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*Editor in Chief*

Dr. Pietro Paolo Falciglia

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# FOREWORD

I am pleased to put into the hands of readers Volume-9; Issue-1, January-February 2024 of “**International Journal of Environment, Agriculture and Biotechnology (IJEAB) (ISSN: 2456-1878)**”, an international journal which publishes peer reviewed quality research papers on a wide variety of topics related to **Environment, Agriculture and Biotechnology**. Looking to the keen interest shown by the authors and readers, the editorial board has decided to release issue with DOI (Digital Object Identifier) from CrossRef also, now using DOI paper of the author is available to the many libraries. This will motivate authors for quick publication of their research papers. Even with these changes our objective remains the same, that is, to encourage young researchers and academicians to think innovatively and share their research findings with others for the betterment of mankind.

I thank all the authors of the research papers for contributing their scholarly articles. Despite many challenges, the entire editorial board has worked tirelessly and helped me to bring out this issue of the journal well in time. They all deserve my heartfelt thanks.

Finally, I hope the readers will make good use of this valuable research material and continue to contribute their research finding for publication in this journal. Constructive comments and suggestions from our readers are welcome for further improvement of the quality and usefulness of the journal.

With warm regards.

Editor-in-Chief

Date: March, 2024

# Vol-9, Issue-1, January - February 2024

(DOI: 10.22161/ijeab.91)

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1

## Reproductive phenology and environmental temperatures in the Smooth Newt *Lissotriton vulgaris meridionalis* (Boulenger, 1882), (Amphibia, Urodela) in a Mediterranean habitat

Author(s): Antonio Pizzuti Piccoli

 DOI: [10.22161/ijeab.91.1](https://doi.org/10.22161/ijeab.91.1)

Page No: 001-007

2

## Comparative Performance of the Developed Mini Tractor Operated Sprayer Cum Weeder with The Various Types of Existing Spraying and Weeding Methods

Author(s): M. V. Jalu, P. S. Ambaliya, Y. H. Modhvadiya

 DOI: [10.22161/ijeab.91.2](https://doi.org/10.22161/ijeab.91.2)

Page No: 008-019

3

## Economic efficiency of human urine as fertilizer in the production of NERICA 4 rice in the Sudano-Sahelian zone of Mali

Author(s): Ousmane Coulibaly, Sory Sissoko, Alou Coulibaly, Yacouba Maiga, Mohamed Dicko, Sidiki G Dembele, Mohamed S Maiga


 DOI: [10.22161/ijeab.91.3](https://doi.org/10.22161/ijeab.91.3)

Page No: 020-025

4

## Effect of priming treatment and storage containers to enhance the seed quality of tomato seeds

Author(s): Nisha, Axay Bhuker, Mukesh Kumar, Rahul and Gagandeep Singh

 DOI: [10.22161/ijeab.91.4](https://doi.org/10.22161/ijeab.91.4)

Page No: 026-033

5

## A Cost-Benefit Analysis of Building a Dam in Pakistan: To Mitigate Floods, Promote Tourism, Boost Agriculture, and Generate Electricity

Author(s): Shahzad Ismail

 DOI: [10.22161/ijeab.91.5](https://doi.org/10.22161/ijeab.91.5)

Page No: 034-049

6

## In Vitro Efficacy of Entomopathogenic Nematodes (EPNS) against Economically Important Insect-Pests of Cauliflower

Author(s): Babita Kumari, Anil Kumar, Sujata, Lochan Sharma

 DOI: [10.22161/ijeab.91.6](https://doi.org/10.22161/ijeab.91.6)

Page No: 050-058

7

*Multi-criteria analysis of the environmental vulnerability of the cotton zone of Mali: Case of the northeast subsidiary of Koutiala*

*Author(s): Tahirou Barry, Hamidou Diawara, Souleymane Sidi Traoré, Rachid Abdourahamane Attoubounou*


 DOI: [10.22161/ijeab.91.7](https://doi.org/10.22161/ijeab.91.7)

**Page No: 059-068**

8

*Green Synthesis of Gold Nanoparticles Using Acacia Modesta*

*Author(s): Adil Khan, Sardar Mohammad, Hakeem Ullah, Sadia Sardar, Sahibzada Muhammad Aqeel, Waleed AL-Ansi, Guochao Xu*

 DOI: [10.22161/ijeab.91.8](https://doi.org/10.22161/ijeab.91.8)

**Page No: 069-077**

9

*Wildlife migration corridors from the Community Forest of Nafadji to the Boucle du Baoulé Biosphere Reserve*

*Author(s): Hady Diallo, Coulibaly Ousmane, Yacouba Maiga, Aly Poudiougou*

 DOI: [10.22161/ijeab.91.9](https://doi.org/10.22161/ijeab.91.9)

**Page No: 078-089**

10

*Effect of Gibberellin and Nitrogen Fertilizer on Growth and Zucchini (Cucurbita pepo L.) Yield In Tropical Regions*

*Author(s): Gandi Warisman, Damanhuri, Eko Widaryanto*

 DOI: [10.22161/ijeab.91.10](https://doi.org/10.22161/ijeab.91.10)

**Page No: 090-096**

11

*Land use dynamics and socio-economic impacts of wetlands in the Oti plain in the face of climate change*

*Author(s): Lardja Lamboni, Maimounata Belem*

 DOI: [10.22161/ijeab.91.11](https://doi.org/10.22161/ijeab.91.11)

**Page No: 097-114**

12

*Evaluating the Effects of Different Sowing Dates and Tillage Practices on Faba bean Yield Based on DSSAT Model*

*Author(s): Ashraf Nour El-Sadek*

 DOI: [10.22161/ijeab.91.12](https://doi.org/10.22161/ijeab.91.12)

**Page No: 115-125**

13

*Remodeling in Microbial Fuel Cell (MFC) design and parameters for the sustained production of electricity*

*Author(s): Mohammad Farooq, Hanif Ul Islam, Adil Khan, Salman Khan, Sardar Mohammad, Waleed AL-Ansi*

 DOI: [10.22161/ijeab.91.13](https://doi.org/10.22161/ijeab.91.13)

