations, data collection, probability distribution Related to the input variables, definition of the form of data registration, and validation of the model.

After collecting the data, the variables can be modified to obtain various results, analyze the various scenarios presented and carry out the evaluation of the business plan. With the evaluation of the factors found through the method, it is possible to identify the factors of greater impact for activity both positively and negatively, which facilitates decision making for project implementation.

III. RESEARCH METHODOLOGY

The objective of this research is to analyze the costs of production and the expectations of return and risks associated with garlic agribusiness. The investments, production costs, profitability and risks inherent to this agribusiness were detailed.

The application was applied in the property of Mr. Américo Yoshio Nagano in the locality of São João dos Cavalheiros, in the municipality of Três Barras, Planalto Norte Catarinense in 01 (a) hectare.

3.1 CHARACTERIZATION OF THE RESEARCH

According to Gil (2010), this research is classified as applied in relation to its nature, since it covers studies designed to solve problems identified within the scope of the societies in which it is being researched.

With regard to its objective, it is classified as descriptive, presenting the investments, costs and main activities necessary to the exploitation of the garlic culture, aiming to study the expectation of return and risk of such culture. This type of research ntends to describe the characteristics of a certain population or phenomenon or to establish relations among variables (GIL, 2010).

It is classified as a survey, since it is characterized by direct interrogation, requesting information from a significant group of people (GIL 2010). As for its periodicity is characterized as a cross-sectional study, because the considerations will be in a period of time.

As for the technical procedures of data collection, it is classified as documentary based on internal controls, since it used documents elaborated with diverse and specific purposes.

The data analysis is classified as quantitative in the case of a research of economic science, with data collection applied to tables, graphs, involving statistical procedures (GIL, 2010).

3.2 COLLECTION, TREATMENT AND ANALYSIS OF THE DATA.

Data collection was initially performed through the internal controls related to the garlic production process, such as the costs incurred with planting, which corresponds to the direct labor costs plus the social charges, the inputs used in the planting and in the course of production And maintenance costs.

The information was published in a spreadsheet with the help of the Excel software, where the indicators Net Present Value (NPV), Annual Net Present Value (NPV), Benefit / Cost Index (IBC), Return on Investment Added (ROIA) , Internal Rate of Return (IRR), Minimum

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

Attraction Rate (TMA) and Investment Recovery Period (Playback).

Regarding the approach to the problem, it is classified as quantitative, since it sought to classify and analyze based on numbers, using resources and statistical techniques (Moresi, 2003).

The characterization was given to one hectare of garlic with the characteristics of productivity, cost structure, storage mechanisms and commercialization, in the locality of Campo São João dos Cavalheiros, in the municipality of Três Barras, Planalto Norte Catarinense.

IV. PRESENTATION OF RESULTS

The present study identifies the production costs of garlic in natura and garlic seed. It initially covers the operational costs of labor and equipment for land preparation, planting and after-care and consumed inputs, which are measured for the production of 1 hectare of garlic in natura and 1 hectare of garlic seed.

For the survey of production and maintenance costs, mechanized and manual operations related to the production itself were segregated.

The mechanized operations were calculated using the tractor, based on the hourly cost, according to Marion (2014), plus depreciation and other inputs according to tables 1.

Table.1: Cost of mechanized operations for garlic production / ha

Mechanized Operations				
Description	Specification	Unit value (R $)	Quantity Hours	Unproductive phase Formation lot 180 days (R $)
Soil preparation				
Gradation	Trator Case 165 Maxxum + Grade Aradora	103,51	3,5	362,29
Ground unpacking	Trator Case 165 Maxxum + Subsolador Asa	105,12	4	420,48
Plant Survey and Planting Fertilization	Trator Case 165 Maxxum + Enxada Rotativa	104,18	8	833,47
			TOTAL	**1.616,24**

Source: Authors (2016)

The manual operations were calculated from the use of working hours, based on the hourly cost man, according to Marion (2014), plus charges. The inputs applied in this step were also aggregated, according to tables 2.

Table.2: Cost of manual operations and inputs for garlic production / ha

Operações Manuais				
Description	Specification	Unit value (R $)	Quantity Hours	Unproductive phase Formation lot 180 days (R $)
Implantation				
Planting	Days / man	46,96	44	2.066,40
	SUBTOTAL			2.066,40
Fertilizers				
Fertilizer planting 09.25.15	kg	1,674	1000	1.674,00
	SUBTOTAL			**1.674,00**
Others				
Seeds of Garlic	kg	40,00	860	34.400,00
	SUBTOTAL			**34.400,00**
	TOTAL			**38.140,40**

Source: Authors (2016)

For the maintenance of the garlic culture the mechanized operations used, such as sprays, cover fertilization, among others, are shown in table 3.

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

Table.3: Cost of mechanized operations for garlic

Mechanized operations				
Description	Specification Machine Hour	Unit Value (R $) Per Hour	Number of hours	Increasing production Training in 180 days Total (R $)
Cultivation				
Spraying	Landine Tractor + Sprayer	85,96	6,08	522,86
rrigation	I Equip.Irrigação Completo	42,43	40,00	1.697,17
SUBTOTAL				**2.220,03**
TOTAL				**2.220,03**

Source: Authors (2016)

Manual operations for the maintenance of garlic, such as thinning / thinning, harvesting and the inputs applied in this step, are shown in Tables 4, 5, 6.

Table.4: Cost of manual operations for maintenance of garlic in natura / ha

Manual Operations				
Description	Specification	Unit Value (R $)	Number of hours	Increasing production Training in 180 days Total (R $)
Implantation				
Raleo / Roughing	Days / man	51,66	25,00	1.291,50
	SUBTOTAL			**1.291,50**
Harvest				
Manual Harvesting	Days / man	51,66	29,00	1.498,14
	SUBTOTAL			**1.498,14**
Fertilizers				
Cloreto	Kg	1,50	100,00	150,00
Nitrabor	Kg	3,92	100,00	392,00
Ureia	Kg	1,50	100,00	150,00
	SUBTOTAL			**692,00**

Source: Authors (2016)

Table.5: Cost of manual phytosanitary operations for garlic (Continua...)

Description	Specification	Unit Value (R $)	Number of hours	Increasing production Training in 180 days Total (R $)
	Fitossanitários			
Fertilizers	Mancozin	Lt	50,00	1,00	50,00
Fertilizers	Microxisto Tek-F	Lt	60,00	0,74	44,58
Fungicide	Academic	Lt	19,00	4,50	85,50
Fungicide	Cabrio Top	kg	59,60	8,00	476,80
Fungicide	Comet	Lt	112,28	1,10	123,51
Fungicide	Echo WG	kg	38,50	4,00	154,00
Fungicide	Galben - M	kg	40,00	7,50	300,00
Fungicide	Nativo SC 300	Lt	76,50	2,00	153,00
Fungicide	Neoran	kg	13,50	3,10	41,85
Fungicide	Porteiro	Lt	117,72	1,00	117,72
Fungicide	Proplant	Lt	104,00	1,00	104,00
Fungicide	Redshield 750	kg	39,15	1,00	39,15
Fungicide	Rovral	Lt	175,00	2,00	350,00

Fungicide	Stimo	Lt	48,66	3,40	165,44
Fungicide	Support	Lt	12,61	7,00	88,27
Fungicide	Vondozeb 800 WP	Lt	17,90	2,00	35,80
Fungicide	Zetanil	Lt	32,90	4,50	148,05
Herbicide	Poquer	Lt	88,56	0,90	79,70
Herbicide	Totril	Lt	184,80	1,67	307,69
Herbicide	Abamectin	Lt	38,00	0,50	19,00
Herbicide	Actara 250 WG	kg	225,00	0,10	22,50
Herbicide	Assist	Lt	9,00	1,10	9,90
Herbicide	Capture 400 EC	Lt	248,14	0,15	37,40
Herbicide	Cefanol	kg	33,90	5,50	186,45
Herbicide	Engeo Pleno	Lt	130,00	0,20	26,00
Herbicide	Kraft 36 EC	Lt	67,90	1,50	101,85
Herbicide	Paradox	Lt	24,00	2,00	48,00
Herbicide	Platinum NEO	Lt	139,20	0,15	20,88
Herbicide	Premio 20	Lt	588,60	0,30	176,58
Herbicide	Produtor	Lt	35,70	1,00	35,70
Herbicide	Sabre	Lt	18,50	4,00	74,00
Herbicide	Talstar	Lt	95,60	0,10	9,56
	SUBTOTAL				**3.632,89**

Source: Authors (2016)

Table.6: Cost of manual operations for maintenance of garlic seed / ha

Manual Operations				
Description	Specification	Unit Value (R $)	Number of hours	Increasing production Training in 180 days Total (R $)
Implantation				
Raleo / Roughing	Days / man	51,66	25,00	1.291,50
SUBTOTAL				**1.291,50**
Harvest				
Manual Harvesting	Days / man	51,66	29,00	1.498,14
SUBTOTAL				**1.498,14**
Storage				
Storage	Days	128,64	120,00	15.436,36
SUBTOTAL				**15.436,36**
Classification				
ClassificationManual	Days / man	51,66	25,00	1.291,50
	SUBTOTAL			**1.291,50**
Packing				
Packing	Un.	3,50	1.742,00	6.097,00
Manual Packaging Manual	Days / man	51,66	15,00	774,90
SUBTOTAL				**6.871,90**
Fertilizers				
Cloreto	kg	1,50	100,00	150,00
Nitrabor	kg	3,92	100,00	392,00
Ureia	kg	1,50	100,00	150,00
SUBTOTAL				**692,00**

Source: Authors (2016)

The production of garlic in natura and garlic seed presents in relation to the cost equal value until the harvesting process, having differentiation after harvest, as shown in tables 4 and 6, in relation to the part of the pesticides the cost is the same as demonstrated In table 5.

The sale price practiced for the commercialization of garlic in natura and garlic seed are shown in table 7, on the value of the revenue occurs incidence of funrural 2.3%.

Table.7: Practical Selling Price of Garlic

Sale lot 180 days - 1 hectare - approximately 7,260 pounds				
Description	Specification	Unit value (R $)	Quantity kg	Total Value (R $)
Garlic In Natura	Kg	12,00	7.260,00	87.120,00
	TOTAL			**87.120,00**
Sale lot 270 days - 1 hectare - approximately 4.355 kilos				
Garlic Seed	Kg	29,50	4.355,00	128.472,50
	TOTAL			**128.472,50**

Source: Authors (2016)

The cash flow in table 8, characterized by garlic in natura, constructed from the information of the intensive system, shows the periods in which cash was disbursed, represents all the operational costs and the necessary inputs for the production. Within an initial disbursement of R $ 39,756.64 for a crop. Be it garlic in natura and garlic seed.

Table.8: Statement of net cash flow from the commercialization of Garlic In Natura

Months	INVESTMENT (R $)	GROSS REVENUE (R $)	OPERATING COST (R $)	CASH FLOW (R $)
0	(39.756,64)			(39.756,64)
1			(698,11)	(698,11)
2			(876,42)	(876,42)
3			(3.441,35)	(3.441,35)
4			(1.903,29)	(1.903,29)
5			(917,24)	(917,24)
6	(39.756,64)	87.120,00	(3.501,90)	43.861,46
7			(698,11)	(698,11)
8			(876,42)	(876,42)
9			(3.441,35)	(3.441,35)
10			(1.903,29)	(1.903,29)
11			(917,24)	(917,24)
12		87.120,00	(3.501,90)	83.618,10

Source: Authors (2016)

Table.9: Statement of net cash flow from the commercialization of Garlic Seed

Months	INVESTMENT (R $)	GROSS REVENUE (R $)	OPERATING COST (R $)	CASH FLOW (R $)
0	(39.756,64)			(39.756,64)
1			(698,11)	(698,11)
2			(876,42)	(876,42)
3			(3.441,35)	(3.441,35)
4			(1.903,29)	(1.903,29)
5			(917,24)	(917,24)
6	(39.756,64)		(5.357,23)	(45.113,87)
7			(3.160,98)	(3.160,98)
8			(4.735,51)	(4.735,51)
9		128.472,50	(18.418,71)	110.053,79

10		(1.903,29)	(1.903,29)
11		(917,24)	(917,24)
12		(5.357,23)	(5.357,23)
13		(3.859,09)	(3.859,09)
14		(3.859,09)	(3.859,09)
15	128.472,50	(14.977,36)	113.495,14

Source: Authors (2016)

4.1 INTERPRETATION OF RESULTS

The risk and return indicators of the Multi-Index Methodology were prepared based on an initial investment of R $ 39,756.64, from an MRA of 0.79% per month for a period of 06 months for fresh garlic and for 9 months For garlic seed, as presented in table 10.

Table.10: Marketing indicators for garlic in natura and garlic seed

		Garlic In Natura	Garlic Seed
RETURN	PRESENT VALUE	102.945,19	94.060,63
	NET PRESENT VALUE	63.188,55	54.303,99
	VALUE PRESENTED LIQUID ANNUALIZED	5.541,47	3.744,52
	INDEX BENEFIT / COST (IBC)	2,59	2,37
	ROIA / YEAR	8,25%	7,44%
RISK	INTERNAL RETURN RATE (IRR)	10,69%	8,93%
	TMA / TIR INDEX	0,89	1,15
	RISK OF MANAGEMENT	0,50	0,50
	RISK OF BUSINESS	0,51	0,51

Source: Authors (2016)

Regarding the return indicators, from a TAM of 0,79% am for the cultivation of garlic seed and garlic in natura, the expectation of recovering the investments made is confirmed, from the Present Value of R $ 94,060 , 63 for garlic seed and a present value of R $ 102,945.19 for garlic in natura, generating a Net Present Value of R $ 54,303.99 and R $ 63,188.55, respectively.

The IBC - Benefit / Cost Index, which is an indicator that measures the expectation of return for each unit of capital invested, a result of R $ 2.37 for each R $ 1.00 investment of garlic seed and of R $ 2,59 for each R $ 1.00 investment in garlic in natura.

The ROIA - Additional Return Due to Investment is estimated at 7.44% a.a for garlic seed and 8.25% for garlic in natura.

Regarding the risk indicators, the IRR - Internal rate of return found was 8.93% for garlic seed and 10.69% for garlic in natura. The TMA / TIR index found was 1.15 for garlic seed and 0.89 for garlic in natura.

In relation to the risk of management that is associated with the experiences and knowledge of the production and marketing process that the producer has on the subject, the same can be considered of 0.50, due to the availability of private technical guidance published in this segment. Return and risk are two variables that go together in the investment world. The greater the possibilities of return the greater the risks involved, as shown in table 11.

Table.11: - Management Risk

Skills	**Perception**
Economic Aspects	0,45
Industry Trends and Segment	0,55
Production Process and Innovation	0,50
Negotiation with stakeholders	0,45
Positioning Strategy	0,50
Average per area	0,41
Perceived Management Risk	0,50

Source: Souza and Clemente (2012, Adapted)

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

In relation to business risk, it is characterized to problems that may affect the project environment, enters the risk market and its possible threats that may occur during the process, such as the weather, if during the process was better favorable for The business succeed, where there are many variations can compromise the outcome of the business. The same can also be considered of 0.51 because it is related mainly to the climate, since the lack of rain can damage the production of the vegetables, as shown in table 12.

Table.12: Business Risk

PEST		5 Porter Forces		SWOT	
Aspect	Perception *	Aspect	Perception *	Aspect	Perception *
Legal policy	0,30	Starter	0,55	Weaknesses	0,45
Economic	0,50	Substitutes	0,50	Threats	0,65
Cultural partner	0,45	Providers	0,60		
Technological	0,30	Customers	0,45		
Demographic	0,30	Competitors	0,65		
Average	0,37	Average	0,55	Average	0,55
Perceived Business Risk =				0,51	

Source: Souza and Clemente (2012, Adapted)

4.2 FEASIBILITY ANALYSIS BY MONTE CARLO SIMULATION

In the simulation, the quantity of 7,260 kilograms of fresh garlic and 4,355 of garlic seed was considered as uncertain variables or input variables for the simulation, and their respective sale price in each situation, called assumptions.

As for the variable sale price, the probability density function was chosen, with a value of R $ 12.00 / kg for the commercialization of garlic in natura and R $ 29.50 / kg for Garlic seed values are averages of the value practiced in the market according to surveys carried out during the course of the study, characterizing as a continuous probability distribution.

For forecast variables, we chose the NPV (Net Present Value), ROIA (Additional Return Due to Investment). The number of replicates considered for the executed result was 5,000.

After the simulation was carried out, it was possible to obtain the frequency graphs with the minimum, mean and maximum values of the variables, median, variance and standard deviation, among other information.

Below you can see the graphs related to the selling price for garlic in natura and garlic seed. Figure 1 shows that the average for NPV (net present value) is R $ 63,314.57 for garlic in natura and 54,510.95 for garlic seed, values very close to those found in the Multi-Index, which are R $ 63,188, 55 and R $ 54,303.99 respectively. The minimum value was R $ 41,121.18 for garlic in natura and 24,586.85 for garlic seed, and a maximum of R $ 85,587.99 for garlic in natura and 88,522.71 for garlic seed.

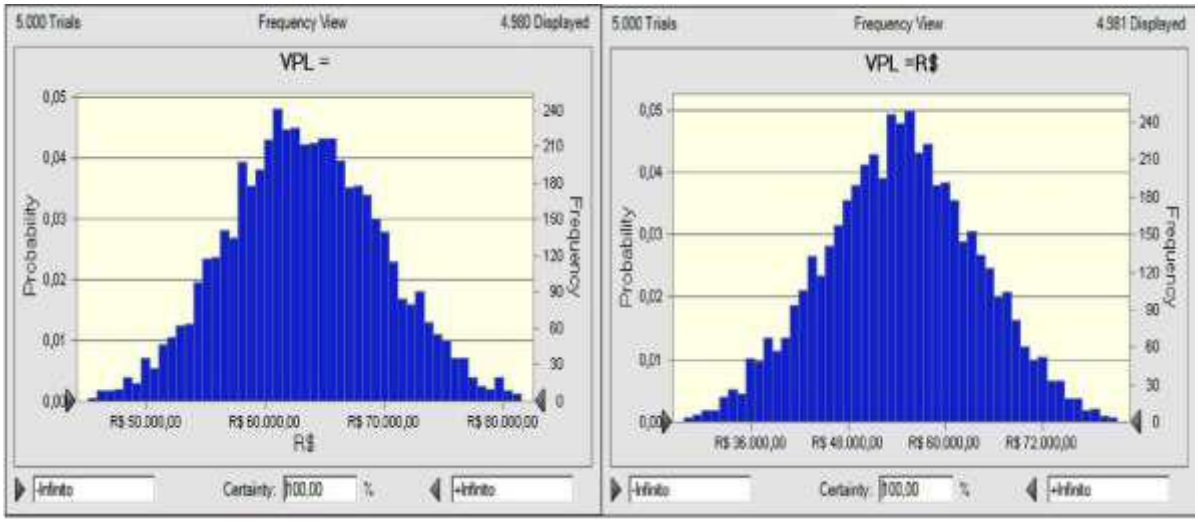


Fig.1: Frequency graph and statistics of the output variable NPV - Net Present Value Garlic In Natura and Garlic Seed

Source: Authors (2016)

http://dx.doi.org/10.22161/ijeab/2.1.64 *ISSN: 2456-1878*

Figure 2 shows that the average for ROIA (Additional Return Due to Investment) is 8.25% for garlic in natura and 7.42% for garlic seed, values very close to those found in the Multi-Index, which are 8 , 25% and 7.44% respectively. The minimum value was 6.10% for garlic in natura and 4.09% for garlic seed, and maximum of 10.04% for garlic in natura and 10.25% for garlic seed.