**Page No: 126-139**

14

*Influence of Foliar Application of Salicylic Acid on Growth and Yield of Chia (Salvia hispanica)*

Author(s): Jashkaran Singh, L. P. Yadava, Raj Nandini Singh

 DOI: [10.22161/ijeab.91.14](https://doi.org/10.22161/ijeab.91.14)

Page No: 140-145

15

*Solid Waste Management- Mismanagement from Houseboats of Dal Lake: Assessing Strategies for Effective Waste Reduction and Resource Recovery*

Author(s): Umer Inayat, Rizwana Malik, Adnan Abubakr, Bilal Ahmad Bhat, Samreena Jan


 DOI: [10.22161/ijeab.91.15](https://doi.org/10.22161/ijeab.91.15)

Page No: 146-152

16

*Amino Acid Profile and Physicochemical Properties of African Locust Bean (Parkia biglobosa) Seeds as affected by Combined Irradiation and Cooking*

Author(s): Alex O Asunni, Stephen A. Fagbemi, Ajibola M. Oyinloye, Catherine B. Gowon, Victor N. Enujiughha

 DOI: [10.22161/ijeab.91.16](https://doi.org/10.22161/ijeab.91.16)

Page No: 153-164

17

*Trichoderma-fortified compost in controlling diseases and increasing yield of tomato*

Author(s): Rezwana Rahman, Md. Khurshed Alam Bhuiyan, Md. Abu Ashraf Khan, M. Mofazzal Hossain, Md. Tanbir Rubayet

 DOI: [10.22161/ijeab.91.17](https://doi.org/10.22161/ijeab.91.17)

Page No: 165-174

18

*The benefits of market gardening in a context of food insecurity in the commune of Djirataoua in Niger*

Author(s): Younoussou Rabo, Inoussa Ali Garba, Ali Mahamane

 DOI: [10.22161/ijeab.91.18](https://doi.org/10.22161/ijeab.91.18)

Page No: 175-187



# Reproductive phenology and environmental temperatures in the Smooth Newt *Lissotriton vulgaris meridionalis* (Boulenger, 1882), (*Amphibia*, *Urodela*) in a Mediterranean habitat

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**Abstract**— The present work aims to contribute to the knowledge of the influence of environmental temperatures on the reproductive dynamics of the population of the Smooth Newt *Lissotriton vulgaris meridionalis* (Boulenger, 1882) at “Bosco di Palo” Natural Park (north of Rome, Central Italy). The data collection took place for a long – term investigation from the breeding season of 1995 – 1996 until the breeding season 2014 – 2015 (with the exception of the seasons 2003 – 2004, 2004 – 2005 and 2005 – 2006); the breeding season is considered the beginning of the filling of temporary ponds until they are completely drained. Samples were taken every fifteen days. During the breeding seasons investigated, the greatest concentration of individuals in water occurs in the period between January and May. The maximum presence of newts in water occurs with a maximum temperature range that oscillates from 14 °C to 17 °C and a minimum temperature that oscillates from 3 °C to 7 °C. From the analysis of the data it would therefore emerge that the minimum atmospheric temperature may constitute one of the most significant environmental parameter for the reproductive phase of the Smooth Newt. This data agrees with the observations reported in the present work and suggests that the identified trend can be extended to populations of Mediterranean environments.



**Keywords**— *Lissotriton vulgaris*, long-term study, Mediterranean habitat, reproductive phenology, temperature.

## I. INTRODUCTION

The present work aims to contribute to the knowledge of the influence of environmental temperatures on the reproductive dynamics of the population of the Smooth Newt *Lissotriton vulgaris meridionalis* (Boulenger, 1882) at “Bosco di Palo” Natural Park.

The smooth newt *Lissotriton vulgaris meridionalis* is a Urodelian Amphibian distributed in the Italian peninsula, with the exclusion of the southern regions (RAZZETTI & BERNINI, 2006). Its ecology, in the Mediterranean, is strictly influenced by environmental parameters. The vitality of populations is closely linked to the conservation

of small wetlands, often temporary, which allow egg deposition and larval development (BELL & LAWTON, 1975; ACCORDI & NOBILI, 1999; PIZZUTI PICCOLI, 2008; PIZZUTI PICCOLI, 2017) .

The newt reproduces both in temporary and perennial waters (ponds, lakes, fountains), never in flowing waters (BELL, 1977; RAZZETTI & BERNINI, 2006). Given the absence of fish, temporary ponds have the advantage of significantly reducing the number of predators present. Temporary ponds, however, are extremely unpredictable habitats, and premature drying can often destroy an entire generation of larvae. Metamorphosed individuals spend



about two years in the undergrowth before reaching sexual maturity and returning to breed in ponds. The adults live a terrestrial life outside the breeding season (GRIFFITHS, 1984).

In the “Bosco di Palo” Natural Park, the populations of *Lissotriton vulgaris meridionalis* have been monitored since 1995 and we have seen how their state and reproductive biology are closely related to rainfall, temperatures and the seasonal filling of temporary ponds (PIZZUTI PICCOLI, 2008 ; PIZZUTI PICCOLI, 2010).

In recent decades, amphibian populations in the world have shown a sharp decline due to global warming and the reduction of rainfall in many sites of the distribution range (METTOURIS *et al.*, 2017; PIZZUTI PICCOLI, 2017).

The study aims to verify, through long-term observations, whether it is possible to identify an optimal temperature range for reproduction of the Smooth Newt in Mediterranean habitat.

## II. STUDY AREA

The “Bosco di Palo” Natural Park (Fig. 1) is located 37 km to the north of Rome (Central Italy - IGM Topographic Map Sheet 149 NE IV) and is situated between the sea and the Via Aurelia in locality of Palo Laziale, in the town of Ladispoli (41 ° 56 'N, 12 ° 05 'E). The study area is part of a narrow coastal plain that extends from the delta of the Tiber River and that was formed during the Quaternary period (BONO *et al.*, 1993).



Fig.1: The “Bosco di Palo” Natural Park.

The territory was divided into three longitudinal strips parallel to the sea, a band made up of silt deposits and marshy black lands, an intermediate band characterized by ancient fossil dunes and a third more recent band formed by coastal dune and beach (currently in strong erosion). The soil wooded area is characterized by clay. The climate is part of the type mesomediterranean with mild winter, a summer period of about three months of dryness and rainfall regime of maritime type (BLASI 1994; BLASI 2018).

The environments that we find in the Park are the Mediterranean scrub, planitial wood and grassland. The planitial wood, characterized by the presence of temporary ponds, consists of a mixed forest of deciduous oaks of about 60 hectares, with the dominance of *Quercus ilex* L., *Quercus cerris* L., *Quercus pubescens* Willd. and *Ulmus minor* Miller (LUCCHESI, 1990; LA MONTAGNA *et al.*, 2023).

The amphibians of the study area are represented by four species: *Bufo bufo* (Linnaeus, 1758), *Hyla intermedia* Boulenger, 1882, *Pelophylax bergeri* (Günther, 1986) / *Pelophylax klepton hispanicus* (Bonaparte, 1839) and *Lissotriton vulgaris meridionalis* (Boulenger, 1882) (PIZZUTI PICCOLI, 2008).

The temporary ponds are temporary water collections whose depth varies between 20 and 150 cm. These environments are extremely precarious because they are influenced by the seasonal weather patterns. Because of the shallow, thermal stratification is absent; the temperature of the water, from surface to bottom, is under the direct influence of the sun and reflects the seasonal and daily variations in air temperature, even if it remains always few degrees below respect to it. The ponds undergo a drying period, from June to September, and freezing at the surface for few days during negative peaks of temperatures in the months of January and February. The oxygen concentration is subject to daily and annual fluctuations and also varies vertically; it is higher in surface for the presence of photosynthetic organisms and less abundant on the bottom for the presence of organisms decomposers. The water pH decreases with the onset of warm weather (GATTA, 1990; MURA & BRECCIAROLI, 2003). The bottom of the ponds is characterized by a strong decomposing activity; the half-submerged trees growing around the ponds and directly into the water (mainly *Fraxinus oxycarpa* Bieb.) release a considerable mass of leaves on the bottom of ponds. Within the ponds, the vegetation is very scarce and characterized by terrestrial grasses that withstand periods of immersion.

For the research were chosen three ponds in the wood that have the following characteristics: Pond 1, called "pond of *Emys*", with a maximum diameter of 20 m, a maximum area of 62.8 sq. m. and a maximum depth of 120 cm; Pond 2, called "pond of newts", with a maximum diameter of 4 m, maximum area of 12.56 sq. m. and a maximum depth of 81 cm; Pond 3, called "pond of reeds", with a diameter of 22 m, maximum area of 69 sq. m. with a maximum depth of 83 cm. The Pond 3 is characterized by the coverage of rushes, *Juncus sp.* and *Typha sp.* in about a third of the surface (LUCCHESI, 1990; LA MONTAGNA *et al.*, 2023).

### III. MATERIAL AND METHODS

The data collection took place from the breeding season of 1995 – 1996 until the breeding season 2014 – 2015 (with the exception of the seasons 2003 – 2004, 2004 – 2005 and 2005 – 2006, in which the study was temporarily interrupted); the breeding season is considered the beginning of the filling of temporary ponds until they are completely drained. Samples were taken every fifteen days.

The meteorological data (maximum and minimum daily temperature, rainfall) were provided by the Survey Station of the Ministry of Agricultural and Forestry Resources present in the “Bosco di Palo” Natural Park and, subsequently, since 2006, by the Survey Station of the Ministry of Agricultural and Forestry Resources of Massimina (Rome).

The capture of the specimens was performed by dipnetting, according to pre-established transects, by using a net square shape with side of 36 sq. cm, with square mesh of 0.5 cm side.

For each sampling has been established dipnetting mode according to the size of the pond (HEYER, 1988); in the “pond of reeds” the research was carried out with an average of 80 dipnetting for sampling, in the “pond of *Emys*” the research was carried out with an average 80 dipnetting for sampling and in the “pond of newts” the research was carried out with an average of 30 dipnetting for sampling.

In each season the specimens were marked through photos of the ventral pattern and was made an estimation of the population density applying the Lincoln - Petersen Method modified by Bailey, suitable for small populations of temporary ponds (ACCORDI & NOBILI, 1999; PIZZUTI PICCOLI, 2017); Bailey's modification is thought to yield a better estimate when sample size is

small (less than circa 20) (BAILEY, 1951; GREENWOOD & ROBINSON, 2006).

The field monitoring was conducted in accordance with applicable laws and authorizations provided for this kind of studies. Handling of individuals was made in compliance with the standards necessary to prevent transmission of pathogens between individuals (RAZZETTI & BONINI, 2001; STOCH & GENOVESI, 2016).

### IV. RESULTS

The graphs in Fig. 2, Fig. 3 and Fig. 4 show the data relating to the comparison between the maximum and minimum daily temperatures and the average number of newt captures in the study period for each sampling day. The graph refers to the total number of newts captured and to the male and female specimens of the population. In the breeding seasons monitored, the presence of water in the pools since autumn has led to the presence of the animals for an average long period (with presences also in autumn) even if the capture frequencies have a spring maximum in the months of March and April. In some seasons, the entry into the water occurs concentrated in a short time if the water in the pools forms late (late winter). The exit from the water generally always occurs in a short time at the end of spring.

In TABLE 1 are shown the number of individuals caught per breeding season and the population size estimated for the site. The data produced can be considered an underestimation of the population of “Bosco di Palo” for the presence of other breeding sites besides those investigated, certainly has been identified the range size of the population.