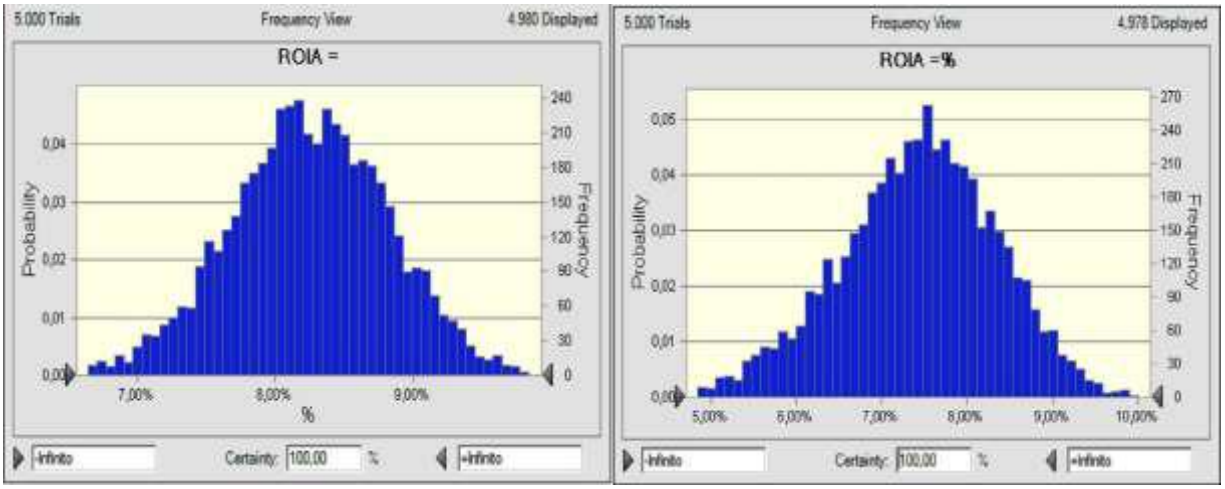


Fig.2: Frequency graph and output variable statistics ROIA - Additional Return from Investment Garlic In Natura and Garlic Seed

Source: Authors (2016)

V. FINAL CONSIDERATIONS

The purpose of this study was to analyze the expectations of return and the risks associated to the implementation of garlic cultivation, bringing the comparison between the commercialization of garlic in natura and garlic seed. The methodology used was the Multi-index, presented and discussed by Souza and Clemente (2008). Monte Carlo simulations were also carried out to verify and confirm the decision to invest in this agribusiness.

In the decision to invest in garlic cultivation there are two variables that determine the choice of garlic production for garlic consumption in natura or garlic seed production, productivity and marketing price.

The risk indicators for marketed garlic in natura are NPV of R $ 63,188.55 and ROIA of 8.25%, confirmed by Crystal Ball. When applied the competitive strategy in the production of garlic seed with expectation of return in 09 months, the impacts are of a NPV of R $ 54,303.99 and a ROIA of 7.44%.

The results show that the production of garlic in natura constitutes a more profitable activity, compared to the production of garlic seed.

Management and business risks were considered medium-sized, since public or private technical guidance is available for the agricultural segment, and such agribusiness is exposed to interference mainly from the climate.

The research concludes that the use of the multi-index methodology, its set of indicators for analysis, the evaluation of the return on investment and the associated risks improve the perception of the rural administrator, contributing with satisfactory results in its investment portfolio (Bendlin 2016).

The case study was carried out in a region in the interior of Santa Catarina that adopts garlic production, aiming to meet the maximum productivity; However, it is advisable to develop this study in other regions or small farms, under the possibility of obtaining different results, due to the possible variations, such as the inputs or the changes, that undergo market changes.

REFERENCES

[1] Association of Horti-Fruti Producers and Distributors of the State of São Paulo. Garlic. 2009. Available at: <http://www.aphortesp.com.br/alho.html>. Accessed on: 13 nov. 2014.

[2] BENDLIN, Luciano; SOUZA, A. ; VICHINHESKI3, K. A. . Methodology for the Construction of the Efficient Border For Selecting Investment Projects: A Multi-Index Approach. International Journal of Modern Engineering Research, v. 6, p. 8-22, 2016.

[3] BENDLIN, LUCIANO; SOUZA, A.; SENFF, C.O.; STAFIN, O. O.; PEDRO, J. J.. Production costs, return expectations and risks associated with eucalyptus plantation in the region of Planalto Norte Catarinense / Brazil .. Costs e @ gronegócio Online, v. 2, p. 2-33-33, 2016.

[4] National Association Of Garlic Producers. Brazilian garlic. Available at:

<http://www.anapa.com.br/simples/?page_id=5>. Accessed on: 06 Aug. 2014.

[5] Supply Centers Of The State Of Santa Catarina. Available at <http://www.ceasasc.com.br/cotacaodeprecosyg> Accessed on: 12 Sep 2015.

[6] GIL, Antonio Carlos. How to design research projects. 5.ed. São Paulo: Atlas, 2010.

[7] Lourenço, Joaquim Carlos. The evolution of Brazilian agribusiness in the current scenario. 29 Aug. 2008. Available at: <http://www.administradores.com.br/artigos/ economia-e-financas / a-evolucao-do-agribusiness-brasileiro-no-cenario-actual / 24824 />. Accessed on: 08 Oct. 2014.

[8] Lucini, Marco Antonio. Culture of garlic. Curitibanos: EPAGRI. Available at: <http://www.anapa.com.br/principal/images/stories/ documentos/Cultura_do_alho_no_sul.pdf>. Accessed on: 13 nov. 2014.

[9] Marion, José Carlos. Rural accounting. 14.ed. São Paulo: Atlas, 2014.

[10] Portal Hortas. How to Plant Garlic. Available at: <http://hortas.info/como-plantaralho>. Accessed on: 13 nov. 2014.

[11] Our Garlic Magazine. Brasília: ANAPA, ed. 1, ten. 2008. Available at: <http://www.anapa.com.br/simples/wp-content/uploads/2013/04/1.pdf>. Accessed on: 20 Oct. 2014.

[12] Our Garlic Magazine. Brasília: ANAPA, n. 19, apr. 2014. Available at: <http://www.anapa.com.br/simples/wp-content/uploads/2013/05/ Nosso_Alho_19_abril_2014-vers% C3% A3o-final-completo.pdf>. Accessed on: 30 Aug. 2014.

[13] Our Garlic Magazine. Brasília: ANAPA, n. 20 Aug. 2014. Available at: <http://www.anapa.com.br/simples/wp-content/uploads/2014/ REVISTA_Nosso_Alho_n20_agosto_2014.pdf>. Accessed on: 24 jun. 2015.

[14] Rosamilha, Nelson. Monte Carlo Simulation Method. Jun.2012. Dimension:

[15] <Http://nelsonrosamilha.blogspot.com.br/2012/06/metodo-de-simulacao-de-monte-carlo.html> Accessed on 18 Aug 2015.

[16] Santos, Daniel Ferreira dos; SANTOS, Renato da Costa dos; CATAPAN, Anderson. Administration of agribusiness in Brazil. 1.ed. Curitiba: CRV, 2014.

[17] Souza, Alceu; CLEMENT, Ademir. Financial decisions and investment analysis. São Paulo: Atlas, 2008.

http://dx.doi.org/10.22161/ijeab/2.1.65 *ISSN: 2456-1878*

Mapping of Planning Land Use Based GIS in Sub-District Kintamani, Bali

Made Sri Sumarniasih

Department of Agroecotechnology, Faculty of Agriculture, Udayana University, Denpasar, Bali-Indonesian

Abstract*—Research land use plans implemented in Kintamani Sub-district, Bangli Regency, Provence of Bali. The Soil samples were collected by overlaying maps of soil types, land use maps, maps of slope, so we get a map of the land unit with 48 sample points. . The scoring method used to analyze slope, soil type and rainfall. The results of the analysis are used to plan the direction of land use in the Kintamani district. Land use is as a buffer zone and protected areas, land outside the forest area. The existing condition of the land is owned by farmers, the use of land in the buffer zone, with intercropping and organic matter or mulching, while in protected areas which are land use under the conditions then existing rules soil and water conservation.*

***Keyword—mapping, land use planning, GIS*.**

I. INTRODUCTION

The use of land in the sub-district of Kintamani is still less attention to the rules of conservation, in areas with a slope of over 25%, is still used for farming seasonal crops. As is known sub-district of Kintamani is upstream of several rivers vital for the region Bali, one of which is the Watershed (DAS) Ayung [4]. Overcome this, is necessary improvement efforts follow the rules of soil and water conservation to reduce damage to the land and can provide benefits for farmers, and increased revenue. The results of this study are expected to provide a positive impact on soil and water conservation, conservation area planted area, the increase in the production of agricultural commodities and sustainably to improve the welfare of farmers [1].

Geographic Information Systems (GIS) is an information management tool that is strongly associated with mapping and analysis of all things and events that occur on earth. GIS is a computer-based system used to store, manipulate, and analyze geographic information, original information earth's surface is presented in the form of a map created manually, then with the presence of GIS, information is processed by a computer, and the results in the form of digital maps [3]. SIG not only function "map maker", but further than that, SIG is able to produce an information system that is applied, which can be used by planners or decision maker for the purposes of processing resources that exist in the region. Developments will update spatial data in the era of science and technology advancement is increasingly needed, but requires accurate data, practical, and efficient. Their GIS applications can act as a substitute for maps wall, which was replaced with the display layers of digital maps (spatial database) with symbols and colors that appeal. Development application SIG is no longer something that is considered expensive, because it has a lot of GIS software which is free and open source, such as Map Window, Quantum GIS, and others [5], [6].

Based on the problems outlined above, it is necessary to study the direction of land use plans based on the assessment of scores of slope, soil type and rainfall. The results of the analysis with the scoring method was presented in the form of digital maps using GIS application.

II. RESEARCH METHODS

2.1. Research Sites

The study was conducted on multiple land use in the district of Kintamani, Bangli, Bali Province. Geographically located at 115º18'27" BT until 115º23'00"BT and 08º10'00"LS until 08º20'00"LS. Tools that be used in this study were: 1) map appearance of the earth (1: 25,000), a map of soil types (1: 25.000), land use maps (1: 25,000) [2], (2) GPS, (3) plastic bags, paper labels, Abney level, meter, ring samples, knives, pens, and drill ground; (4) Photo camera for creation of documentation; and (5) A set of PC computer for data processing

2.2. Research Procedure

Research procedures are as follows:

1) Digitizing a map of the study area.
2) Making a map overlay of the land unit based of map soil type, slope maps and land use maps, in order to obtain a map of the land unit "Fig 1". [8].
3) Based on the map of the land unit gained 48 points soil samples to be analyzed in the Laboratory of Soil and Environment Faculty of Agriculture, University of Udayana.

http://dx.doi.org/10.22161/ijeab/2.1.65 *ISSN: 2456-1878*

4) Analyze slope, soil type and daily rainfall with the scoring method.

2.3. Analysis Method

Determining the direction of the use of land use by methods proposed by Sukartiko [7]. The system is based on three criteria: the field slope, soil type according to sensitivity to erosion and precipitation daily average. Each of these factors in value/ the weight in accordance with the level of influence relative to the sensitivity of the area concerned on erosion.

Classification for each factor is as follows:

1. Slope Field
   A. = 0 to 3% (flat)
   B. = 3 to 8% (ramps or wavy)
   C. = 8 to 15% (slightly sloping or bumpy)
   D. = 15 to 30% (sloping or hilly)
   E. = 30 to 45% (rather steep)
   F. = 45 to 65% (steep)
   G. = more than 65% (very steep)
2. Soil type according to their susceptibility to erosion
   Class 1 = Alluvial Land Glei, Planosol, Hidrimorf gray, Laterik ground water. (not case sensitive)
   Class 2 = Latosol (somewhat sensitive)
   Class 3 = Brown Forest Soil, Non Calsic Brown, the Mediterranean (less sensitive)
   Class 4 = Andosol, laterite, Grumusol, Podsol, Podsolic (sensitive)
   Class 5 = Regosol, Litosol, Organosol, Renzina (very sensitive).
3. The intensity of the rainfall average
   Class 1 = 0 to 13.6 mm / day (very low)
   Class 2 = 13.6 to 20.7 mm / day (low)
   Class 3 = 20.7 to 27.7 mm / day (medium)
   Class 4 = 27.7 to 34.8 mm / day (high)
   Class 5 = > 34.8 mm / day (very high).

The weights of each class is 20 to slope factor field, 15 to soil types and 10 for an average rainfall intensity, thus for each class factors are as follows:

1. Slope field
   Class 1 = 20
   Class 2 = 40
   Class 3 = 60
   Class 4 = 80
   Class 5 = 100
2. The type of soil according to sensitivity to erosion
   Class 1 = 15
   Class 2 = 30
   Class 3 = 45
   Class 4 = 60
   Class 5 = 75
3. The intensity of the average daily rainfall
   Class 1 = 10
   Class 2 = 20
   Class 3 = 30
   Class 4 = 40
   Class 5 = 50

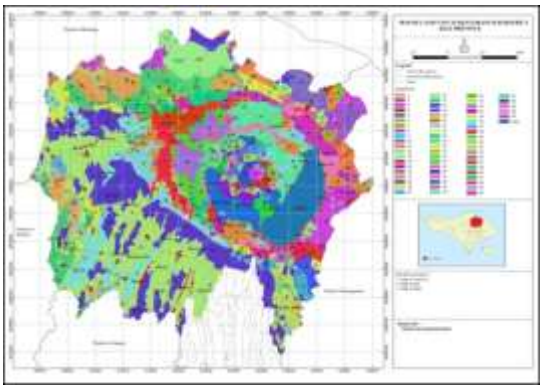


Fig 1. Unit Maps of Land and Soil Samples

Furthermore, the determination of the direction of land use for each unit of land is done by showing the values score above three factors by observing the status of the land area. Differentiated land area of land within the forest and land outside the forest area. In accordance with its designation, land within the forest can be classified into: protected forests, production forests, forest preserves and forests. Land outside the forest area is classified as a protected area, buffer area, cultivated area of crops and seasonal crops cultivation areas.

A). In the Forest Area

1. Forest Preserve
   Based on the sum of the value of the score, then an area declared to be a forest area, fostered and maintained as protected forests if the total value of the score is equal to or greater than 175.
2. Production Forest
   Production forests are classified into: a limited production forest logging with a total score of 125-174 and production forests free total score equal to or less than 124, outside the forest area of nature reserves, forests and other conservation tour.
3. Forest nature reserve and Tourism
   Determination of nature reserves and tourist assessment is not based on these factors, but more focused on the interests of culture, germ plasm conservation and recreation.

B). Beyond the Forest Area

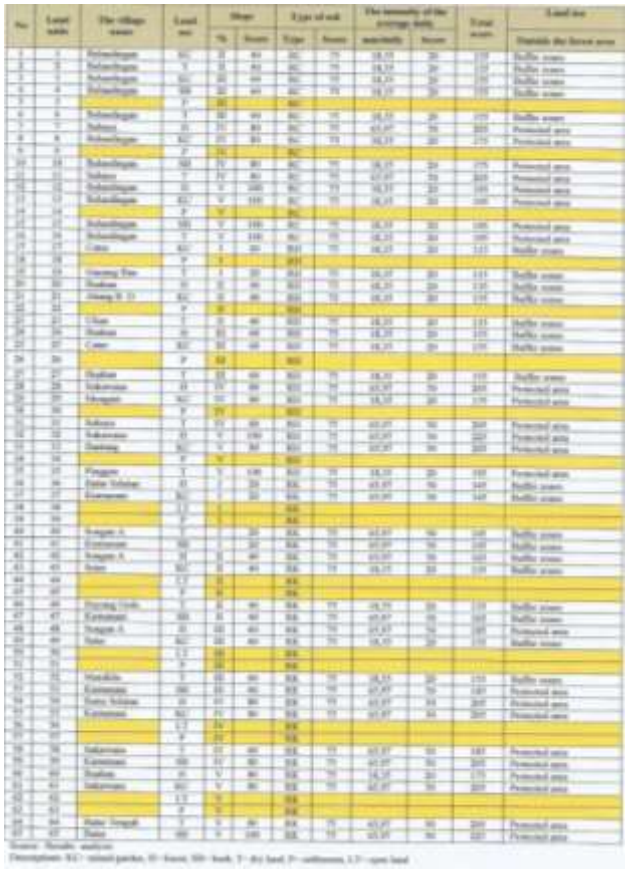
1. Protected Areas
   Establishment of protected areas in the same way that the establishment of protected forests if the

http://dx.doi.org/10.22161/ijeab/2.1.65 *ISSN: 2456-1878*

amount of the value of a score equal to or greater than 175.

2. Buffer Zone
   An area is declared as a buffer zone when a score is 125 -174 and or meet some general criteria as follows:
   - The physical state it is possible to do farming region economically.
   - Economically convenient location developed as a buffer zone.
   - No adverse aspects of ecology/ environment.
3. Region Annual Crop Cultivation
   An area designated as an area of cultivation of annual crops if the region have a total score of equal to or less than 124 and is suitable or annual crop farming should be developed (timber, plantation crops, and industrial plants).
4. Plant of Annuals
   Areas with criteria such as the determination of annual crop cultivation area, but the area is located on land owned, indigenous lands and state lands that should be developed farming seasonal crops.

Table 1. Land Use Planning in Sub-District Kintamani



III. RESULTS AND DISCUSSION

Research was conducted on different land use in the district of Kintamani Bali. This region is upstream of the watershed (DAS) Ayung, one of the longest river basin in the province of Bali, then in determining the direction of land use must pay attention to the rules of soil and water conservation. Under these conditions, the factors that influence erosion are also factors that need attention, and can be broken down as follows:

1) Climate is a factor that affects hydrological processes and is an important element in the process of hydrology. Elements of the climate that affect most of the hydrological processes that are rainfall. Data rainfall in the district of Kintamani obtained from Tuban Meteorology and Geophysics Agency (BMKG). At the observation post in the village of Kintamani, the amount of rainfall average was 68.97 mm / day and for the observation post Catur 38.73 mm /day.
2) The type of soil in the district of Kintamani (BPDAS, 2009), is a type of Regosol Humus soil, Regosol Regosol Brown and Gray each covering an area of 8729, 74 ha; 12014.61 ha and 412.90 ha. This type of soil is sensitive to erosion, if the land use does not follow the rules of soil and water conservation. If it rains with high intensity because of the type of soil susceptible to erosion, the surface soil layer (top soil) is easy to carry rainwater runoff into the form of erosion or landslides.
3) Factors topography of research showing the shape of the area, including differences in slope steepness or slope. Increasingly steep slope, the greater the rainfall becomes runoff or erosion caused by rainwater was not given the opportunity infiltrated. Slope area of research is the slope of class I to class V.
4) The land use is upland area of, 55 ha; 994.73 ha mixed farms; 318.51 ha of forest; bush 175.64 ha; open land and undeveloped land 163.46 ha and 78.34 ha.

The results of the analysis with the scoring method using the data of slope, soil type and rainfall daily average, then the use of land in the district of Kintamani directed to the protected area and the buffer zone is presented in Table 1. Referral land use for 48 sample points vary according to the value of scores obtained from summing the values score above three factors with pay attention ownership status of the land.

Status distinguished tenure on the land in the forest area and land outside the forest area. Land in the forest area can be classified into: protected forests, production forests, forest preserves, and forests. Land outside the forest area is classified into: protected areas, buffer zones, annual crop cultivation area and cultivation area of seasonal crops.

(A) Land in the area is divided into:

1. Forest Preserve: 175- value or more

http://dx.doi.org/10.22161/ijeab/2.1.65 *ISSN: 2456-1878*

2. Production Forest: value 125-174
3. Forest Nature Reserve and forest tour

(B) Land outside the forest area

1. Protected Areas: equal to 175 or greater.
2. Buffer Zone: value 125-174
3. The annual crop cultivation area: the value is less than 124
4. Region cultivation of seasonal crops: lands, indigenous lands and state lands

Fig.2: Land Use Planning in Sub-Distrct Kintamani

The total scores obtained from the analysis are:
135-225, because the land is outside the forest area and owned by farmers, then the direction of land use are:
1. Protected areas
2. Buffering Region

Land unit: 1, 2, 3, 4, 6, 17,18, 20, 21, 23, 24, 25, 27, 36, 37, 40, 41, 42, 43, 46, 47, 49, and 52. Have a total score of above 125 is (135 to 175). That is all geared land use as a buffer zone. The research location is outside the forest area and land belonging to the community or farmer, the land use to prevent land degradation due to erosion, is the use of conservation land. Communities in land use pattern suggested by intercropping and addition of organic materials or mulching. In addition to preventing erosion also maintain the fertility and quality of the land, so that high productivity can be achieved land unit that scored above 175 ie (175-225) is a unit of land: 7, 8, 10, 11, 12, 13, 15, 16, 28, 29, 31, 32, 33, 35, 48, 52, 53, 54, 58, 59, 60, 62, 64 and 65 as a protected area, but because of the study sites land ownership dominated owned by the community, then the direction of land use adapted to the land use existing. If previous already used for mixed farms it should be recommended to the conservation cropping pattern: the pattern of intercropping with organic matter or mulch. Whereas if the conditions existing is bush or forest, then referral to a protected area of forest remains, while in the bush land used for mixed garden high density, or planting timber trees by observing the rules of soil and water conservation. planning of land use is presented in "Fig 2".

IV. CONCLUSION

Based on the results of the analysis can be concluded:

1) Land use in Kintamani are buffer zones and protected areas
2) In the buffer zone should be done planting intercropping (seasonal plant with annual crops or perennial plants with annual crops) and added organic matter or mulching.
3) In the protected area because the farmer's land as the existing condition of land use, cropping patterns which can be directed attention to the rules of land conservation.

ACKNOWLEDGEMENTS

Thanks to Ministry of Research and Technology who gave Grant Leading Universities so that this research can be done, and the Institute for Research and Community Service (LPPM) for all facilities provided.

REFERENCES

[1] Arsyad, S. 2000. Soil and Water Conservation. Press Publisher IPB, Bogor.
[2] Bakosurtanal. 2000. Map Rupa Bumi Indonesia. Jakarta.
[3] Barus B., and U.S. Wiradisastra, 2000, Geographic Information Systems, Laboratory of Remote Sensing and Cartography, Soil Department, Faculty of Agriculture, IPB, Bogor.
[4] BPDAS. Unda Anyar. 2009. Integrated Watershed Management Plan Watershed SWP Pangi Ayung. Report Watershed Management Center Anyar Unda. Denpasar.
[5] Darmawan Setiadi, I Made Nyoman Piarsa, Ni Made Ika Mandenni Marini. 2015. Mapping Geographic Information System Web-Based Population Growth Rate. In PIGEON Journal VOL. 3, NO. December 3, 2015. ISSN: 2252-3006. P. 180-189.
[6] Ramadona, A.L and Kusnanto.2010 Day. Quantum GIS Applications for Environmental Information System. Publisher BPFE Yogyakarta.
[7] Sukartiko, B. 1988. Land Capability Assessment System for Determination Tutorial Land Use Planning in the Framework of Watershed Management. Technical Meeting Standardization System of Classification and Land Survey Methods, Cibinong, Bogor
[8] Sumarniasih, Made Sri and Made Antara. 2016. Conservation planning on eroded land based of local wisdom in Kintamani sub-district.

http://dx.doi.org/10.22161/ijeab/2.1.66 *ISSN: 2456-1878*

Photocatalytic Degradation of Azo Dye (Methyl Red) In Water under Visible Light Using Ag-Ni/TiO_2 Sythesized by γ - Irradiation Method

Vo Thi Thu Nhu[1,2], Do QuangMinh[1], Nguyen Ngoc Duy[3], Nguyen QuocHien[3*]

[1]Ho Chi Minh City University of Technology, 268 Ly ThuongKiet, District 10, Ho Chi Minh City, Vietnam
[2]University of Technical Education Ho Chi Minh City, 1 Vo Van Ngan Street, Thu Duc District, Ho Chi Minh City, Vietnam
[3]Research and Development Center for Radiation Technology, 202A Street 11, Linh Xuan Ward, Thu Duc District, Ho Chi Minh City, Vietnam

*Corresponding author.
E-mail address: hien7240238@yahoo.com (N.Q. Hien), Phone: 84 8 38975922, Fax: 84 8 38975921

***Abstract*—** *Commercial TiO_2 (P25) co-doped with bimetallic silver and nickel nanoparticles (Ag-Ni/TiO_2) was prepared by γ-irradiation method. The properties of Ag-Ni/TiO_2 were characterized by X-Ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), diffuse reflectance spectroscopy (DRS), energy dispersive X-ray spectroscopy techniques (EDX) and surface area measurement by Brunauer-Emmett-Teller (BET) method. The size of silver and nickel nanoparticles was determined by TEM to be of 1-2 nm.The photo-catalytic degradation of azo dye methyl red in the aqueous suspensions of TiO_2 and Ag-Ni/TiO_2 under visible light was carried out to evaluate the photo-catalytic activity. Results showed that Ag-Ni/TiO_2 was found to enhance photo-degradation efficiency of azo dye metyl red compared to commercial TiO_2. The results showed that Ag 3% (w/w) and Ni 1.5% (w/w) co-doped TiO_2 had the highest photoactivity among all studied samples under visible light. Thus, γ-irradiation method can be suitably applied to prepare photo-catalyst of Ag-Ni/$TiO_2$with highly photocatalytic activity.*

***Keywords*—TiO_2, silver, nickel, nanoparticles, photocatalytic,γ-irradiation.**

I. INTRODUCTION

Photocatalytic reactions at the surface of titanium dioxide have been attracting much attention in view of their practical applications to environmental treatment [1]. The material TiO_2 is a well-known photocatalyst for its high efficiency, low cost, physical and chemical stability, widespread availability, and noncorrosive property [2].NanoTiO_2 shows relatively high reactivity and chemical stability under ultraviolet light (<387nm), whose energy exceeds the band gap of 3.2 eV in the anatase crystalline phase. The development of photocatalysts with high reactivity under visible light (> 400 nm) should allow the main part of the solar spectrum, even under poor illumination of interior lighting, to be used [3]. Several approaches for nanoTiO_2modification have been reported [4]. These included dye sensitization, semiconductor coupling, impurity doping, use of coordination metal complexes, and metal deposition. A combination of two or more kinds of metals has been widely applied in various materials to enhance the performance and reliability of the materials. The incorporation of metals in the titanium dioxide crystal latticemay result in the formation of new energy levels between valence bandand conduction band, inducing a shift of light absorption towards the visible light region. Possible limitations are photocorrosion and reduce charge recombination at metal sites[5].

The use of Ag and Ni for bimetallic catalyst has been reported as the effective method to improve the efficiency of various reactions[6, 7]. Ag-Ni/cacbonnanotube for glucose oxidation[6]. Silver and nickel doped TiO_2 by sol-gel method applied against bacteria under UV and visible light irradiations[7].

Various methodsfor the synthesis of modified TiO_2photocatalyst included precipitation [8] , hydrothermal, solvothermal[9], chemical vapour deposition[10], and electrospinning[11] , radiolysis [12]. Among several methods for modified nanopowder TiO_2, radiolysis method usingγ-irradiation is advantageous because the experiment can be

http://dx.doi.org/10.22161/ijeab/2.1.66 *ISSN: 2456-1878*

carried out at very mild conditions, ambient pressure and room temperature with high reproducibility and it is the unique method [13, 14, 15]. In addition, Zhang et al. (2010) [16] also synthesized Ag-Ni alloy nanoparticles by radiolytic method. So, in this study, we reportedthe preparation of Ag and Nico-doped on TiO_2 (Ag-Ni/TiO_2)byγ-irradiation method andstudied of its photoactivityof degradation of methyl redin water. The properties of the catalysts were characterized by X-ray diffraction(XRD), diffuse reflectance spectroscopy (DRS), surface area measurement by Brunauer-Emmett-Teller (BET) method, transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy techniques (EDX), X-ray photoelectron spectroscopy (XPS). The photocatalytic performance, reaction kinetic, and reusability of the catalysts were also investigated.

II. EXPERIMENTAL

2.1. Materials

Silver nitrate ($AgNO_3$)and nickel nitrate hexahydrate($Ni(NO_3)_2{\cdot}6H_2O$)were purchased from China used as dopant metal salts. Titanium dioxide, TiO_2 (Degussa P25)wasfrom Germany used as the supportand ethanol was from China. Methyl red from China was used as the model of organic pollutant for photocatalyticdegradation study.

2.2. Sample preparation

A series of TiO_2 samples was prepared by codoping with silver and nickel in the range of 0.75–3% (w/w) by γ-irradiation method. 2 g TiO_2 and 10 ml ethanol were added into 90 ml distilled water and stirred. The required amount of $AgNO_3$ and $NiNO_3$ were then added to the TiO_2 suspension mixture with variousmass ratios of Ag and Ni. The reduction of Ag^+ and Ni^{2+} was carried out by γ-irradiation on a γ- ^{60}Co sourceusing gamma chamber GC – 5000, BRIT, India at the Nuclear Research Institute, Da Lat, Vietnam with dose rate of 2.5 kGy/h and absorbed dose range from 15.8 to 46.5kGy measured by a dichromate dosimetry system [17] at room temperature and under atmospheric pressure. The TiO_2 doped with Ag and Ni photo-catalyst was separated by centrifugation, washed by distilled water and dried at 60ºC. The detailed parameters of experiments were listed in Table 1.

2.3. Characterization of TiO_2 doped with Ag-Ni nanoparticles

The size of Ag and Ni nanoparticles doped on TiO_2 catalystwas characterized by TEM images on a JEM 1010, JEOL, Japan and XRD patterns were measured on D8 Advanced, Brucker, Germany using a Cu Kα (λ = 0.15418 nm). The specific surface areas of samples were determined by nitrogen adsorption at 77K using Quantachrome 1994-2010 instrument of Germany using BET method.The solid UV–vis DRS was carried out using JASCO V550 model UV-vis spectrometer. XPS analyses were obtained with a ULVAC PHI instrument, equipped with Al KαX-ray source. The morphology and the elemental content of the catalyst were investigated with SEM (Hitachi SEM S-4800) coupled with a Genesis 4000 EDX spectrometer.

2.4. Photocatalytic degradation activity

0.025 g of photocatalystswas added to 50 mL methyl red(10^{-5}M). The solution with the catalyst was stirred in the dark for 1hour for the solution to attain absorbed equilibrium.It was then irradiated using the 150 W halogen lamp (the visible light source) at a distance of 40 cm from the solution level and the temperature of the reactor was controlled at 30± 2°C. After period of time of 20, 40, 60, 80, 100 and 120 min, the aqueous suspension was filtered through centrifugation to remove catalyst particles. Each set of experiment was performed three times. MR concentration was estimated by colorimetric method using UV-vis spectrophotometer (Biochrom, Libra S32).

The photo-catalytic kinetic of methyl red degradation was described by the pseudo-first-order kinetic as follows:

$$v = -dC/dt = -kC \text{ (or } C = C_o e^{-kt}) \quad (1)$$

Where v is the reaction rate, C is the concentration of MR dye at certain reaction time, C_o is the initial concentration of MR, k is rate constant and t is reaction time.

After the integration of the equation (1), the model can be expressed by the following equation (2):

$$\ln(\frac{C_o}{C}) = kt \quad (2)$$

A plot of $\ln(\frac{C_o}{C})$ with time will yield a linear plot with slope k.

http://dx.doi.org/10.22161/ijeab/2.1.66 *ISSN: 2456-1878*

III. RESULTS AND DISCUSSION

Table 1.Composition and absorbed dose for preparation of different Ag-Ni/TiO_2 samples

Sample name	Weight of precursor	V(H_2O): V (C_2H_5OH)	Dose (kGy)	Irradiation time (min)
Ag0.75-Ni1.5/TiO_2	m$AgNO_3$ =0.0236 g m$Ni(NO_3)_2.6H_2O$ = 0.1488 g	90 ml: 10 ml	23.3	1398
Ag1.5-Ni0.75/TiO_2	m$AgNO_3$ = 0.0472 g m$Ni(NO_3)_2.6H_2O$ = 0.0744 g	90 ml: 10 ml	15.8	948
Ag1.5-Ni1.5/TiO_2	m$AgNO_3$ = 0.0472 g m$Ni(NO_3)_2.6H_2O$ = 0.1488 g	90 ml: 10 ml	26.0	1560
Ag1.5-Ni3.0/TiO_2	m$AgNO_3$ = 0.0472 g m$Ni(NO_3)_2.6H_2O$ = 0.2976 g	90 ml: 10 ml	46.5	2790
Ag3.0-Ni1.5/TiO_2	m$AgNO_3$ = 0.0944 g m$Ni(NO_3)_2.6H_2O$ =0.1488 g	90 ml: 10 ml	31.6	1896

The absorbed doses presented in Table 1 were calculated based on the dose of ~1.67 kGy for reduction of 1 mM Ag^+[18], but in excess for 20% .

3.1. Characterization of catalysts

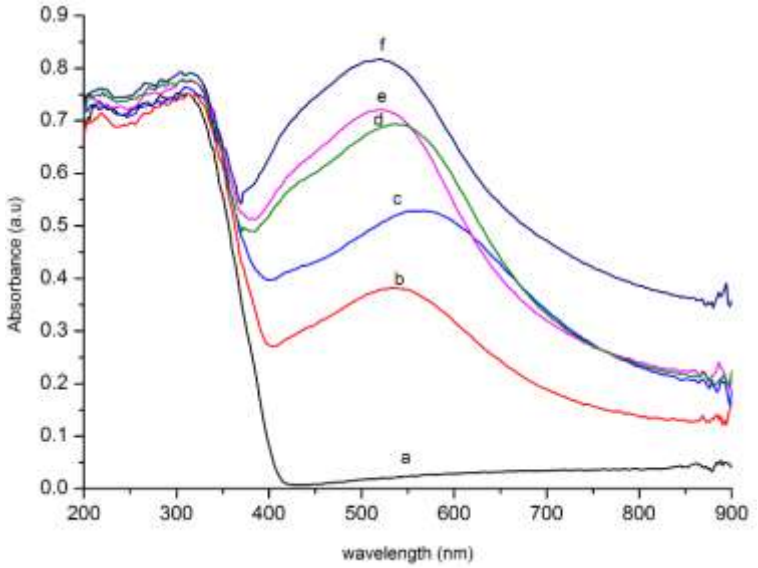


Fig.1:UV-vis Diffuse reflectance spectra (DRS) of pure TiO_2 and modified TiO_2: (a) Pure TiO_2; (b) Ag0.75-Ni1.5/TiO_2; (c) Ag1.5-Ni0.75/TiO_2; (d) Ag1.5-Ni3.0/TiO_2; (e) Ag1.5-Ni1.5/TiO_2; (f) Ag3.0-Ni1.5/TiO_2

The UV-visible spectral of the pure TiO_2 and modified TiO_2 by Ag and Ni in the range of 200 to 900 nmwere shown in Fig. 1. It was found that the absorbance of Ag-Ni/TiO_2 in the visible region was always higher than that for pure TiO_2. The absorption peak of Ag-Ni/TiO_2 shifted towards the visible region. The visible-light photo absorption of Ag3.0-Ni1.5/TiO_2 was the highest among studied samples. The absorption of the modified TiO_2 samples in the range of 510−570 nm was probably due to Ag and Ni nanoparticles which absorbed in this spectral range.

http://dx.doi.org/10.22161/ijeab/2.1.66 *ISSN: 2456-1878*

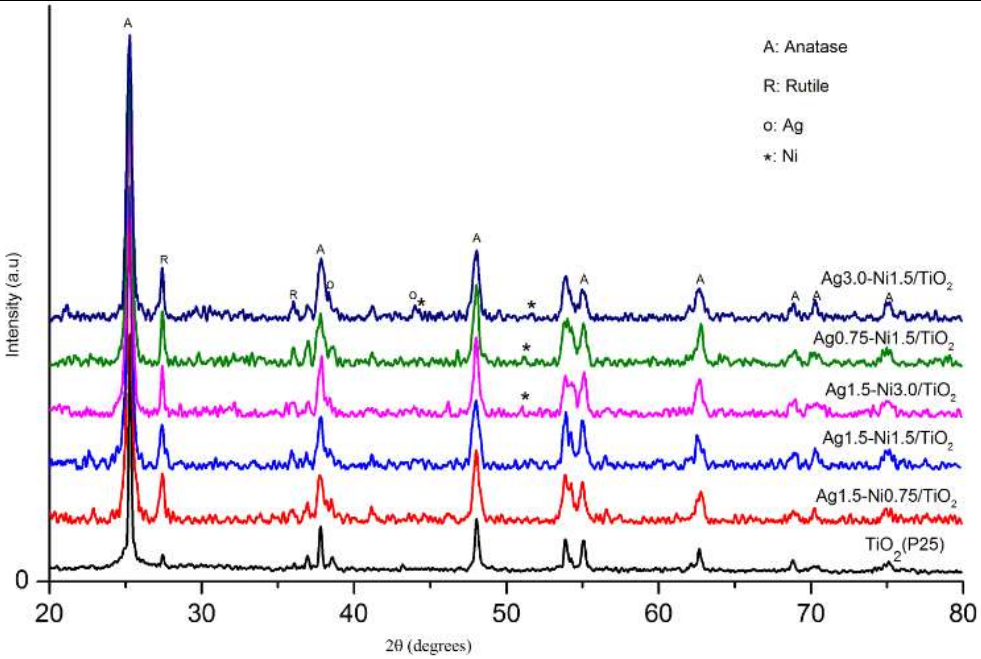


Fig. 2.XRD patterns of TiO_2 (P25) and$Ag-Ni/TiO_2$with various content of Ag and Ni.