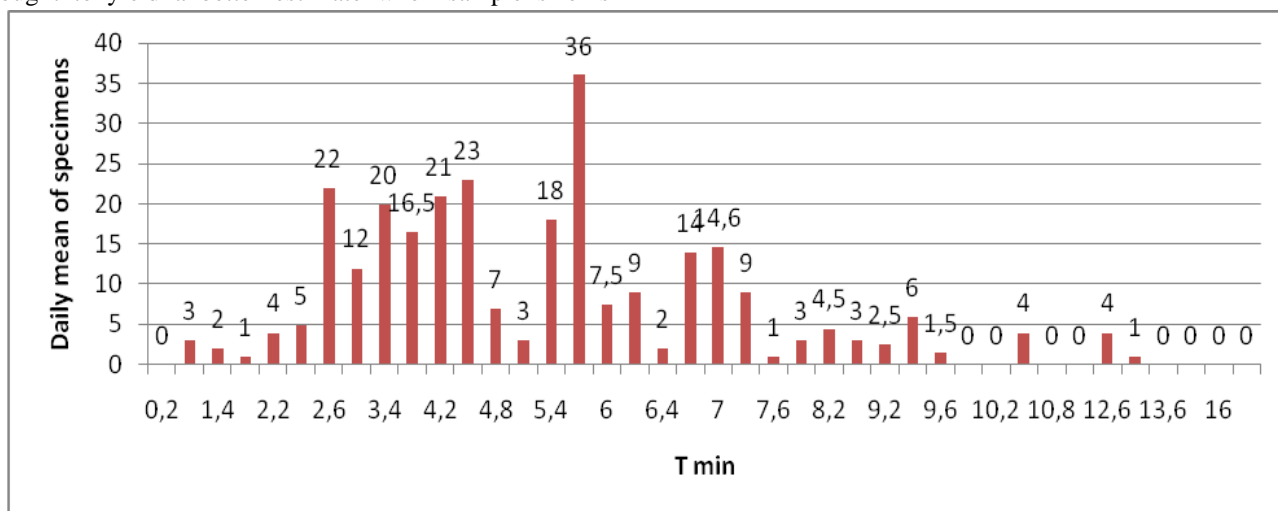


Fig.2: Daily mean of total specimens captured in the study seasons at the minimum temperatures recorded.

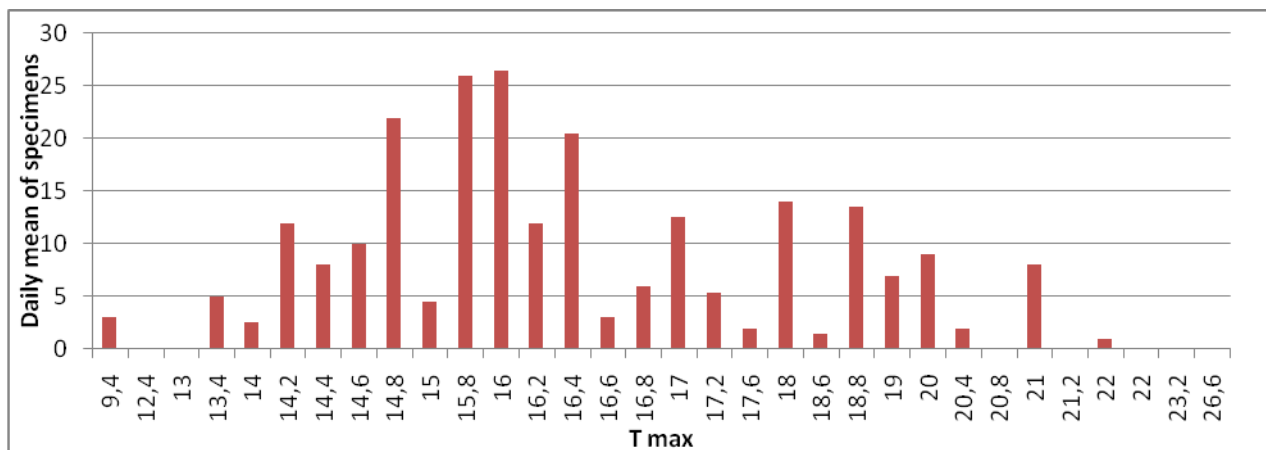


Fig.3: Daily mean of total specimens captured in the study seasons at the maximum temperatures recorded.