Fig. 2 showed the typical XRD patterns of the pure TiO_2 (P25), and TiO_2 doped with various content of Ag and Ni. It is clearly from Fig. 2 that original TiO_2 powder exhibits typical pattern that indicate for the phases of anatase and rutile. The XRD pattern of $Ag3.0-Ni1.5/TiO_2$ consisted of peaks at 25.2 º; 37.6º; 48.0º; 53.9º; 55.1º; 62.4º; 68.7º; 70.2º and 75.1º correspond to the crystal planes [101], [004], [200], [105], [211], [204], [220], [220] and [215] respectively; this is indicate for phase anatase of TiO_2, whereas rutile crystallites structure has peaks at 27.4º and 36.1º correspond to the crystal planes [110] and [101].Peaks at 2θ values of 38.1º, 44.1º that reflect the cubic Ag phase which can be attributed to the crystal planes of metallic silver [111] and [200], respectively.Peaks at 2θ values of 44.5º and 51.7ºthat indicated for crystal planes of metallic nickel [111] and [200]. All peaks for Ag and Ni were weak because of the low content of silver and nickel.

Table.2:θºvalues of [101] plane and [200] plane of TiO_2after doping by Ag and Ni with different content

Ag content , %(w/w)	0.0 (TiO_2)	0.75	1.5	1.5	1.5	3.0
Ni content, %(w/w)	0.0 (TiO_2)	1.5	0.75	1.5	3.0	1.5
2θº [101] plane	25.5	25.37	25.34	25.21	25.42	25.22
2θº[200] plane	48.29	48.16	48.13	47.98	48.17	48.02

In addition, from the results of XRD inTable 2, it can be seen that the position of TiO_2 plane [101] and [200] change the angle by doping with Ag and Ni. According to Bragg's law: $n\lambda=2d\sin\theta$[19], can be drawn that the lesser is the value of sinθ,the larger is the d spacing. Opposite, the larger is the value of sinθ, the lesser is the d spacing. So we can conclude that the value of d spacing change with Ag and Ni doping, which implies that nickel and silver ions diffused into the lattice of TiO_2.

Table.3:Sample, BET surface area of pure TiO_2 and Ag-Ni doped TiO_2 samples

Sample	BET surface area (m^2/g)
TiO_2	69.417
$Ag0.75-Ni1.5/TiO_2$	53.083
$Ag1.5-Ni0.75/TiO_2$	55.991
$Ag1.5-Ni1.5/TiO_2$	56.200
$Ag1.5-Ni3.0/TiO_2$	53.747
$Ag3.0-Ni1.5/TiO_2$	51.800

http://dx.doi.org/10.22161/ijeab/2.1.66 *ISSN: 2456-1878*

The surface areas of pure TiO_2 and Ag-Ni/$TiO_2$with various content of Ag and Ni were determined by the nitrogen gas adsorption method and shown in Table 3. Theresults in Table 3 showed that the surface area of Ag-Ni/TiO_2samples decreased compared to that of TiO_2.

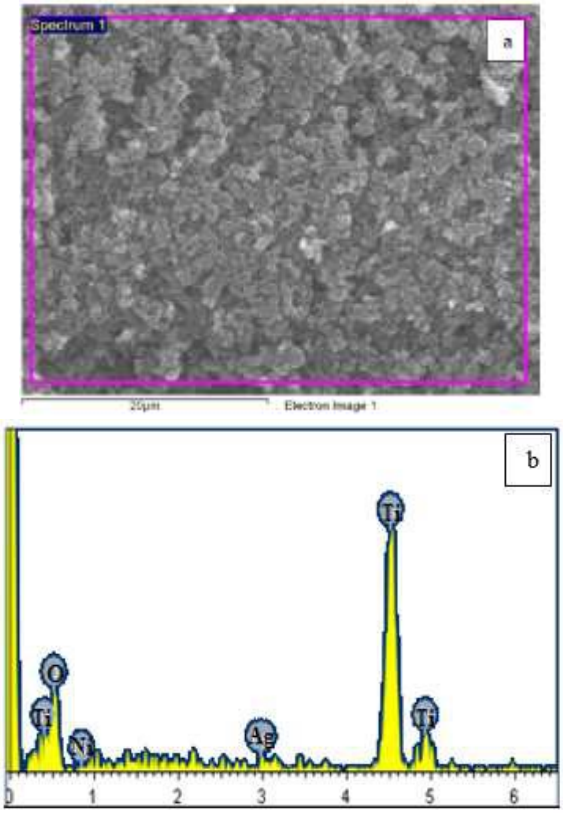


Fig. 3:SEM image (a) and EDX diagram (b) of Ag3.0-Ni1.5/TiO_2 catalyst

The SEM micrograph and EDX spectrum of Ag3.0-Ni1.5/TiO_2 catalyst were shown in Fig. 3. The SEM micrograph showed catalyst particles with spherical morphology. The composition of the Ag3.0-Ni1.5/TiO_2 catalyst was determinedby EDX analysis. The EDX spectrum was recorded in the binding energy region of 0 - 6 eV which was shown in Fig. 3b. The existence of Ag and Ni atoms on the TiO_2 was confirmed.

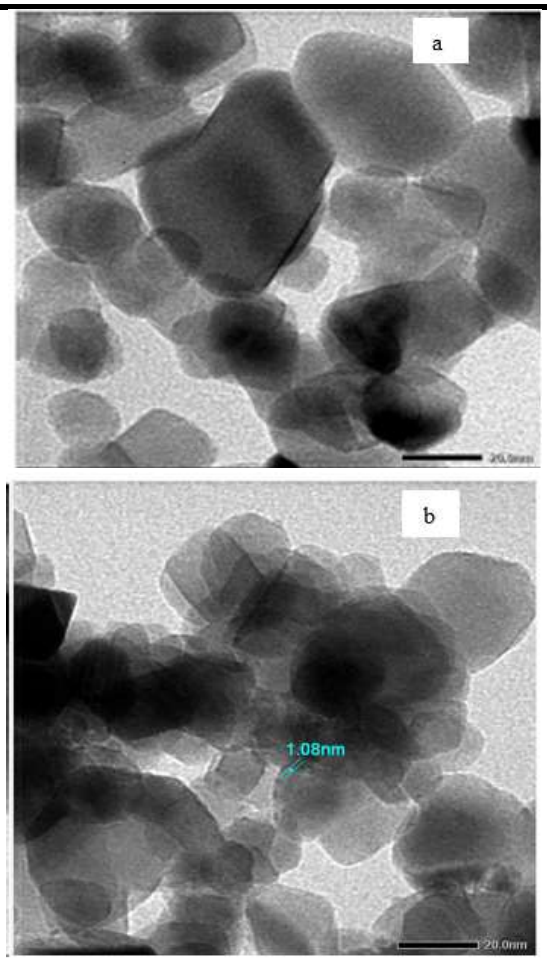


Fig. 4:TEM images of TiO_2 (P25) (a) and Ag 3.0-Ni1.5/TiO_2 (b) photocatalyst

The morphology and metal distribution of the catalysts were then examined by TEM images. Fig. 4 showed the TEM images of TiO_2 (P25) and Ag3.0-Ni1.5/TiO_2. TEM image of TiO_2in Fig. 4a indicated that the TiO_2 particles have not agglomerated. The average size of particles of TiO_2 was estimated to be 10 – 40 nm. TEM image of Ag3.0-Ni1.5/TiO_2 in Fig. 4b indicated that Ag and Ni nanoparticles with size of about 1-2 nm were dispersed on the surface of TiO_2.The size of TiO_2 particles was almost unchanged for TiO_2 and Ag3.0-Ni1.5/TiO_2sample.

http://dx.doi.org/10.22161/ijeab/2.1.66 *ISSN: 2456-1878*

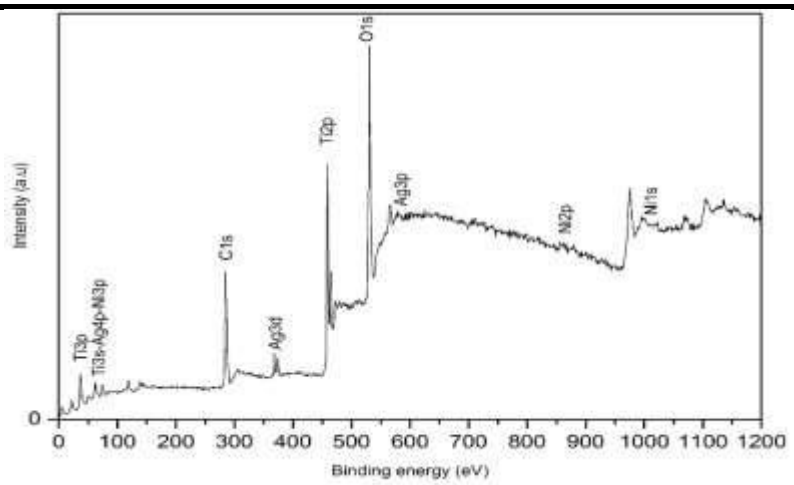


Fig. 5: XPS full survey of Ag3.0-Ni1.5/TiO_2

XPS analysis of silver and nickel co-doped TiO_2 sample (Ag3.0-Ni1.5/TiO_2) was performed and shown in Fig. 5. The XPS full spectrum showed the presence of different elements on the surface of the catalyst. XPS analysis of Ag3.0-Ti1.5/TiO_2 sample detected peaks of Ti, O, C, Ni and Ag. The presence of C was attributed to carbon contamination existed on the sample rack.

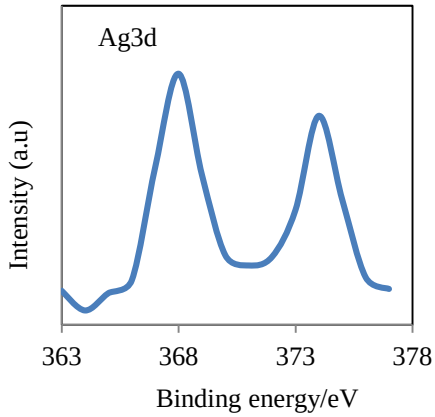


Fig. 6: XPS survey for Ag3d

Fig. 6 showed that the catalyst exhibited their Ag3d level two peaks (at around 368 and 374 eV), which indicated for the Ag 3d5/2 and 3d3/2. The binding energy of peak Ag 3d5/2 maximum at 368.1 eV was close to the value reported for metallic Ag(0) [12].

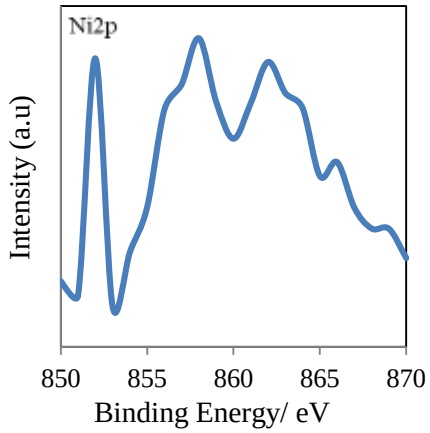


Fig. 7: XPS survey for Ni2p

The Ni 2p3/2regions of the Ag3.0-Ti1.5/TiO_2catalystshowed severalpeaks in the range of 850–870 eV (Fig. 7). The first peak at 852.1 eV resulted for metallic nickelNi(0)[20]. The second peak at 858.1 eV was attributed to Ni^{2+} ions within the composite oxide structure [21], whereas the peak at 862 eV was assigned to its corresponding shake-up satellite lines. The detection of metallic nickel Ni(0) clearly indicated a reduction of the ion Ni^{2+}on the surface of the catalyst.

3.2. Photocatalytic degradation of methyl red

3.2.1.Effect of dopant content

http://dx.doi.org/10.22161/ijeab/2.1.66 *ISSN: 2456-1878*

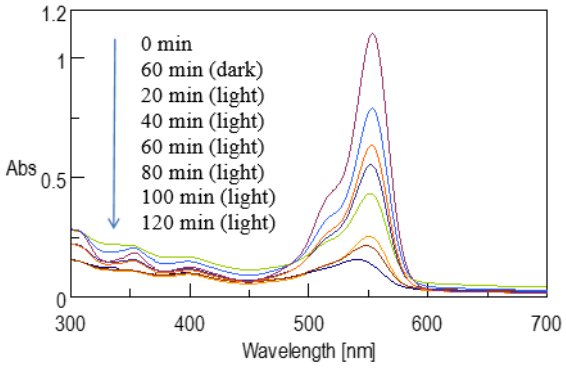


Fig. 8:Absorption spectra of MR at different time interval degraded by the Ag1.0-Ni0.75/TiO₂catalyst under visible light.

Fig.8 showed the absorption spectra of MR before and after irradiating under the visible light for different time interval using Ag1.0-Ni0.75/TiO_2 as a photocatalyst. The intensity of the peak was found to decrease with increasing irradiation time during photocatalytic degradation of MR. It proves that the concentration of MR decreased with increasing degradation time.

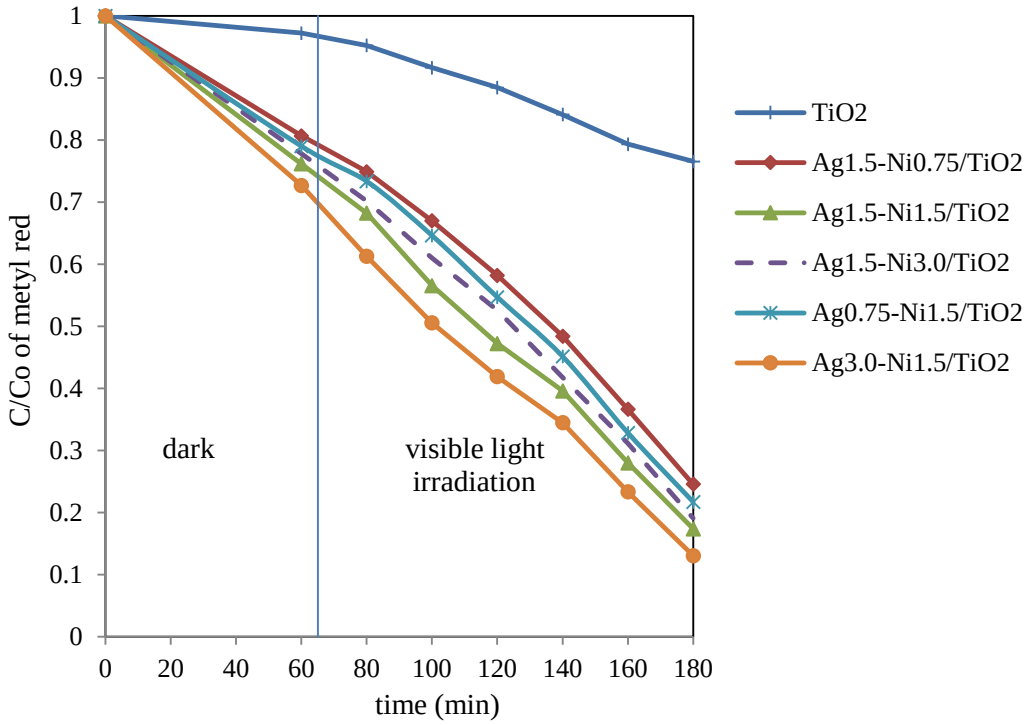


Fig.9: Degradation of MR under visible irradiation by TiO_2 and Ag-Ni/TiO_2 with different dopant (Ag and Ni) content. The initial concentration of MR: 1×10^{-5}M, amount of catalyst: 0.5 g/L.

Photocatalytic degradation of MR by pure TiO_2 and Ag and Ni co-doped on TiO_2 catalysts with various dopant concentrations under visible light was presented in Fig. 9. The effect of dopant on the percentage of methyl red degradation was studied with different amount of Ag and Ni varying from 0.75 to 3.0 % (w/w), with MR solution concentration of 10^{-5}M and amount of catalyst of 0.5 g/L. All the Ag-Ni/TiO_2 samples showed higher photocatalytic activity than that of commercial TiO_2 (Degussa P25) under visible light irradiation. Thus modified TiO_2 by Ag and Ni nanoparticles resulted in higher photocatalytic activity. Among catalysts, the sample containing 3% Ag and 1,5% Ni (w/w) performanced the highest photodegradation efficiency.

http://dx.doi.org/10.22161/ijeab/2.1.66 *ISSN: 2456-1878*

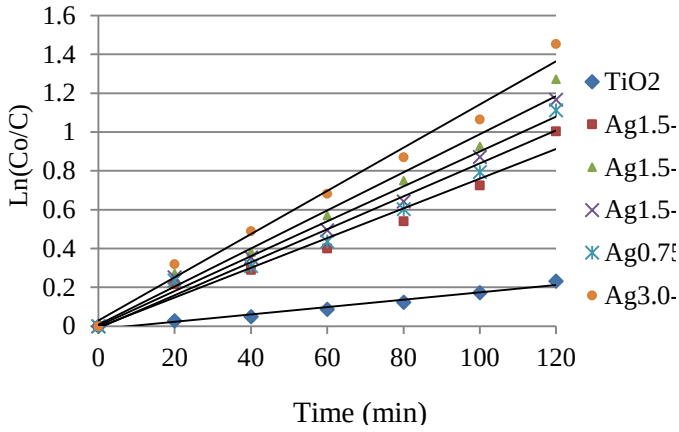


Fig.10: Ln(C_o/C) versus irradiation time for MR under visible light by TiO_2 and Ag-Ni/TiO_2 catalysts with initial concentration of MR: 10^{-5}M, amount of catalyst: 0.5 g/L.

As a result, the photodegradation kinetics fitted well with the pseudo first-order model that showed in Fig. 10. Degradation rate constants calculated from the results in Fig. 10 were of 0.0019; 0.0077; 0.0085; 0.009; 0.0098 and 0.0111 min^{-1} for TiO_2, Ag1.5-Ni0.75/TiO_2, Ag0.75-Ni1.5/TiO_2, Ag1.5-Ni3.0/TiO_2, Ag1.5-Ni1.5/TiO_2 and Ag3.0-Ni1.5/TiO_2, respectively. The sample with 3 % Ag (w/w) and 1.5 % Ni (w/w) doped on TiO_2exhibited the highest rate constant.

3.2.2. Effect of pH

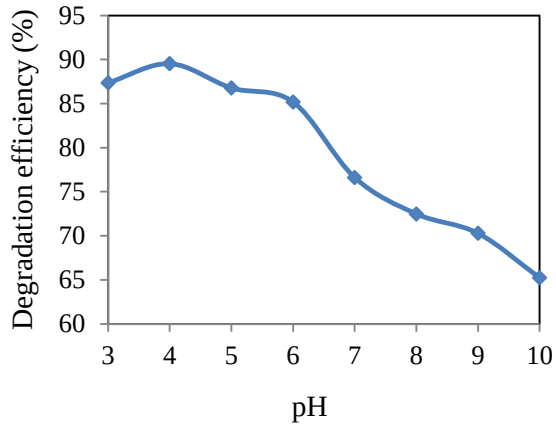


Fig. 11: The effect of pH on photodegradation of MR (amount of catalyst: 2.0 g/L; irradiation time: 60 min; MB initial concentration: 10^{-5}M;)

The pH of a dye solution is an important parameter that affects the rate of degradation. The effect of pH on photo catalytic degradation of MR was investigated with content of catalyst (Ag3.0-Ni1.5/TiO_2) of 0.5 g/L, concentration of MR of10^{-5} M,irradiation time of 60 min andthe range of pH from 3 to 10. PH was adjusted by 1N HNO_3 and 1N NaOH. Fig. 11 showed the degradation efficiency of MR at different pH values.The results clearly showed that photo catalytic degradation of the MR dye increased to pH 4 and then decreased significantly to pH 10. The maximum MR degradation of 89.18% was observed at pH 4. This may be explained that the higher degradation extent of MR occurred in acidic medium rather than alkaline. Hence, at acidic pH values,the particle surfaces of catalysts are positively charged and at basic pH values, they are negatively charged [22]. In acidic environment, the adsorption of anionic dye molecules on the surface of the catalyst particle increased.Moreover,in acidic pHthe photo excited electrons in the photo catalyst could be fast abstracted from the surface by the numerous protons of the medium [23].

3.2.3. Effect of catalytic content

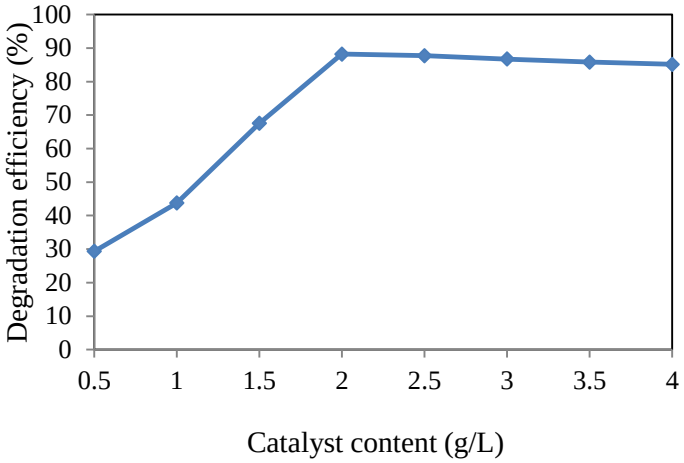


Fig. 12: Degradation at different content of Ag3.0-Ni1.5/TiO_2 catalyst (MR initial concentration: 10^{-5}M; pH: 4; irradiation time: 60 min).

The effect of catalyst content (Ag3.0-Ni1.5/TiO_2) on the degradation efficiency of MR was investigated with different catalytic content varying from 0.5 to 4.0 g/L, at dye solution concentration: 10^{-5}M for 60 min and pH at 4.The results were shown in Fig.12. The degradation efficiency significantly increased up to 2 g/L of catalytic content. When catalytic content was more than 2.0 g/L, the degradation efficiency of dye was found to bealmost unchanged. Therefore, an optimum catalytic dose of 2 g/L was selected for further experiment.

http://dx.doi.org/10.22161/ijeab/2.1.66 *ISSN: 2456-1878*

3.2.4. Effect of irradiation time

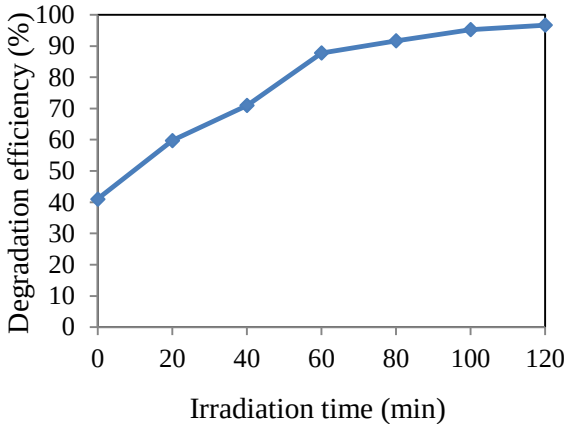


Fig. 13: The effect of irradiation time on photodegradation of MR (catalyst content: 2.0 g/L;pH: 4; MR initial concentration: 10^{-5}M).

In order to study the effect of irradiation duration on the degradation efficiency on MR, the experiments were carried out with Ag3.0-Ni1.5/TiO_2 catalyst amount of 2.0 g/L; pH of dye solution at 4, initial concentration of MR of 10^{-5}M and irradiation time of the dye solution in the range from 0 to 120 min. The results in Fig. 13 indicated that the degradation percentage increased with the increase of irradiation time. At the irradiation time of 120 min, the percent degradation of MR achieved 96.7%.

3.2.5. Reuse of the photocatalyst

To determine the ability of reuse of Ag-Ni/TiO_2as a photocatalytic, reuse experiment of the photocatalytic activity of Ag3.0-Ni1.5/TiO_2 catalyst was performed. After degradation of MR, the photocatalyst was then washed with distilled water and dried. Then the catalyst was reused for degradation of MR solution. The results in Fig. 14 showed that after four times of reuse, the catalyst was still active with a slight decrease in the degradation efficiency from 96.7% (first cycle) to 89.1% (fifth cycle). Hence, it can be confirmed that the photocalytic activity of Ag-Ni/TiO2 catalyst was almost stable during degradation of MR.

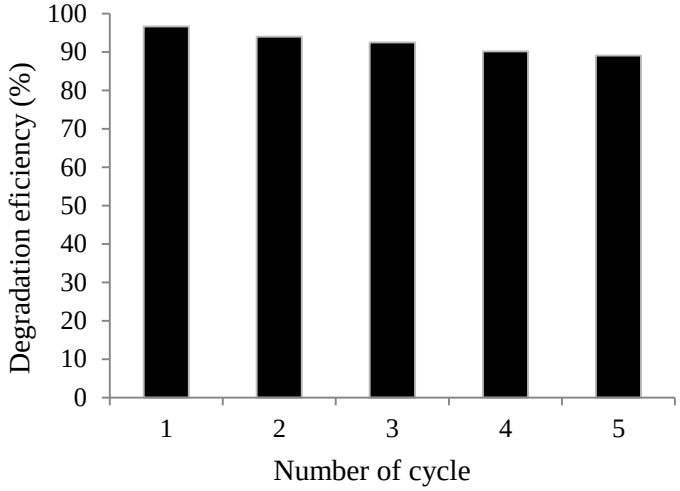


Fig. 14: Regeneration of Ag3.0-Ni1.5/TiO_2photocatalyston degradation MR (amount of catalyst: 2.0 g/L; pH: 4; MR initial concentration: 10^{-5}M; irradiation time: 120 min).

IV. CONCLUSION

Co-doping Ag and Ni nanoparticles on $TiO_2$with different amount of Ag and Niwas carried out byγ-irradiation method. The presence of Ag and Ni in the crystal lattice of TiO_2 was confirmed. The size of Ag and Ni nanoparticles on the surface of TiO_2 was of 1-2 nm. Ag-Ni/TiO_2photocatalysts displayed higher photocatalytic activity for pure TiO_2. Among all synthesized catalysts, the TiO_2 modified with 3.0%Ag (w/w) and 1.5%Ni (w/w) exhibited the highest photocatalytic activity under visible light. In addition, the Ag 3.0-Ni 1.5/TiO_2photocatalystcan be reused many times with almost unchangeof photocatalyticactivity. Thus, Co-doping $TiO_2$with silver and nickel by γ-irradiation can besuitably usedas photocatalyt for degradation of organic pollutants in water.

REFERENCES

[1] Fujishima X., Zhang C. R. 2006. "Titanium dioxide photocatalysis: Present situation and future approaches". *Chimie* 9, 750–760.

[2] Di Paola A.,Cufalo G., Addamo M. et al. 2008. "Photocatalytic activity of nanocrystalline TiO_2 (brookite, rutile and brookite-based) powders prepared by thermohydrolysis of $TiCl_4$ in aqueous chloride solutions". *Colloid Surface* A 317 (1–3), 366–376.

[3] Seery M. K., George R., Floris P., Pillai, S.C. 2007. "Silver doped titanium dioxide nanomaterials for enhanced visible light photocatalysis". *J. Photochem. Photobiol. A: Chem.* 189 (2–3), 258–263.

[4] Hariprasad N., Anju S. G., Yesodharan E. P., Suguna Y. 2013. "Sunlight induced removal of Rhodamine B from water through semiconductor photocatalysis: effects of adsorption, reaction conditions and additives". *Res. J. Mater. Sci*. 1, 9–17.

[5] Demeestre K., Dewulf J., Ohno T., Salgado P. H., Langenhove H.V. 2005. "Visible light mediated photocatalytic degradation of gaseous trichloroethylene and dimethyl sulfide on modified titanium dioxide". *Appl. Catal. B: Environ*. 61 (1–2), 140–149.

[6] Ouyang R., Zhang W., Zhou S., Yang Y., Ji Y., Feng K., Liang X., Xiao M., Miaoa Y. 2016. "Single walled carbon nanotube sandwiched Ni-Ag hybrid nanoparticle layers for the extraordinary electrocatalysis toward glucose oxidation". *Electrochimic. Acta*188, 197–209.

[7] Ubonchonlakate K., Sikong L., Tontai T., Saito F. 2010. "*P. aeruginosa* inactivation with silver and nickel doped TiO_2 films coated on glass fiber roving". *Adv. Mater. Res*. 150-151, 1726–1731.

[8] Dvoranova D., Brezova V., Mazur M., Malathi M.A. 2002. "Investigations of metal-doped titanium dioxide photocatalysts". *Appl. Catal. B: Environ*. 37, 91–105.

[9] Zhu J., Deng Z., Chen F. et al. 2006. "Hydrothermal doping method for preparation of Cr^{3+}-TiO_2photocatalysts with concentration gradient distribution of Cr^{3+}". *Appl. Catal. B: Environ*. 62 (3–4), 329–335.

[10] Wu G., Nishikawa T., Ohtani B., Chen A. 2007. "Synthesis and characterization of carbon-doped TiO_2 nanostructures with enhanced visible light response". *Chem. Mater*. 19 (18), 4530–4537.

[11] Patil K.R., Sathaye S.D., Khollam Y.B., Deshpande S.B., Pawaskar N.R., Mandale A.B. 2003. "Preparation of TiO_2 thin films by modified spin-coating method using an aqueous precursor". *Mater. Lett*. 57 (12), 1775–1780.

[12] Grabowska E., Zaleska A., Sorgues S., Kunst M., Etcheberry A., Colbeau-Justin C., Remita H. 2013. "Modification of titanium(IV) dioxide with small silver nanoparticles: Application in photocatalysis". *J. Phys. Chem*. C 117 (4), 1955−1962.

[13] Khatouri J., Mostafavi M., Amblard M., Belloni J. 2016. "Radiation induced copper aggregates and oligomer". *Chem. Phys. Lett*. 191, 351–356.

[14] BelloniJ . 1996. "Metal nanocolloids".*Curr. Opin. Colloid Interface Sci*. 1, 184–196.

[15] HayesD., Micic O. I., Nenadovitt M. T., Swayambunathan V., Meisel, D. 1989. "Radiolytic production and properties of ultrasmall cadmium suldide particles". *J. Phys.Chem*. 93, 4603–4608.

[16] Zhang Z., Nenoff T. M., Leung K., Ferreira S. R., Huang J. Y., Berry D.Y., Provencio P. P., Stumpf R. 2010. "Room temperature synthesis of Ag-Ni and Pd-Ni alloy nanoparticles". *J. Phys. Chem*: C 114, 14304–14318.

[17] ASTM International, 2004. "Standard practice for use of a dichromate dosimetry system, ISO/ASTM 51401:2003(E)". Standards on dosimetry for radiation processing, pp. 69–74.

[18] Remita S., Fontaine P., Rochas C., Muller F., Goldman M. 2005. "Radiation induced synthesis of silver nanoshells formed onto organic micelles". *Eur. Phys. J. D* 34, 231–233.

[19] Suwarnkar M. B., Dhabbe R.S., Kadam A. N., Garadkar K.M. 2014. "Enhanced photocatalytic activity of Ag doped TiO_2 nanoparticles synthesized by a microwave assisted method". *Ceram. Int*. 40, 5489–5496.

[20] Galtayries A., Grimblot J. 1999. "Formation and electronic properties of oxide and sulphide films of Co, Ni and Mo studied by XPS". *J. Electron Spectrosc. Relat. Phenom*. 98–99, 267–275.

[21] Wang H., . Xiang X., Li F., Evans D. G, Duan X. 2009. "Investigation of the structure and surface characteristics of Cu-Ni-M(III) mixed oxides (M = Al, Cr and In) prepared from layered double hydroxide precursors", *Appl. Surf. Sci*. 255, 6945–6952.

[22] Guillard H., Lachheb A., Houas M., Ksibi C., Elaloui E., Herrmann J.M. 2003. "Influence of chemical structure of dyes, of pH and of inorganic salts on their photocatalytic degradation by TiO_2 comparison of the efficiency of powder and supported TiO_2". *J. Photochem. Photobiol. A: Chem*. 158, 27–36.

[23] Lydia, I.S., Merlin, J.P., Dhayabaran, V.V., Radhika, N. 2012. Photodegradation of methyl red using Ag-doped Fe_3O_4 nanoparticles. Int. J. Chem. Environ. Eng. 3(4), 209–216.

http://dx.doi.org/10.22161/ijeab/2.1.67 *ISSN: 2456-1878*

An Assessment of Indoor Air Quality (IAQ) in Metal industries of Delhi

Malvika Khullar, Dr. Puja Gupta

Department of Resource Management and Design Application, University of Delhi

***Abstract*—** *With growing realization and concern for our health, the focus on Indoor Air Quality has increased. Exposure to indoor air pollution is responsible for nearly 2 million excess deaths in developing countries and for some 4% of the global burden of disease. Today it is critical that the industry be familiar with the environmental hazards that employees are subjected to in the workplace. Iron & steel and other manufacturing industries, foundries and forges produce a lot of pollutants in the indoor environment. Exposures to mineral dusts, metal fumes, products of combustion, resin bonding systems, physical noise and heat and vibration hazards may seriously impact the health of workers in foundries.*

The study aimed at assessing Indoor Air Quality in Metal industries of Delhi in Mayapuri industrial area. The study was carried out in 3 phases. In the first phase, the study collated awareness about Indoor Air Quality and related health effects amongst the owners and employees working in the enterprises. In the second phase, the study involved monitoring of the randomly selected enterprises in terms of CO_2, $PM_{2.5}$, PM_{10} and presence of dampness and molds in the enterprises. The third phase involved spreading awareness regarding Indoor Air Quality amongst the sample.

Highlights of the study are:

Majority of the owners were aware about the concept of Indoor Air Quality and its relationship with productivity and health of a person. They could also cite some of the health impacts caused due to poor Indoor Air Quality. In spite of their wisdom on Indoor Air Quality, there were no monitoring and maintenance provisions in their enterprises.

Also, none of the owners provided their employees personal protective equipment (PPE) and any information or training regarding Indoor Air Quality and its health hazards.

Majority of the employees were unaware of the concept of Indoor Air Quality. The employees could neither relate health with Indoor Air Quality nor showed any interest in improving the same.

It was seen that there was a moderately high level of CO_2 concentrations in the enterprises, mainly due to insufficient ventilation in the enterprises.

$PM_{2.5}$ concentrations were found to be poor and were almost double than its acceptable limits. While it was seen that PM_{10} concentrations were within their acceptable limits.

It was observed that in majority enterprises presence of molds and dampness was observed. It was observed on walls and ceilings. The areas near the walls and places with molds were surrounded by a bad odor.

This study has been helpful in providing clear direction towards the Indoor Air Quality in Metal industries operating in Mayapuri. It also highlights the awareness regarding Indoor Air Quality amongst the sample. The study concludes that there is a sense of knowledge regarding Indoor Air Quality amongst the owners/managers of the enterprises but a huge scope in monitoring and maintenance provisions. The need for spreading awareness about IAQ and its related health effects were highlighted which would improve Indoor Air Quality in industries. The study showcased a clear scope for awareness generation and training amongst the owners and employees of the enterprises regarding Indoor Air Quality. The study suggests surveying of health status in metal industries and similar research in other industries. The findings were also shared with competent authorities to sensitize them towards poor Indoor Air Quality in the metal industries.

***Keywords*—Awareness, Exposure, Indoor Air Quality, metal industries, Pollutants**

I. INTRODUCTION

Buildings create shelter and conditions for working, learning, leisure and comfortable living. A built environment should be safe with no health hazards for its users either due to poor design and construction, or due to poor operation, maintenance and performance. Negligence and/or compromise of any of the actions required to achieve high criteria set for the conditions indoors can bring about serious problems resulting in the substantial costs and numerous undesirable consequences. The holistic approach for creating indoor environmental quality in buildings is hence required involving different disciplines and harmonization with policies and regulations. Numerous determinants of healthy, productive and comfortable indoor environments must be

considered comprising among others outdoor air pollution and climate, as well as the expectations and behavior of buildings' users to name just few (Achieving IAQ supporting health and comfort in highly energy efficient buildings, 2013) .