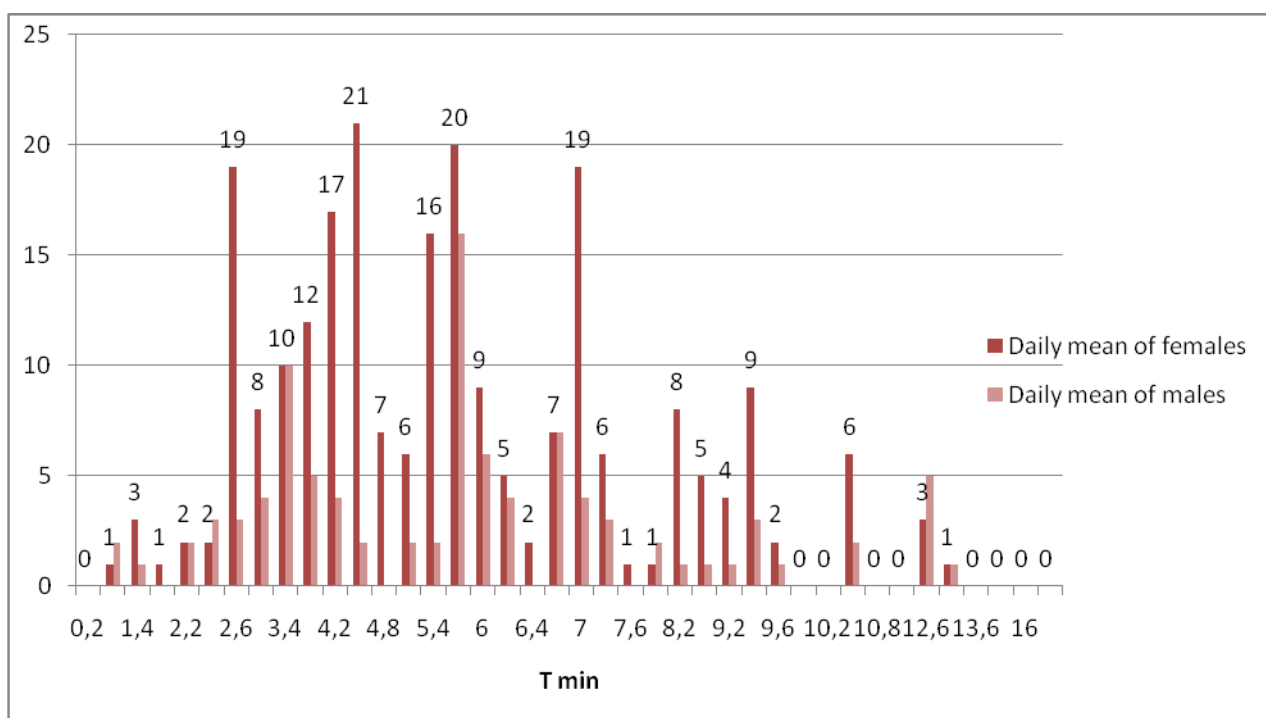


Fig.4: Daily mean of male and female specimens captured in the study seasons at the minimum temperatures recorded.

Table 1. The data of the breeding seasons from 1995 – 1996 to 2014 – 2015.

Breeding season	Number of caught individuals	Males	Females	Male percentage	Female percentage	Population estimate (numbers of individuals)
1995 – 1996	100	43	57	43%	57%	257
1996 – 1997	172	52	120	30%	70%	246
1997 – 1998	80	29	51	36%	64%	221
1998 – 1999	145	52	92	48%	52%	267
1999 – 2000	131	61	70	46%	54%	329

2000 – 2001	104	53	51	51%	49%	295
2001 – 2002	114	55	59	48%	52%	387
2002 – 2003	134	47	87	35%	65%	256
2003 – 2004	-	-	-	-	-	-
2004 – 2005	-	-	-	-	-	-
2005 – 2006	-	-	-	-	-	-
2006 – 2007	154	51	103	33%	67%	294
2007 – 2008	165	63	102	38%	62%	232
2008 – 2009	136	56	80	41%	59%	267
2009 – 2010	145	63	82	45%	55%	360
2010 – 2011	109	46	63	42%	58%	342
2011 – 2012	130	48	82	37%	63%	259
2012 – 2013	112	52	60	46%	54%	212
2013 – 2014	147	71	76	48%	52%	324
2014 – 2015	138	50	88	36%	64%	287

**V. DISCUSSION AND CONCLUSIONS**

During the breeding seasons investigated, starting from the 1995-1996 season, the greatest concentration of individuals in water occurs in the period between January and May. The maximum presence of newts in water occurs with a maximum temperature range that oscillates from 14 °C to 17 °C and a minimum temperature that oscillates from 3 °C to 7 °C.

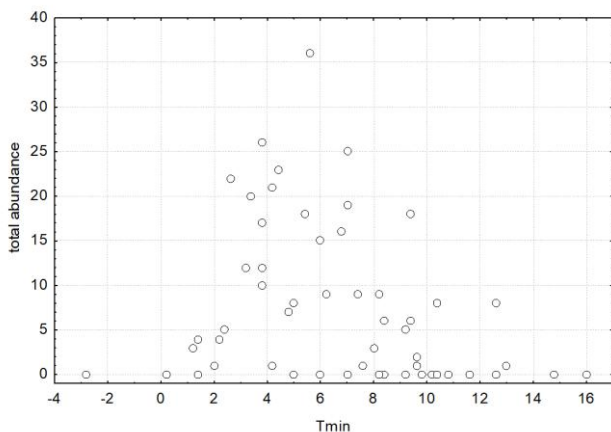


Fig.5: Significant correlations between the total abundance of smooth newts in the ponds and the minimum air temperature in the reproductive seasons.

The aggregated abundance data for the whole reproductive seasons show that the only covariate really negatively influencing the abundance of the smooth newts in the ponds was the minimum temperature (Spearman’s rho = -0.307, P = 0.022) (Fig. 5). All the other covariates showed no significant association with the abundance of

newts (P always > 0.05). On the other hand, while a negative correlation was observed between female abundance and both mean (Spearman’s rho = -0.279, P = 0.039) and minimum temperatures (Spearman’s rho = -0.353, P = 0.008) (Fig. 6 and Fig. 7), abundance of males was not significantly correlated with any covariate.

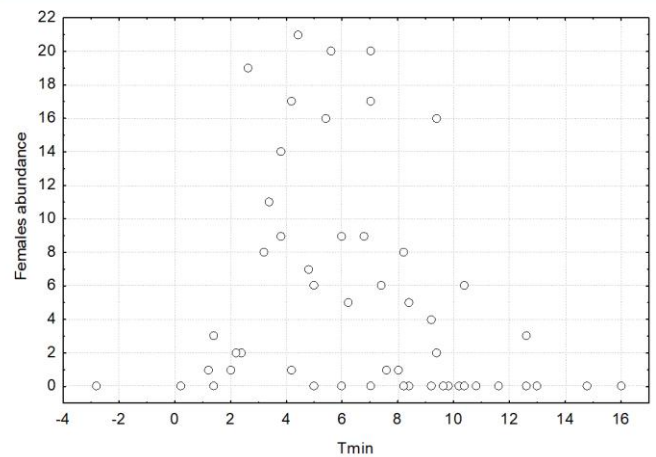


Fig.6: Significant correlations between the female abundance in the ponds and the minimum air temperature in the reproductive seasons.

From the analysis of the data it would therefore emerge that the minimum atmospheric temperature may constitute one of the most significant environmental parameter for the reproductive phase of the Smooth Newt.

This trend was already highlighted in previous studies on the population of the “Bosco di Palo” Natural

Park (PIZZUTI PICCOLI, 2008); the strong point of the present study consists precisely in the long-term study of the population and its reproductive phenology as a function of the temperatures recorded.