Metal industries are the indispensable part of an economy; they form the backbone of industrial development of any country. Iron and steel industry is by nature a heavy industry. Besides, iron and steel industry, heavy engineering and machine tools industries are the main dealers of metals. These industries have witnessed a phenomenal growth and produces a whole range of capital goods and consumer durables. The capital goods required for textile industry, fertilizer plants, power projects, cements, steel and petro-chemical plants, mining, construction and agricultural machineries such as equipment for irrigation projects, diesel engines, pumps and tractors, transport vehicles, etc. are being produced indigenously (Metal Industry in India, 2015).

In Iron & Steel and other manufacturing industries, foundries and forges produce a lot of pollutants in the environment – both indoor and ambient environment. Processes for molding, melting and castings etc. are accompanied by evolution of heat, noise, dust fines, fly-ash, oxides of Nitrogen, Sulphur and metals. Particulate matters are generated in large quantities when preparing mold core sands and molds melting metals, pouring metal, knocking out poured molds and loading and unloading raw materials. Here metals are given a specific shape by metal castings for various engineering purposes. Pollutants are also emitted in sintering, pelletizing, rolling mills, coke-oven plants, refractories etc. in steel making and by-products manufacturing (Health hazards of Foundries and Forges, 2009).

Many people are exposed to common air pollutants in their occupations e.g. smoke, dust, SPM, RSPM, carbon mono-oxide, sulphur dioxide, oxides of nitrogen (N_{ox}), hydrocarbons, and heavy metals like Pb, Cd, Cr, As, Ni etc. Their prolonged exposure causes various health hazards. Heavy metals cause acute and chronic poisoning. Some disastrous episodes have focused attention upon air pollution as a health hazard (Health hazards of Foundries and Forges, 2009).

1.1 Indoor Air Quality

Indoor air quality (IAQ) refers to the quality of the air inside buildings as represented by concentrations of pollutants and thermal (temperature and relative humidity) conditions that affect the health and performance of occupants. The growing proliferation of chemical pollutants in consumer and commercial products, the tendency toward tighter building envelopes and reduced ventilation to save energy, and pressures to defer maintenance and other building services to reduce costs have fostered IAQ problems in most of the buildings. As a result, occupant's complaints of stale and stuffy air, and symptoms of illness or discomfort breed undesirable conflicts among occupants/owners/tenants/building managers. Therefore, it has become one of the most important issues of environment and health worldwide considering the principle of human rights to health that everyone has the right to breathe healthy indoor air (Air Pollution, 2014) .

Some general health hazards are caused as a result of contact between the pollutants and the body. These hazards are as follows:

- Eye irritation
- Headache
- Noise and throat irritation
- Irritability of respiratory tract
- Gases like hydrogen sulphide, ammonia and mercaptans cause odor nuisance even at low concentrations
- High temperature can cause fatigue and dehydration
- Chronic pulmonary diseases like Bronchitis and asthma are aggravated by a high concentration of SO_2, NO_2, and particulate matter.
- Carbon monoxide combines with the hemoglobin in the blood and consequently increases stress on those suffering from cardiovascular and pulmonary diseases
- Dust particles cause respiratory disease (Health hazards of Foundries and Forges, 2009).

Indoor Air Quality (IAQ) refers to the air quality within and around buildings and structures, especially as it relates to the health and comfort of building occupants. Understanding and controlling common pollutants indoors can help reduce risks of indoor health concerns. Health effects from indoor air pollutants may be experienced soon after exposure or, possibly, years later. Some health effects may show up shortly after a single exposure or repeated exposures to a pollutant. These include irritation of the eyes, nose, and throat, headaches, dizziness, and fatigue. Such immediate effects are usually short-term and treatable. Sometimes the treatment is simply eliminating the person's exposure to the source of the pollution, if it can be identified. Soon after exposure to some indoor air pollutants, symptoms of some diseases such as asthma may show up, be aggravated or worsened. The likelihood of immediate reactions to indoor air pollutants depends on several factors including age and preexisting medical conditions. In some cases, whether a person reacts to a pollutant depends on individual sensitivity, which varies tremendously from person to person. Some people can become sensitized to biological

or chemical pollutants after repeated or high level exposures.

Certain immediate effects are similar to those from colds or other viral diseases, so it is often difficult to determine if the symptoms are a result of exposure to indoor air pollution. For this reason, it is important to pay attention to the time and place symptoms occur. If the symptoms fade or go away when a person is away from the area, for example, an effort should be made to identify indoor air sources that may be possible causes. Some effects may be made worse by an inadequate supply of outdoor air coming indoors or from the heating, cooling or humidity conditions prevalent indoors. Other health effects may show up either years after exposure has occurred or only after long or repeated periods of exposure. These effects, which include some respiratory diseases, heart disease and cancer, can be severely debilitating or fatal. It is prudent to try to improve the indoor air quality in your home even if symptoms are not noticeable.

While pollutants commonly found in indoor air can cause many harmful effects, there is considerable uncertainty about what concentrations or periods of exposure are necessary to produce specific health problems. People also react very differently to exposure to indoor air pollutants. Further research is needed to better understand which health effects occur after exposure to the average pollutant concentrations found in homes and which occurs from the higher concentrations that occur for short periods of time.

1.2 Causes of Indoor Air pollution

Indoor pollution sources that release gases or particles into the air are the primary cause of indoor air quality problems. Inadequate ventilation can increase indoor pollutant levels by not bringing in enough outdoor air to dilute emissions from indoor sources and by not carrying indoor air pollutants out of the area. High temperature and humidity levels can also increase concentrations of some pollutants (Indoor Air Quality, 2015).

Some general health hazards are caused as a result of contact between the pollutants and the body. These hazards are as follows:

- Eye irritation
- Headache
- Nose and throat irritation
- Irritability of respiratory tract
- Gases like hydrogen sulphide, ammonia and mercaptans cause odor nuisance even at low concentrations
- High temperature can cause fatigue and dehydration
- Chronic pulmonary diseases like Bronchitis and asthma, are aggravated by a high concentration of SO2, NO2, particulate matter and photochemical smog
- Carbon monoxide combines with the hemoglobin in the blood and consequently increases stress on those suffering from cardiovascular and pulmonary diseases
- Dust particles cause respiratory disease. Diseases like silicosis, asbestosis etc. result from specific dust
- Carcinogenic agents like PAH's, Cr(VI), Cd etc. cause cancer
- Hydrogen fluoride causes diseases of bone (fluorosis) and mottling of teeth
- Certain heavy metals like lead, cadmium, mercury, chromium, nickel, manganese etc. enter into body by inhalation, skin absorption and through food chain. They cause acute and chronic poisoning.

1.3 Pollutants in Indoor Environment

Dust, SPM, noise and gaseous pollutants pose a potential threat to health of workers in industries and populations residing in the surrounding areas. Dust also absorb gases and in such a combination prove to be a more serious health hazards due to synergism. It has recently been demonstrated that SO2 absorbed on submicroscopic particles penetrate deep into the lungs and this is a greater danger to health. Silicosis and siderosis are common diseases in foundry-men and forge shops workers. Most of the cases of silicosis, however, have arisen in the manufacture of silica containing materials and in foundry workers. Pottery industry got such a bad reputation for silicosis. Grinders in the cutlery industry using sandstone wheels died in large numbers from silicosis. Pneumoconiosis and black lung diseases are caused due to coal dust. Asbestosis is common in asbestos workers in industries.

Direct IR radiation poses a risk to sight. Contact with hot metal or hot water may result in severe burns. Workers exposed to gamma rays and related ionizing radiations suffer from several hazards. Explosion and fire hazards occur during handling of liquid metal and the presence of flammable chemicals & liquid fuel. Iron foundry slag may be highly reactive if calcium carbide is used to de-sulphurize the iron (Shrivastava, 2009) .

Total Suspended Particulate Matter

TSP is mostly a primary pollutant, but some of it is formed as secondary pollutant. It consists of soot, dust, tiny objects of liquid, and other material. An increase in the incidence of respiratory diseases and gastric cancer has been linked with the increase in particulate level. The natural sources include volcanoes, forest fires, and desert land. Some manmade sources are steel industry, power plants, and flour mills. Agricultural activities also

contribute to TSP loading. Particulate gradually settle back to earth and can cause people to cough, get sore throats, or develop other more serious breathing problems. Particulate matter also causes discoloration of buildings and other structures

A. *Carbon Dioxide*

Carbon dioxide emissions have increased significantly during 19th century because of the use of coal, oil and natural gas. It finds uses as a refrigerant, in fire extinguishers and in beverage carbonation. Higher concentrations can affect respiratory function and cause excitation followed by depression of the central nervous system. Contact with liquefied CO_2 can cause frostbite. Workers briefly exposed to very high concentrations have effects like damage to the retina, sensitivity to light (photophobia), abnormal eye movements, constriction of visual fields, and enlargement of blind spots (Kumar) .

1.4 Significance

In recent years, with growing realization, people have become more aware of potential health and comfort problems that may be associated with poor indoor air quality.

In Iron & Steel and other manufacturing industries, foundries and forges produce a lots of pollutants in the environment – both working and ambient environment. In the processes involved in foundries and forges, metals are extracted and produced from ores by various metallurgical processes and processes for moulding, melting and castings etc. are accompanied by evolution of heat, noise, dust fines, fly-ash, oxides of Nitrogen, Sulphur and metals. Particulate matters are generated in large quantities when preparing mould core sands and moulds melting metals, pouring metal, knocking out poured moulds and loading and unloading raw materials. Here metals are given a specific shape by metal castings for various engineering purposes (Health hazards of Foundries and Forges, 2009).

Health plays an important role in increasing the productivity of an individual. Therefore, it becomes important to study such sectors. The study can be used by government and other officials to know the current Indoor Air Quality status in metal industries operated in the study locale. It will also be helpful for the competent authorities to know the current status of IAQ in their enterprises. The study would also provide the definite direction to future researchers in the related fields.

1.5 Objectives

To assess Indoor Air Quality (IAQ) of Metal Industries and study occupant awareness.

1. To study awareness amongst sample related to Indoor Air Quality (IAQ) and related health hazards
2. To assess Indoor Air Quality of the enterprises in terms of:
    - Insufficient ventilation (CO_2 concentrations)
    - PM_{10} levels
    - $PM_{2.5}$ levels
    - Observe the presence of dampness and molds in the factory premise
3. To design and implement an awareness generation campaign to capacitate the sample about Indoor Air Quality and its health hazards

II. CONCLUSION

Buildings create shelter and conditions for working, learning, leisure and comfortable living. The holistic approach for creating indoor environmental quality in buildings is hence required involving different disciplines and harmonization with policies and regulations. Numerous determinants of healthy, productive and comfortable indoor environments must be considered comprising among others outdoor air pollution and climate, as well as the expectations and behavior of buildings' users to name just few.In Iron & Steel and other manufacturing industries, foundries and forges produce a lot of pollutants in the environment – both indoor and ambient environment. Processes for molding, melting and castings etc. are accompanied by evolution of heat, noise, dust fines, fly-ash, oxides of Nitrogen, Sulphur and metals. Particulate matters are generated in large quantities when preparing mold core sands and molds melting metals, pouring metal, knocking out poured molds and loading and unloading raw materials. Here metals are given a specific shape by metal castings for various engineering purposes. Pollutants are also emitted in sintering, pelletizing, rolling mills, coke-oven plants, refractories etc. in steel making and by-products manufacturing.

Indoor air quality (IAQ) refers to the quality of the air inside buildings as represented by concentrations of pollutants and thermal (temperature and relative humidity) conditions that affect the health and performance of occupants. The growing proliferation of chemical pollutants in consumer and commercial products, the tendency toward tighter building envelopes and reduced ventilation to save energy, and pressures to defer maintenance and other building services to reduce costs have fostered IAQ problems in most of the buildings. As a result, occupant's complaints of stale and stuffy air, and symptoms of illness or discomfort breed undesirable conflicts among occupants/ owners/ tenants/ building managers.

Indoor pollution sources that release gases or particles into the air are the primary cause of indoor air quality

problems. Inadequate ventilation can increase indoor pollutant levels by not bringing in enough outdoor air to dilute emissions from indoor sources and by not carrying indoor air pollutants out of the area. High temperature and humidity levels can also increase concentrations of some pollutants.

In recent years, with growing realization, people have become more aware of potential health and comfort problems that may be associated with poor indoor air quality.

Health plays an important role in increasing the productivity of an individual. Therefore, it becomes important to study such sectors. The study can be used by government and other officials to know the current Indoor Air Quality status in metal industries operated in the study locale. It will also be helpful for the competent authorities to know the current status of IAQ in their enterprises. The study would also provide the definite direction to future researchers in the related fields.

The present research 'An assessment of Indoor Air Quality in Metal industries of Delhi' is an endeavor to assess Indoor Air Quality in metal industries. The study also focused on assessing the awareness of the employees and owners of the enterprises regarding the concept of Indoor Air Quality.

The study was conducted in Mayapuri Industrial area, Phase 1, New Delhi. The study was carried out in 3 phases. For conducting the present study, 10 enterprises were selected randomly. In the first phase, awareness of the employees and owners was assessed by the researcher regarding Indoor Air Quality and related health effects. In the first phase, owner/managers and employees of the selected enterprises served as the sample. Owners were selected as they are the decision makers in an enterprise and employees as they are the ones who work in the enterprises and are affected the most by the decisions made by the management of an enterprise. They are the affected partners and were of a great help in gaining insight into the knowledge amongst them regarding IAQ. One owner/manager and 5 employees from each unit, served as a sample for this phase, making the sample size of 60. Tool used in the first phase was an interview schedule to Check the awareness amongst the owners and employees.

In phase 2, monitoring of the enterprises was carried out in terms on CO2 concentrations, $PM_{2.5}$ and $PM_{10.}$ 8 hour monitoring was carried out with the help of IAQ tools. The sample size for this phase was 10 enterprises, The enterprises were selected randomly. Tool used in phase 2 for monitoring of the enterprises was IAQ monitor.

The data was collected by the researcher through personal visits to the enterprises with the help of proposed tools. Number of visits were done to gather the complete data and each interview took around 25-30 minutes. The responses obtained from the managers/owners were analyzed both qualitatively and quantitatively with the help of tables and graphs. The information gathered was studied and analyzed keeping in mind the objectives of the study.

General profile of the enterprises: These enterprises were established between the years 1980 and 1996. These enterprises either run in partnership or have one proprietorship form of ownership pattern. These enterprises majorly involve Metal molding, casting, manufacturing grills, cutlery and window panels. 94% of the employees working in these units were males, while only 6% of the employees were females. These 6% females were employed for administrative purposes only.

Awareness of the Owners/managers: Majority of the owner/managers were aware about the term Indoor Air Quality and its concept. They were aware that it affected their as well as their employees' productivity and were aware that there was a relationship between Indoor Air Quality and Health of the occupants. All of them also felt that it was important for them to improve Indoor Air quality of their enterprises. On the contrary, no one knew the type of pollutants that were emitted out of their processes. Even after having a good share of knowledge, majority of them took no measures to reduce impact of emissions indoors. Also, none of them gave any training or provided any information to the employees to aware them regarding Indoor air quality and its health effects.

Awareness of the employees: Majority of the employees did not know anything about the concept and told the researcher that they were listening it for the first time. They were unaware of Indoor Air Quality and its related health effects and that it could affect their productivity. These results also give the researcher a scope in educating the employees about importance of improving Indoor Air Quality of a building. But on the contrary, a majority of the employees were aware about molds and its related health risks. All the employees knew that controlling moisture in the building or the factory could help control the growth of molds.

Enterprise monitoring: Out of 10 randomly selected enterprises, 6 of them had relatively high concentrations of CO_2 mainly due to inadequate ventilation. Almost all the enterprises recorded the $PM_{2.5}$ concentrations as much as double the threshold values whereas PM_{10} concentrations were found out to be within the limits.

REFERENCES

[1] (2013). Achieving Iaq Supporting Health And Comfort In Highly Energy Efficient Buildings, 1-2.
[2] Air Pollution. (2014, June). Retrieved From Cpcb: Http://Www.Cpcb.Nic.In/Proto-Ind_Airpollution_June2014.Pdf
[3] Health Hazards Of Foundries And Forges. (2009).

http://dx.doi.org/10.22161/ijeab/2.1.67 *ISSN: 2456-1878*

[4] Indoor Air Quality. (2015, October 15). Retrieved February 2016, From United States Environmental Protection Agency: Http://Www.Epa.Gov/Indoor-Air-Quality-Iaq/Indoor-Air-Quality-Building-Type

[5] Kumar, D. A. (N.D.). Introduction To Air Polution. Retrieved From Http://Www.Eng.Utoledo.Edu/~Akumar/Iap1/Introduction.Htm

[6] Metal Industry In India. (2015). Retrieved From Indiabizclub.

[7] Shrivastava, A. K. (2009). Health Hazards Of Foundries And Forges. Kolkata.

http://dx.doi.org/10.22161/ijeab/2.1.68 *ISSN: 2456-1878*

Environmental Impact Evaluation of the Industry of *Panela* by Life Cycle Analysis

Dr. C. Walter Francisco Quezada Moreno[1]., Dra. C. Ana Margarita Contreras Moya[2], Dra. C. Elena Rosa Domínguez[2], Dr (asp.). David Quezada Torres[2], Mg. Franklin Antonio Molina Borja[1], Mg. Jaime Orlando Rojas Molina[1]

[1]Department of research, Technical University of Cotopaxi.
[2]Department of Chemical Engineering. University "Marta Abreu" of Las Villas. Carr. a Camajuani Km 5½. Santa Clara, Villa Clara. Cuba.

Abstract— *The objective of the study is to evaluate the environmental impacts generated by the industry of the panela in Ecuador, using the Life Cycle Analysis methodology. The in situ information gathered allowed the identification of the sensitive environmental factors that are affected in all the operations that are carried out in the agroindustry of panela.*

The results show that the craft activities prevail rather than the industrial activities, due to the low industrial development, application and control of regulations, rudimentary manufacturing practices, among others; which have led to insufficient productivity, competitiveness, quality and safety of their products. The results of the Environmental Impact Assessment, using the Eco-Indicator 99 method, according to the Life Cycle Analysis technique, show that the industrial stage has the greatest contribution to the impact, being more representative the impact to the effects by respiration of inorganic compounds, acidification / eutrophication, climate change and land use. The impact level of this type of industry according to Ecuador's environmental legislation, places it as an industry that generates moderate environmental impacts, it does not require intensive protective or corrective practices. However, it requires environmental executive actions oriented to the control and prevention to mitigate these impacts.

Keywords— Agroindustry of Panela, Life Cycle Analysis, Environmental Impact.

I. INTRODUCTION

As societies progress and develop, the energy demands and their environmental effects grow. A balance between production and consumption can be achieved if all the agents acting on the market do so in a responsible way[1], where quality and competitiveness must be fully justified by actions of continuous improvement and innovative efforts for clean and safe productions.

The agroindustry of *panela* in Ecuador has the purpose of developing productive activities from the cultivation, processing and commercialization of sugar cane for *panela*, natural sugar and honey. Its effects in terms of employment multiplication, value addition, and nutrients are high [2] [3]. It is estimated that there are 519 agro-industries, production units of *panela* in the country that use fuel materials for the process such as bagasse, firewood and tires alone or combined [4]. The lack of quality, harmlessness, diversification of production, assessment of productive yield and use of by-products are some of the problems that have generated its lag. In the agroindustry of *panela* the activities of handcrafted character prevail over the activities of industrial character. However, efforts are being made in technological improvements in the *panela* production, natural sugar and recent studies with cane honey, for commercial purposes [5]. In spite of the importance of the sector and the existence of environmental legislation, its execution with technical and environmental obligations has not yet materialized, affecting the productivity, quality and harmlessness of its products and environment.

In the industry of *panela*, the cleaner productions must be a reality and of business fulfillment and awareness. Clean Productions are safe and ultimately economical and necessary for the sustainability of a technological process. Innovative efforts are required from a technical-economic and environmental point of view to achieve improvements and higher productivity with lower resources, which must be valued environmentally to favor their investments and secure the market.

Environmental assessments are valid tools that have become in actions in the business and governmental world to understand and manage the risks and opportunities of the stages of the life cycle of a product or process towards the introduction of scientific knowledge to describe associated environmental impacts.

1. 1 Agroindustry of *Panela*

Panela activity is a subsector of the agro industrial sector, where the *panela* is the main sweetener obtained by extracting, cleaning and concentrating the juice of the

cane. It is a fundamental pillar for rural social, economic, cultural development and for the sovereignty and food and nutritional security of many countries in Asia, the Caribbean and South America [4] [6]. This activity in most factories is developed neglecting technical, environmental and quality standards throughout the process line.

The activities are more handcrafted than industrial; due to its poor development, application and control of regulations, rudimentary manufacturing practices, among others; which have led to insufficient productivity, competitiveness, quality and harmlessness of their products. However, it plays a key and an important role for food sovereignty and security, allowing safe and permanent access to sufficient and nutritious food in all markets, especially in rural and marginal urban areas.

This activity uses different materials for the production and commercialization: water, energy, cane and vegetal and chemical mucilages in the clarification of juice as clarol or sodium hydrosulfite, carbonates, vegetable fats, fuels and others [7] [8].

Deforestation for firewood and the establishment of cane cultivation, the preparation of the land and the application of agrochemicals for its management, coupled with the low efficiency of combustion and heat transfer processes in the burner, are activities that generate negative changes in environmental quality.

In many cases, the sustainability of the activity to concentrate juice to obtain *panela* is at risk, due to the lack of environmental awareness. The inefficiency of the *panela* burners, the use of wood that affects the forests, tires and greenhouse plastics to generate energy and that give off toxic gases such as carbon monoxide (CO) and nitrogen to the atmosphere, are aspects that weaken its activity. CO is a pollutant; it is the product of the incomplete combustion of any type of fuel. The tires have sulfur in the form of sulfides that in the burning they are oxidized and released into the air as a pollutant. This type of reaction is characterized by the presence of combustible substances or also called unburned in the combustion gases, such as carbon in the form of ash, CO and H_2. Bagasse, firewood and tires generate compounds such as CO_2, CO, NOx, SOx, H_2, H_2O and other gases and particles, energy and ash. In the stage of clarification, evaporation and concentration the negative effects are evidenced by the generated and untapped water vapor, the use of flocculants and the presence of wastes [9].

In spite of the multiple advantages of cane in the agroindustry of *panela* in Colombia and Ecuador, it has an undesirable impact due to the consumption of large amounts of fuel wood and used tires and the energy inefficiency of the burners. Up to three tons of firewood per ton of panela are used, generating problems of deforestation, soil erosion and pollution [10] [4], so its environmental effect is highly negative. The calorific power of a tire is 8300 Kcal / kg and the gases that are derived are compounds of carbon, nitrogen and sulfur. Carbon black creates disorders in the respiratory system.

Energy efficiency in the operation of the burner, good manufacturing practices and safety and good use and waste management, is determinant to avoid negative impacts to the environment in the activity of the agroindustry of *panela*. However, environmental degradation and overexploitation of natural resources can weaken a country's competitive base [11]. For this reason, a concept is needed that manages access to raw materials and imposes controls on improper processing practices in order to maintain the integrity of the environment and protect natural resources, and another that on the other hand, it is required an understanding of the competitive nature of the agroindustrial sector and the entrepreneur's unique role [12]. Planning is required for efficient performance in the technological and economic systems of the activity of the *panela* that does not adversely affect society and the natural environment. Consumers show and demand a growing interest in knowing what is behind each product, since, apart from the quality and safety of food, the health and welfare of the community and the quality of the environment are ahead.

1.2 Environmental analysis

Cities require food, paper, water, and other products every minute, so as by-products of their processes emit large quantities of particulate material, CO, SO_2, NOx and other gases as well as large volumes of solid and liquid wastes [13]. Therefore, despite a great effort in research and action aimed at avoiding environmental pollution, it remains a worldwide concern.

Life Cycle Analysis (LCA) is an environmental management technique to evaluate the potential environmental aspects and impacts associated with a product [14] [15]. It is a tool to examine the environment of the serious consequences of the manufacture and use of products or the provision of services [16]. It allows the systematic evaluation of the environment and aspects of a product or service system through all stages of its life cycle [17] [18]. The LCA is positioned as a key tool for the evaluation of potential environmental impacts, as well as for the generation of useful scientific and technical information for the orientation of environmental policy and legislation; the establishment and strengthening of integrated waste management programs and management plans among other policy instruments, as well as the decision-making in the industrial and social sectors [19].

The LCA means recognizing how our choices influence each stage of the process and thus weighing the advantages and disadvantages contributing to the

http://dx.doi.org/10.22161/ijeab/2.1.68 *ISSN: 2456-1878*

economy, the environment and society [20]. It is essential for the sustainable development, to go beyond traditional production to one with responsibility, where economic, environmental and social aspects prevail [21]. The application of the LCA technique in the industry of *panela* constitutes a viable and applicable tool for the entire chain of production, considering internal and external effects.

The greatest contribution is to think and act to mitigate adverse effects to the environment, caused by industrial processes, market or consumption. It is not only generating work, development or food, is to produce thinking about the welfare of all. It is health and good living. It is economic growth, ecological balance and social progress [22]. The global implementation plan for Sustainable Development calls for: "improving products and services while reducing health and environmental impacts, using scientific models such as life-cycle analysis where appropriate" [23].

The execution of LCA studies to the sector, analyzing the productions of the activity of *panela* including the natural sugar, honey and byproducts that it generates, will allow a comprehensive diagnosis of the environmental situation of this activity in the countries of the world and in Ecuador especially.

1.3 Environmental Legislation

Due to its level of pollution, industry in general has worldwide been permanently under the eyes of the community. FAO General - Director José Graziano said in Santiago de Chile that climate change is not a problem of the future but the present. He has warned of its impact in South America, he said that the impacts are being greater than what was thought and yet it is not known how it will affect food production [24].

Concern about the environmental deterioration due to industrialization at a global level has determined that the sustainability of the sector in the country is determined to comply with the current environmental legislation in the sector and country. However, its application and control has not had results, its breach is visible and notorious. According to Annex I of the National Environmental Categorization Catalog (CCAN), for the construction and / or operation of factories for the production *of panela*, it is classified in group II, considered of low impact and requires an environmental file [25].

The government of Ecuador has implemented demanding environmental legislation with respect to the environment, but, these provisions are not fulfilled in the sector. In Ecuador, Local and Provincial Governments known as GAD have legislations that try to regulate certain activities, but with unsatisfactory results for the community. The existing normative instruments of environmental legislation under the present Constitution of the Ecuador Republic grouped in the Unified Text of Environmental Legislation TULAs and the Organic Law of Municipal Regime guides the way of control of productive activities [26]. Considering the impact that the whole production chain has on the environment in a holistic manner will be decisive for the sustainability process of the sector [27].

II. MATERIALS AND METHODS

A bibliographical review about the industry of *panela* was carried out and data were collected through an in situ diagnosis to the agroindustries of *panela* in the center and north of the country. It is estimated that there are 519 productive units. In three production units, the gas produced by combustion was measured using the E-instrument 4400 equipment. The first unit of *panela* operates with bagasse, the second with firewood and the third uses pneumatic tires. In addition, the control in the diesel engine was carried out to operate the equipment of cane juice extraction.

For the environmental evaluation, applying the methodology of Life Cycle Analysis was considered as a case study the unit of *panela* that operates with bagasse only. It has a processing capacity of 20 t cane / day, where noise was also evaluated using the EXTECH Instruments sound level meter, ranging from 26 to 130 dB (A). Controls of wastewater volume, pH and soluble solids (brix) were performed on the liquid wastes from the cleaning, clarifying, concentrating and washing tubs of molds and materials. The environmental effects were analyzed according to the limits of the system shown in figure 1.

http://dx.doi.org/10.22161/ijeab/2.1.68 *ISSN: 2456-1878*

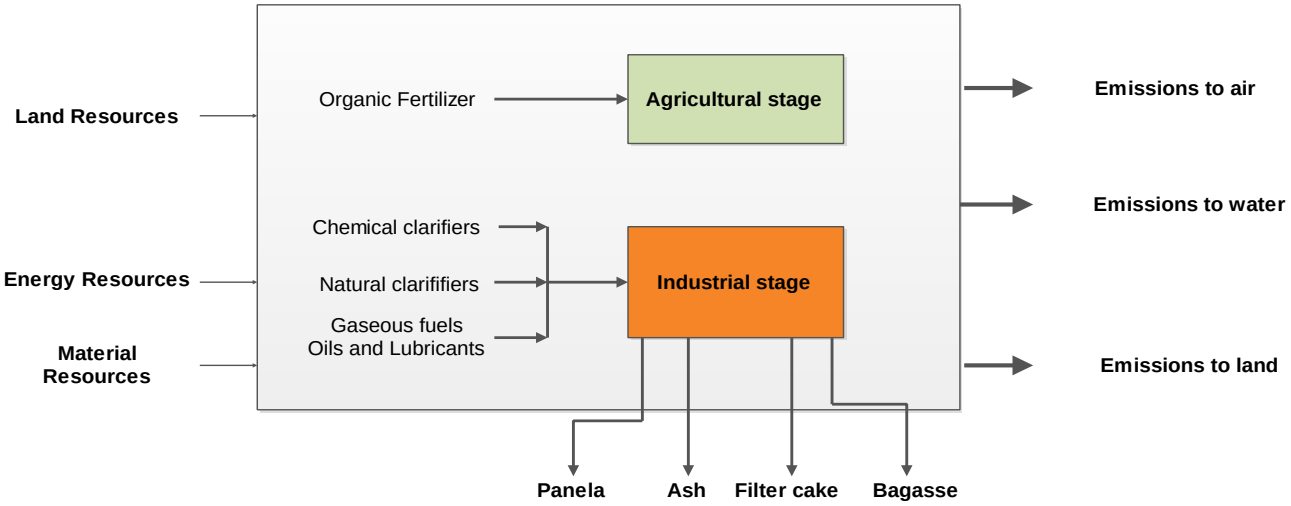


Fig.1: System limits for LCA in the production of panela.

The general inventory of the unit of production was carried out according to information of the entire productive chain of the activity of *panela* (cane production, processing and marketing).

The results are plotted and the environmental assessment is performed using the Life Cycle Analysis (LCA) methodology according to the Sima Pro7.3 software and the Eco-indicator 99 methodology, according to the system limits shown and taking as a functional unit the daily production of *panela*, which corresponds to 2485 kg.

III. RESULTS

The balance of material and energy applied through stoichiometric calculations according to the inputs and outputs of products generated the results indicated in figure 2.

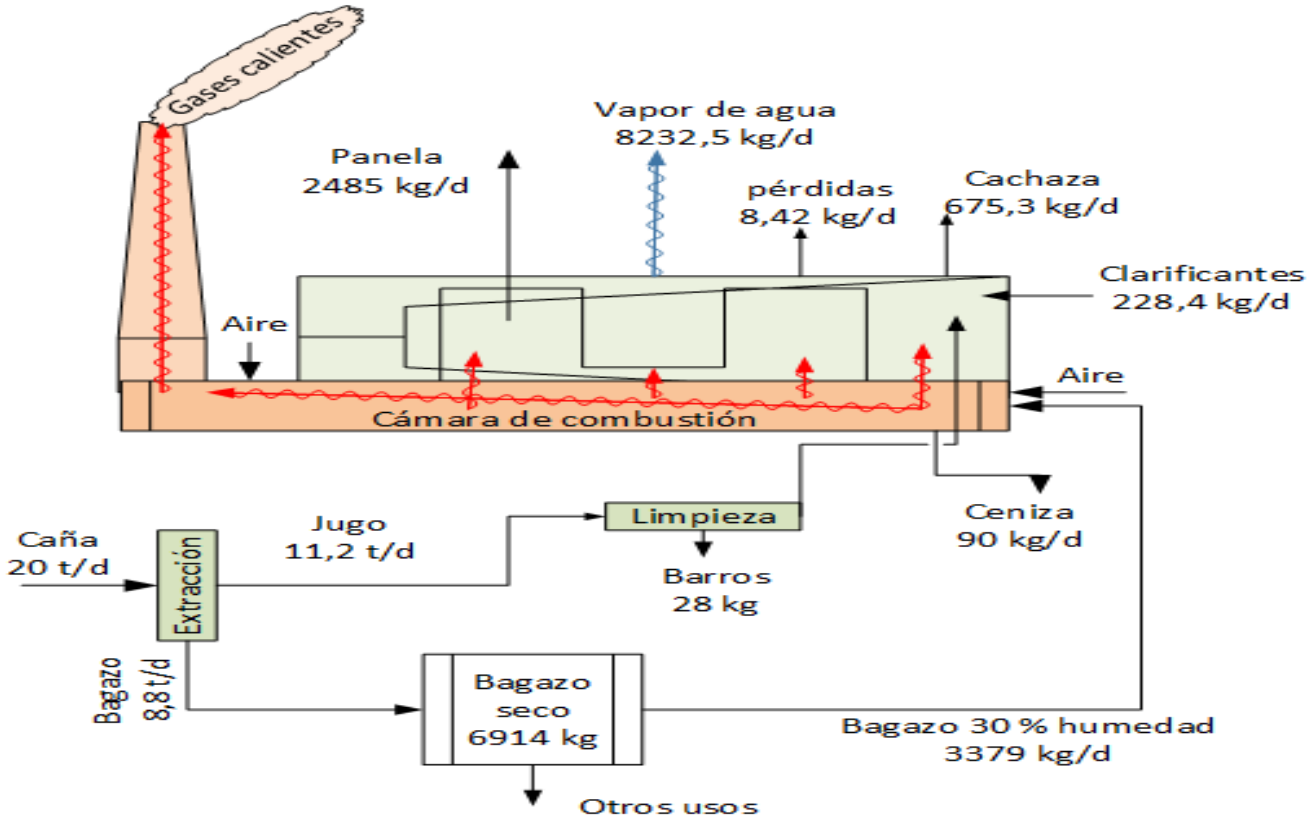


Fig.2: Mass balance and contaminant identification

http://dx.doi.org/10.22161/ijeab/2.1.68 *ISSN: 2456-1878*

The balance of mass and contaminants generated by the 20 tons of cane per day produces 2485 kg of *panela*, by-products and wastes, such as: 8800 kg of bagasse for combustion (energy needed to concentrate juice to *panela*) and 1530 liters of wastewaters produced in the different containers which are discharged to the ground and that have natural watercourses as destination. The pH of the wastewaters and the solids soluble in the solution in the different containers reach values between 4.64 - 5.11 and 2.10 to 11.3 brix degrees, respectively.

The analysis of the permissible limits of noise levels for fixed sources, mobile sources and vibrations is done according to the Unified Text of Environmental Legislation (TULAs), which establishes that the sound pressure limit within an industrial zone, is 75 dB (A) in an eight-hour noise exposure per day [26]. The results of noise indicate that the point A = 83.42 dB (A), very close to the engine (machine that drives the mill or cane juice extraction equipment), exceeds the permissible limit of noise emissions.

In Figure 3, the average results of 14 measurements performed every 30 minutes on the two burner combustion processes are shown: the first one using bagasse only, the second one operating with wood or firewood (sawmill waste and boxes used to transport fruit) and in the engine. In the burner using bagasse, high temperature values are obtained in the flue gases and the combustion is better, since fewer gases (CO and NO) are obtained in front of the burner that uses wood in the combustion.

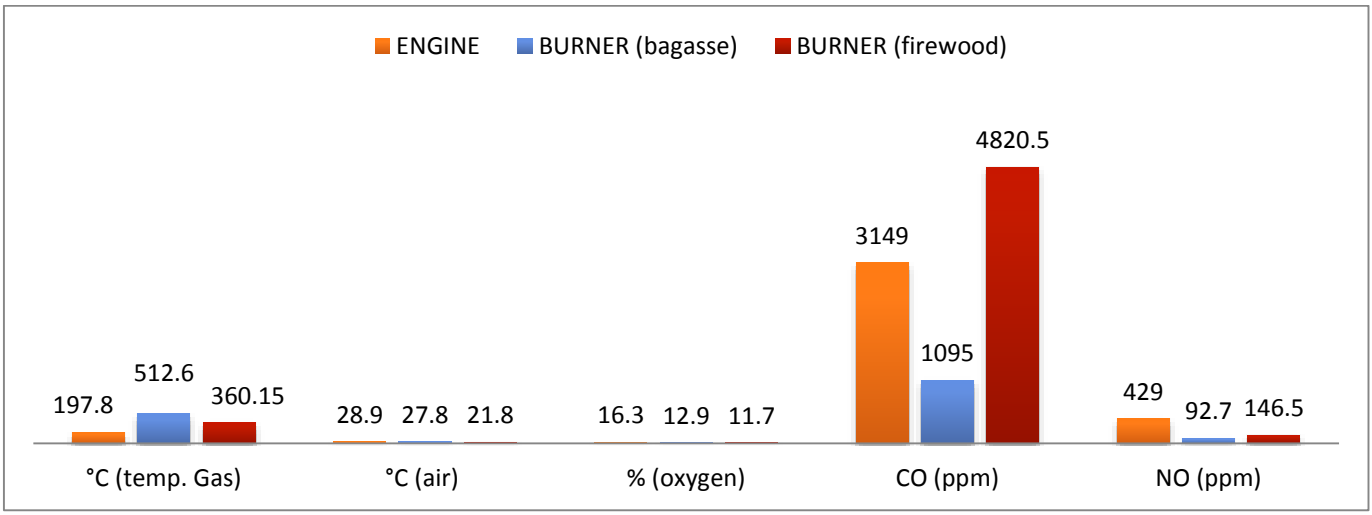


Fig.3: Parameters of the combustion of bagasse, firewood and in the engine.