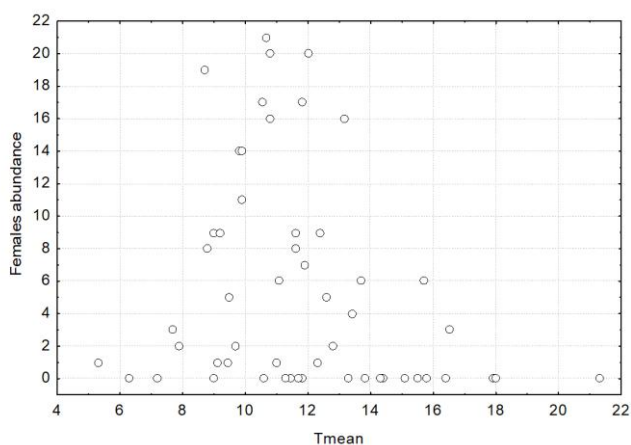


Fig.7: Significant correlations between the female abundance in the ponds and the mean air temperature in the reproductive seasons.

Furthermore in a study carried out in a site about 20 km away from the study area, from 2006 to 2008, it is indicated between 1°C and 11°C the minimum temperature range most suitable for the reproductive phase of the Smooth Newts. The maximum peak of presence of individuals in water occurs with a maximum temperature of 13.2°C and a minimum temperature of 6.9°C (DI GIUSEPPE, 2012).

This data agrees with the observations reported in the present work and suggests that the identified trend can be extended to populations of Mediterranean environments.

In conclusion, it is believed that the present work, based on a long-term study of the population of *Lissotriton vulgaris meridionalis*, can constitute an important contribution in defining the optimal environmental parameters for the reproductive phenology in a Mediterranean environment and can be of support in actions of conservation of the species.

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# Comparative Performance of the Developed Mini Tractor Operated Sprayer Cum Weeder with The Various Types of Existing Spraying and Weeding Methods

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**Abstract**— *Insects, diseases and weeds are the three main biological elements that have the greatest influence on agricultural output losses and farmer profits. The two most crucial practises in agriculture for maximum yielding are chemical application and weeding. In the past, spraying was performed using a knapsack sprayer, and weeding was carried out manually and with a bullock-drawn weeder, both of which required a lot of time and effort. For spraying and weeding in modern agriculture, farmers employ a variety of power-operated tools. However, because each task was carried out independently, it took more time and effort to finish the weeding and spraying tasks. Multioperational equipment or machinery is required to decrease operating time, cost, and the number of passes. As a result, efforts have been made to create a machine that can complete both tasks in a single pass. Considering these points, mini tractor operated sprayer cum weeder was developed. By using the developed sprayer cum weeder time saving of 95.79 %, 90.42 % and 38.71 % could be achieved as compared to existing manual methods, animal drawn machine and power operated machine and developed machine could also save 91.50 and 8.84 % operational cost as compared to existing manual methods and power operated machines in spraying and weeding operations respectively. The weeding efficiency of the developed machine for combined operations was found 84.53 % as compared to power operated weeder (86.12 %) which is more or less equal while it is only for weeding operation.*



**Keywords**— *Sprayers; Weeders; Cost saving; Time saving; Weeding efficiency*

## I. INTRODUCTION

The technological improvements in Indian agriculture since mid-sixties have brought about revolutionary increase in agricultural production. Total farm power availability on India farms is around 2.761 kW/ha and food grain productivity were about 2.42 t/ha around the year 2021-22. In last 50 years, farming has undergone great evolution in spraying mechanism to control various diseases on plants. Pesticides are widely used for controlling diseases, insects and weeds in the crops. They are able to save a crop from pest attack only when applied in time. Historically, pesticides were known as economic poisons. Pesticides can be categorized into

insecticides, fungicides, herbicides (weedicides) and plant-growth-regulators based on their activity and target groups. In modern agriculture, the use of sprayers has become indispensable for crop protection and management. The effectiveness and efficiency of sprayers are essential to achieving optimal yields. There are various types of sprayers available, such as boom sprayers, air blast sprayers, and electrostatic sprayers, each with its own advantages and limitations (Jalu *et al.* 2023). Weeds are always associated with human endeavours and cause huge reductions in crop yields, increase cost of cultivation, reduce inputs use efficiency, act as alternate hosts for several insect pests, diseases and nematodes. Weeds

decrease crop yields from 15 to 50 % depending on the species, density and weeding time through competition with main crop for light, water and nutrition (Hasanuzzaman *et al.* 2009). The total economic losses will be immense if indirect effects of weeds on health, loss of biodiversity, nutrient depletion, grain quality, etc., are taken into consideration.

Mostly in the forming process chemical spray a taking a critical role due to poison properties of chemical. So, now there is need to make something unique and useful machine for spraying and inter cultivation. Agricultural implement and machinery program of the government has been one of selective mechanization with a view to optimize the use of human, animal and other sources of power. Earlier agriculture was more dependent on the nature and all the operations were carried out by using human and animal power. For profitable agriculture timely operations are the most important. Second important point is the cost of operation (Ambaliya *et al.* 2022). In order to reduce labour costs and working hours, a mini tractor operated sprayer and weeder was developed, and its performance compared with existing methods.

## II. VARIOUS TYPES OF SPRAYERS

Spraying agricultural chemicals is a useful way to control insects, diseases and weeds and is important for growing high-yielding, quality crops and pasture. Applying the right amount of chemical at the right time is a major factor in ensuring successful control. Sprayers are the equipment used for applying liquid substances to plants or crops. These substances could be fertilisers, herbicides, or pesticides all of which are important for the maintenance of crop health during the crop growth cycle.



(a) Foot Sprayer/Pedal Pump Sprayers



(b) Lever operated Knapsack Sprayer

### 2.1 Manual operated sprayers

In India, we primarily used old techniques and tools for farming. In order to rid the agricultural area of insects, pesticides and water are primarily needed after a period of time. Some manually operated sprayers are given below.

#### Foot sprayer/pedal pump sprayers

This foot-operated sprayer is frequently used to apply CPP. It can be equipped with one or two long delivery hoses that include lance or two to six nozzle booms. This sprayer has the benefit of covering a vast area with a high volume of spray.

#### Hydraulic knapsack sprayer

This manually powered sprayer features a 15-liter tank and operates by using a hand lever to maintain continuous pressure. Using this sprayer specifically for spot treatments.

#### Pneumatic or compressed system knapsack

It is used sparsely to spray on weeds in rice and jute, as pumping is not essential with this sprayer. The tank is pressurised when the liquid has filled it to about two-thirds of its capacity.

#### Motorized pneumatic sprayer

It is a low volume sprayer that works well for spraying concentrated spray liquid. Spray liquid is ejected in the blast of air that passes between the delivery hose and nozzle tube during the spraying process. Spray liquid is turned into tiny droplets by an air blast. Air serves as a carrier, and the faster it is compressed, the more atomization occurs. These sprayers can be used as blowers as well. Herbicides, pesticides, and fungicides used in crop protection products are significantly lost via mist blowers to the wind.





(c) Manual pneumatic knapsack sprayer



(d) Motorized pneumatic knapsack sprayer

Fig.1 Manually operated sprayers

## 2.2 Animal drawn sprayers

Gupta *et al.* (2003) designed and developed a bullock drawn fraction sprayer. At a pressure of  $3.5 \text{ kg/cm}^2$ , the sprayer's performance was evaluated for various parameters in both lab and field condition. In laboratory and field circumstances, the average boom discharge was  $2.47 \text{ l/min}$  and  $2.53 \text{ l/min}$ , respectively. At  $400 \text{ mm}$  height, the spray pattern was uniform across all nozzles. At  $400 \text{ mm}$  height, the spray pattern for the central nozzles grows wider. The machine required  $0.486 \text{ HP}$  on average to operate. The sprayer's average field capacity was  $0.704 \text{ ha/h}$ , or nearly seven times as much as the backpack sprayer. For the sprayer to cover a  $1 \text{ ha}$  area, only  $1.44$  man hours were needed.

Anibude *et al.* (2016) developed the prototype of an animal drawn hydraulic boom sprayer considering the

agronomical and functional requirement for application of chemicals on field crops. The major components include;  $100 \text{ litres}$  spray tank capacity, mainframe, operator seat,  $3 \text{ Hp}$  petrol engine, piston pump, boom, ten flat fan nozzles, wheel and axle shaft. The petrol engine was used as the power source for operating the piston pump during spraying and pair of bullocks was used for hauling purpose. Application rate of  $260 \text{ l/ha}$  was achieved, theoretical field capacity of  $1.16 \text{ ha/h}$ , effective field capacity of  $1.04 \text{ ha/h}$  and  $89.6 \%$  field efficiency. Comparing the results with what was obtained using the manually operated knapsacks sprayer represents  $62 \%$  and  $37 \%$  increase in effective field capacity and field efficiency respectively.



Fig.2 Animal drawn hydraulic boom sprayer

### 2.3 Power operated sprayers

Padmanathan and Kathirvel (2007) evaluated the performance of power tiller operated rear mounted boom sprayer for cotton crop. A power tiller operated rear mounted boom sprayer was developed for spraying cotton and other crops planted in rows and to produce uniform spray pattern using minimum amount of spray materials. Test was carried out on the developed sprayer both in laboratory and in the field. The spray boom has sixteen hollow cone nozzles, placed 40 cm apart. It has a swath width of 3.2 m for a forward speed of 2 km/h. The effective field capacity of the sprayer was 0.72 ha/h. The performance of the power tiller operated boom sprayer was satisfactory at a pressure of 3 kg/cm<sup>2</sup> and can be adopted by the farmers for spraying cotton crop and other row crops. Cost economics of power tiller operated rear mounted boom sprayer was found to be 88.25 ₹/ha and 76.45 ₹/h. The entire boom assembly fixed at the rear of the power tiller, behind the operator seat. Even in adverse wind conditions, by the time the power tiller would have moved through considerable distance, the chemical would be deposited on the canopy, thereby reducing the effect of chemical inhalation by the operator almost too nil. To facilitate for the convenience of the operator the design of the entire controls was provided near the operator seat so that very efficient spraying can be achieved without affecting the health of the operator.

Babasaheb and Omkar (2015) conducted experiment, on comparative performance of tractor operated boom type field sprayers on cotton crop. Two 12 m tractor operated boom type field sprayers of ASPEE make, one of the existing designs and other of new design (developed)

having similar specifications, were selected for the study. Comparative performance showed that the liquid distribution of developed boom sprayer improved. Discharge and pressure of the developed boom sprayer was nearly uniform for all nozzles, droplet size, droplet density and uniformity coefficient of the existing sprayer ranged from 130.9 to 206.39 µm, 11 to 27 drops/cm<sup>2</sup> and 1.18 to 1.31, respectively, whereas for developed sprayer it was ranged from 155.44 to 181.55 µm, 17 to 29 drops/cm<sup>2</sup> and 0.99 to 1.23, respectively.

Udaybhaskar *et al.* (2018) developed and evaluated low HP tractor operated wiper sprayer. Among all the crop protection methods, chemical protection usage growing effectively as of its immediate action, low cost and reduces human drudgery. To spray pesticides on crop, low hp tractor operated wiper sprayer was developed instead of using conventional equipment to reduce operating cost, time and drudgery. In laboratory condition, uniformity coefficient of developed wiper sprayer was found to be 89.9 %. The average effective field capacity, field efficiency, fuel consumption and application rate of developed wiper sprayer in the field of groundnut was found to be 0.9072, 1.4899, 2.0618 ha/h, 80, 78.83, 77.92 %, 1.513, 1.018, 0.815 l/ha and 423, 253, 181 l/ha at forward speeds of 1.5, 2.5 and 3.5 km/h. Cost economics of developed wiper sprayer was found to be 310.2, 197.61 and 150 ₹/ha at forward speeds of 1.5, 2.5 and 3.5 km/h. Saving of labour cost (%) and time (%) over conventional method of spraying found to be 101.5, 216.27, 316.67 % and 1714, 2880, 4023 % at forward speeds of 1.5, 2.5 and 3.5 km/h. Operating speed of 3.5 km/h was given best performance.