The generation of gases is within the limit allowed in both, carbon monoxide (CO) and nitrogen (NO); being greater the amounts generated in the wood burner than in the one that uses bagasse. The emission of combustion gases, CO and NO, in the engine shows high values and is due to the years of work of the equipment (superior to 15 years), very common in most productive units of *panela* of Ecuador. When assessing measurements on a burner using bagasse and tires, in all measurements the CO values exceed the measurements reached in the burner using bagasse and firewood, which values are higher than the Environmental Standard of Colombia and Ecuador [26] [27].

Figure 4 shows the results of the life cycle assessment in the production of *panela* using bagasse in the burner (case study), by means of Sima Pro7.3 software and the methodology of Eco-indicator 99, from the data of the inventory. The network of the process shows that the industrial stage is the one that contributes most to the contamination. Figure 5 shows that the greatest contribution in all impact categories is in the industrial process, with a more representative impact to the respiratory effects of inorganic compounds, acidification / eutrophication, climate change and land use.

http://dx.doi.org/10.22161/ijeab/2.1.68 *ISSN: 2456-1878*

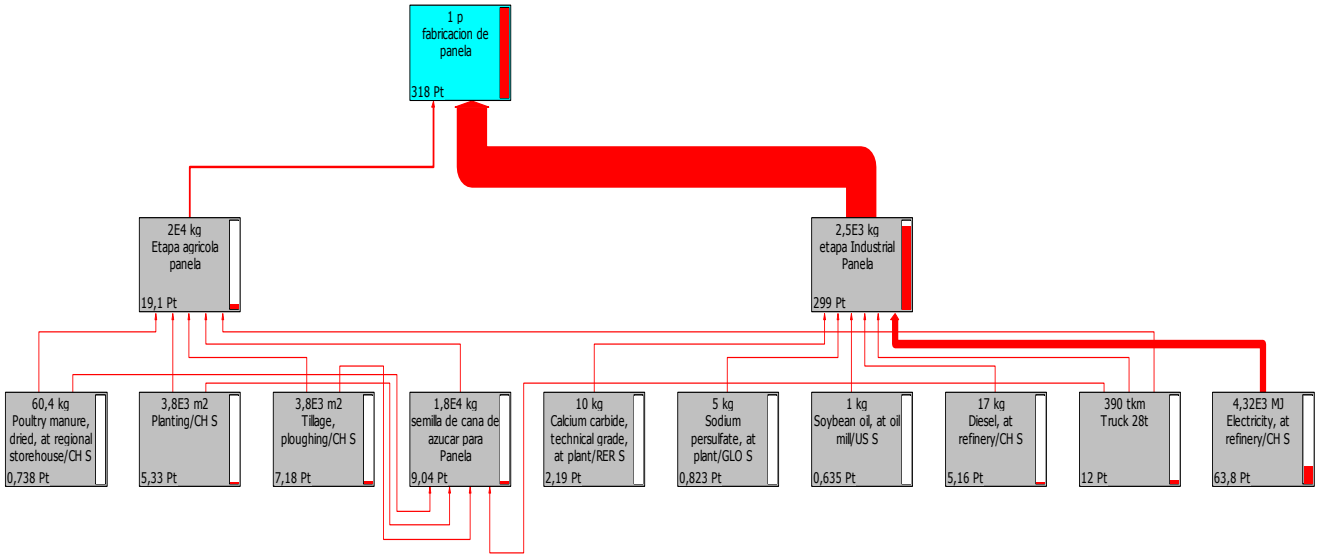


Fig.4: Tree of impacts obtained for the production of panela

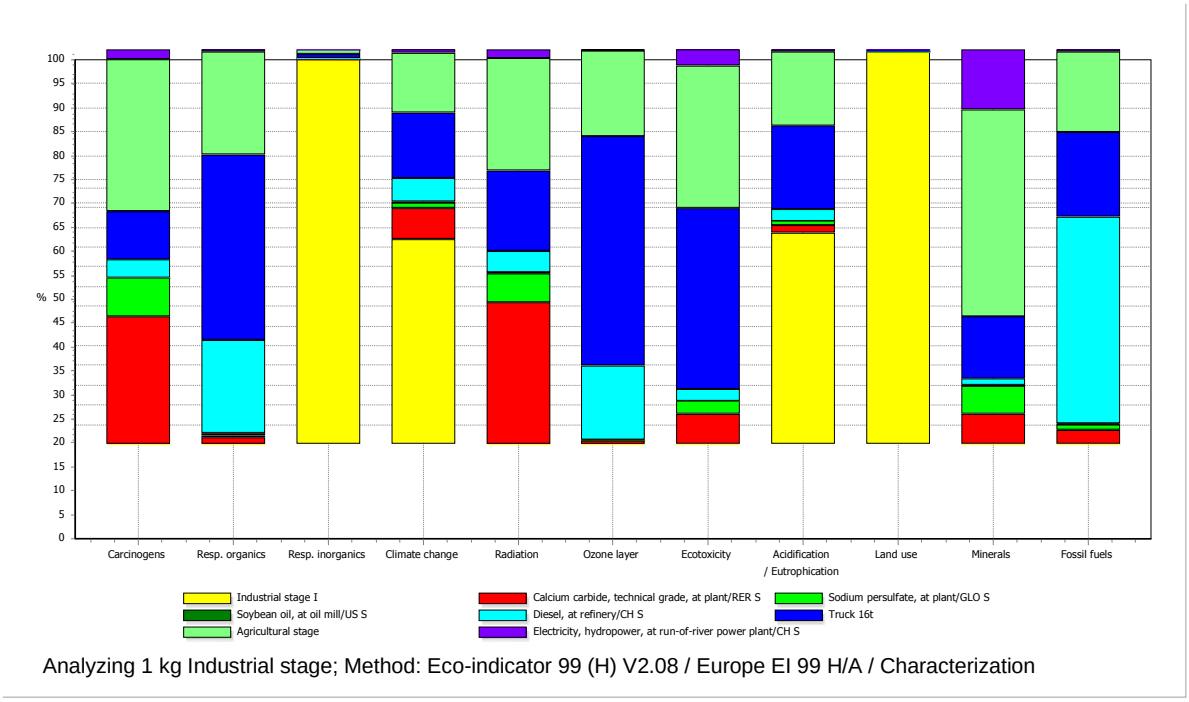


Fig.5: Contribution results by impact category.

From the analysis of the contribution to the categories of damage that is offered in the graph 6, it is seen that the process has a greater incidence in the damages to the ecosystem, mainly due to the categories of land use and acidification / eutrophication. The effects on human health are related to respiration of inorganic compounds and climate change, and the effects on resources are concentrated on the use of fuel for transportation and electricity in the industrial stage. The environmental impact generated by this production affects the sustainability of the process, especially when it is not given a treatment and proper use to the main waste, such as ash, filter cake and bagasse.

On the other hand, in order to mitigate the affectations, work must be done on improving worker protection and hygiene. It is necessary to dispose a wastewaters collection tank to proceed with treatment before its discharge into tributaries or other uses.

Although at the level of factories, the Ecuadorian agroindustry of *panela* is artisan and rudimentary, there

http://dx.doi.org/10.22161/ijeab/2.1.68 *ISSN: 2456-1878*

are opportunities for improvement by studies to intensify the process, since they allow raising efficiency, quality and care of the environment. Globalization and markets demand quality and harmless products, under the slogan of cleaner production.

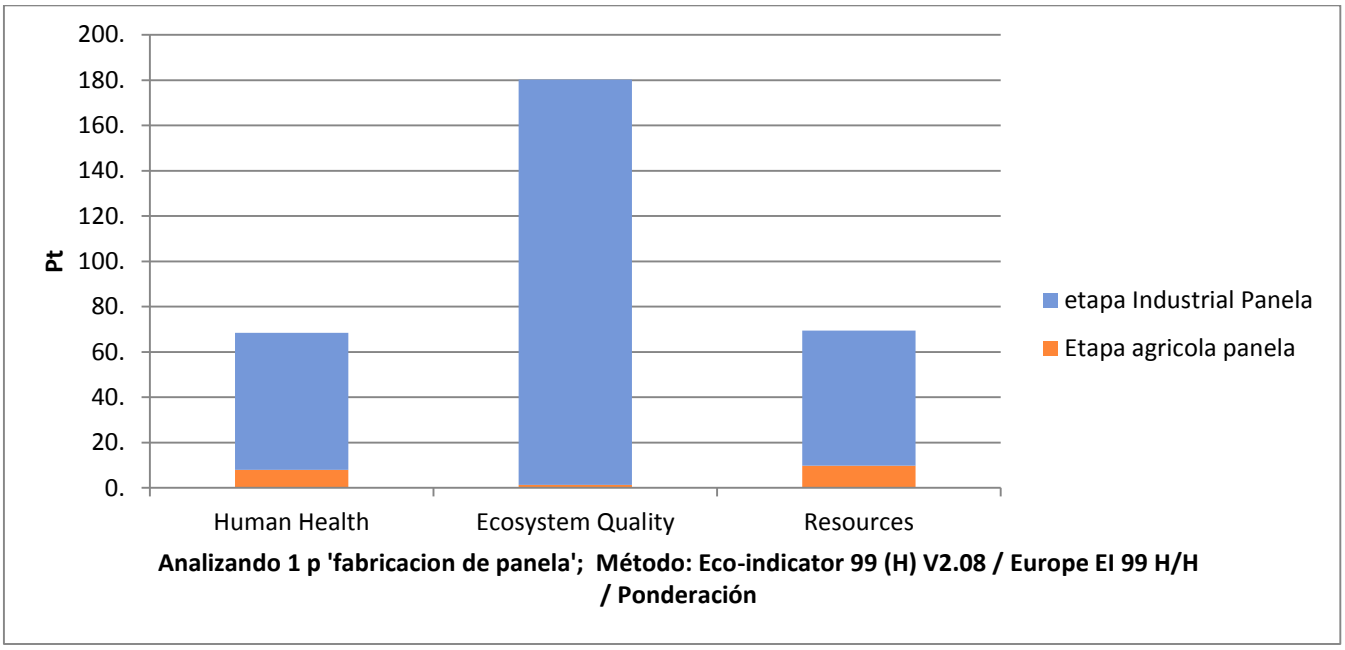


Fig.6: Contribution of the agricultural and industrial stages to the damage categories of the Eco-Indicator 99 expressed in points

IV. CONCLUSIONS

The Ecuadorian agroindustry of *panela*, being a more artisan activity than industrial is categorized in group II, it is considered of low impact and requires an environmental record, but because of its productive linking it generates problems that affect the environment despite being a fundamental pillar for rural social, economic, cultural and important development for the sovereignty and food and nutritional security of the country.

The use of bagasse as fuel for the generation of energy in this type of burners to the production of panela is better and less polluting than the use of firewood or tires, so it is necessary to focus on studies of optimization of burners and efficient use of fuels.

The Life Cycle Analysis of the process showed that the greatest impact is originated in the industrial stage with 94% of contribution and a more representative impact is exhibited on the effects of respiration of inorganic compounds, acidification / eutrophication, climate change and land use.

The contribution to the categories of damage of the Eco-Indicator 99 expressed in points is superior for the Quality of the Ecosystem, with respect to Human Resources and Health; therefore, reveals the need to look for executive solutions that ensure its progress, productivity, quality and safety for sustainability.

REFERENCES

[1] Goedkoop Mark; Effting Suzanne; and Collignon Mercel, 1999. Manual Práctico de ecodiseño. Anexo Eco-indicador 99. Método para evaluar el impacto ambiental a lo largo del ciclo de vida. Holanda, p. 5. Pdf.

[2] Kumar, A. 2010. An Empirical Study on Gur (Jaggery) Industry. Indian Institute of Management Ahmedabad-380 015. India. W.P. http://ssrn.com/abstract=1783403. (Accessed 03 January 2016), p.1-19.

[3] FAO, 2013. Agroindustria para el desarrollo. Food and agricultural organization, Roma. http://www.fao.org/docrep/017/i3125s/i3125s00.pdf. (Accessed 02 January 2016), p. 283.

[4] Quezada, W, 2015. Procedimiento para la intensificación y reconversión de instalaciones paneleras. Tesis presentada en opción al Grado Científico de Doctor en Ciencias Técnicas, Especialidad Ingeniería Química en la Universidad Central Marta Abreu de Las Villas, Cuba.

[5] Quezada Walter; González Erenio; Quezada Walter David and Mera Cristina, 2016. Cane Honey: Process, Quality and Harmlessness. International Journal of Engineering Research. doi: 10.17950. Volume No.5, Issue No.7. 589-593.

[6] Rodríguez Gonzalo, García Hugo, Roa Zulma, Santacoloma Pilar, 2007. Panela production as a

strategy for diversifying incomes in rural area of Latin America. Roma.

[7] Quezada, W., and Gallardo, I, 2014a. Obtención de extractos de plantas mucilaginosas para la clarificación de jugos de caña. Revista Tecnología Química. RTQ. 34(2):114/123. Cuba.

[8] Quezada, W., and Gallardo, I, 2014b. Clarificación del jugo de caña mediante el empleo de plantas mucilaginosas. Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar. ICIDCA. 3:41-48. Cuba.

[9] García María, 2004. Hornillas Paneleras. Evaluación de su Impacto ambiental. Revista Innovación y Cambio Tecnológico, pp. 20-29. Corpoica. Colombia. www.corpoica.org.co - Artículos científicos.

[10] Rodríguez Gonzalo, 2005. La agroindustria rural de la panela en Colombia. Roles, Problemática y Nuevos Retos. Corpoica. Colombia. www.corpoica.org.co - Artículos científicos.

[11] ChidiK Martina y Murmis M., 2003. Gestión ambiental en la agroindustria, competitividad y sustentabilidad, p. 7.

[12] da Silva Carlos, Doyle Baker, Andrew W. Shepherd, Chakib Jenane y Sergio Miranda da Cruz, 2013. Agroindustrias para el desarrollo. FAO. Italia, 2, 254.

[13] Zaror C. 2013. Introducción a la ingeniería ambiental para la industria de procesos. Universidad de Concepción. Chile, pp. 3-7.

[14] Norma Cubana NC ISO 14040. 2009a. Gestión Ambiental-Análisis del Ciclo de Vida-Principios y Marco de Referencia. [ISO 14040:2006 IDT. Habana, Cuba. Oficina Nacional de Normalización.

[15] NC-ISO 14044. 2009b. Gestión Ambiental-Análisis de Ciclo de Vida-Requisitos y Directrices (ISO 14044: 2006, IDT). Oficina Nacional de Normalización. Cuba.

[16] Renee L., 2002. Cornelissen and Gerard G. Hirs. The value of the exergetic life cycle assessment besides the LCA. Energy Conversion and Management 43. Elsevier. Science Ltd. Netherlands. PII: S0196-8904(02) 00025- 0; Pp.1417–1424.

[17] Contreras Ana; Rosa Elena; Van Langenhove Herman; Santos Herrero Ronaldo; Pérez Gil Maylier; Casas Ledón Yannay; Dewulf Jo. 2013. Exergetic analysis in cane sugar production in combination with Life Cycle Assessment. Journal of Cleaner Production. ELSEVIER. 59 (15)- 43-50.

[18] Contreras Moya Ana; Rosa Domínguez Elena; Pérez Maylier; Van Langenhove Herman, 2009. Comparative life Cycle of four alternatives for using by-products of cane sugar production. Journal of Cleaner Production. ELSEVIER. 17 (8). 772-779.

[19] Aguilar G.; Campos A.; Forcada A.; Sören Björn Rüd and Martínez Segio, 2013. Estudio de Análisis de Ciclo Vida (ACV) del manej o de envases de bebidas de polietileno tereftalato (PE T) en la fase de pos-consumo Instituto Nacional de Ecología y Cambio Climático, INECC-Cooperación Alemana al Desarrollo. GIZ., p.4. 1-57.

[20] UNEP, (2004). ¿Por qué Adoptar un enfoque de ciclo de vida?. Life Cycle. Una publicación de las Naciones Unidas. ISBN: 92-807-24500-9, p. 6.

[21] Arne Remmen, Allan Astrup Jensen Jeppe Frydendal, and Danish Standards, 2007. Life Cycle Management A Business Guide to Sustainability. UNEP. *United Nations Environment Programme, EEUU, p.12.*

[22] Norris Gregory; Suppen Nydia; Paula M. do Nascimento Ana and Lie Ugaya Cássia Maria, 2005. ACV – conceitos para o desenvolvimento industrial na américa latina. Impactos socio-económicos en el análisis de ciclo de vida de productos: de un análisis ambiental de ciclo de vida (ACV) a un ACV sustentable en latino-america. Brasil, p. 40.

[23] Suppen Nydia and van Hoof Bart, 2005. Conceptos básicos del Análisis de Ciclo de Vida y su aplicación en el Ecodiseño. México, pp. 1-40.

[24] Cámara de Industrias y Producción, 2015. Boletín de Ambiente y Seguridad Ambiental. Boletín No 25. Ecuador. Pdf.

[25] Ministerio del ambiente, 2014. Catálogo de categorización nacional ambiental CCAN. Anexo 1. http://www.cip.org.ec/attachments/article/2285/ANEXO%201%20CCAN.pdf. [consulta: 11 de agosto 2014].

[26] Ministerio del Ambiente, anexos 1, 2 y 5., 2002. Norma de calidad ambiental del recurso suelo y de descarga de efluentes: recurso agua-libro vi-anexo 1. Criterios de remediación para suelos contaminados-libro vi-anexo 2. Límites permisibles de niveles de ruido ambiente para fuentes fijas y fuentes móviles, y para vibraciones-libro vi-anexo 5. Quito Ecuador.

[27] Sánchez O.; Cardona C. and Sánchez D., 2007. Análisis de ciclo de vida y su aplicación a la producción de bioetanol: Una aproximación cualitativa. Revista Universidad EAFIT. Vol. 43. No. 146. Colombia, pp. 59-79.