Fig.3 Developed low HP tractor operated wiper sprayer

Basavaraj *et al.* (2020) developed and evaluated solar operated sprayer. The performance evaluation of the sprayer was carried out for spraying in sugarcane and

paddy. The walking speed of the operator is about 2.5 km/h and which corresponds to a theoretical field capacity of about 0.6 ha/h. The effective field capacity of the

sprayer was observed to be 0.5 ha/h and field efficiency was 83.33 % was observed. The maximum flow rate obtained for four hole adjustable nozzles with a flow rate of 2.1 l/min and minimum flow rate was obtained for hallow cone nozzle with a flow rate of 1.021 l/min . The discharge rates for sugarcane and paddy were 110.81 and 101.26 l/h respectively. The application rates for sugarcane and paddy were 195.25 and 154.75 l/ha respectively. This equipment does not use any other external source of power for spraying and is operated by the user only; it reduces drudgery, economical and eco-friendly as it uses the solar energy which can be easily affordable by the farmers.

### III. VARIOUS TYPES OF WEEDERS

Weeding is the removal of weeds from the field. It is an effective pre-harvesting method of crop protection and crop production management. Weeds act as competitors of the crop for various resources required for growth like nutrients, light, water, etc. so they have to be removed as they may cause interference and decrease the yield. Weeds can be controlled in many ways. Weed management includes land preparation, water management, hand weeding, hand hoeing, crop rotation, and herbicides. Land preparation helps in the removal of seeds and uprooting of weeds before sowing seeds of the main crop. Hand weeding is done manually which is very tiresome and time-consuming.

### 3.1 Manual operated weeders

Controlling weeds by hand-pulling them may be all that is necessary if you practice regular and proper maintenance procedures. Hand-weeding is particularly important to prevent infestations of the some weeds because they are difficult to manage once they have invaded. Tools and implements for weed control can be machine operated, manual operated or animal operated. Though manually operated weeders are slow in operation but they are the most effective methods among all methods. Some of the tools and implements which are suitable for manual weeding and interculture operations are shown in Fig.4.

Manjunatha *et al.* (2014) developed and evaluated the manually operated sprocket weeder. The sprocket weeder can be easily fabricated by farmers themselves with low cost by using inexpensive bicycle materials. The weeding efficiency of the sprocket weeder was found to be 94.5 %. The sprocket weeder could work up to 4 cm depth. No plant damage was occurred during weeding operation with the sprocket weeder. The field capacity of the sprocket weeder was found to be 0.032 ha/h. The operational cost was found 375 ₹/ha. The saving in time and cost was 84 % and 79.16%, respectively. It can be operated easily by farmers or unskilled labours. It is most economical and effective for marginal farmers who are affordable to maintain bullocks.



Fig.4 Manual operated weeding tools



Fig.5 Manually operated sprocket weeder

### 3.2 Animal drawn weeders

Sims, B. G. (2000) evaluated the performance of the animal drawn weeder. Performance of weeder was depended on the categories of information required for a particular purpose and include both technical and socio-economic parameters. The maximum field efficiency of 75 % was obtained at 2.8 km/h speed. The mean effective field capacity was found 0.11 ha/h.

Biweta and Endeblhatu (2008) tested and evaluated animal drawn weeder. Weeding with pair of oxen using traditional plough at weeding time is much faster and saves labour and time by about 79 % compared with this hand

hoe method. If weeding is performed at early stage, about two weeks after emergence, the plough throws enough soil on the crop rows which was burry and suppress small weeds without harming the crop. Weeding efficiency was found as 81 %. During weeding or cultivating using a pair of oxen, one of them was probably pass on the free space between rows of plant while the other was ride on planted rows, causing breakage on germinated crop. Besides, cultivating depth, which is about 12 cm, is greater than the required depth.

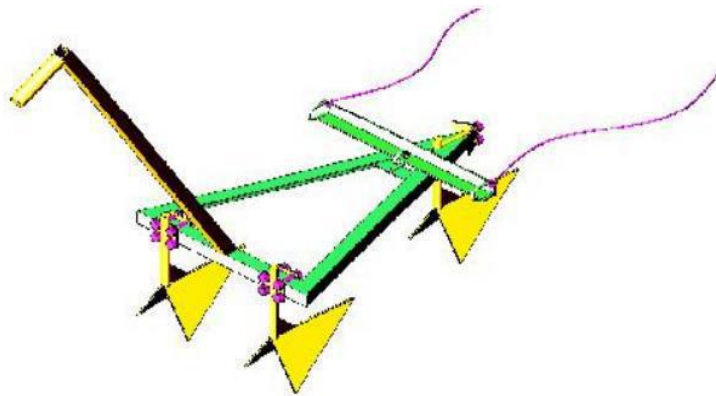


Fig.6 Triangular tool bar animal drawn weeder

### 3.3 Power operated weeder

Rathod *et al.* (2010) developed a tractor drawn inter-row rotary weeder keeping in view the crop, soil and machine parameters and made a performance evaluation. They conducted field tests with inter row rotary weeder at three forward speeds i.e., 1.1, 1.2 and 1.5 km/h. The developed weeder was evaluated at different test fields for different crops. The maximum weeding efficiency of 81.39 was obtained at 1.1 km/h speed and at 13.00 % moisture

content. The mean effective field capacity was 1.43 ha/day. The average field efficiency was found to be 92.50 %. The field efficiency decreased with increase in speed of operation. While the minimum weeding efficiency of 69.04 was obtained at 1.5 km/h of speed and at 13.75 % moisture content hence the weeding efficiency decreased with increase in speed of operation, weeding efficiency increased with increase in depth of operation.



Fig.7 Tractor drawn inter-row rotary weeder

Chandel *et al.* (2014) investigated performance of rotary power weeder in vegetable crop. The self-propelled rotary power weeder was used in wide row line sown vegetable crops tomato, yard long bean and okra. At forward speed of 2.3 km/h, 2.0 km/h and 2.4 km/h the effective field capacities were 0.092, 0.08, and 0.096 ha/h in tomato, yard long bean and okra, respectively. With the average effective working width of 400 mm, the depth of weeding was observed as 53, 46, and 50 mm for tomato, yard long bean and okra, respectively. Weeding efficiency in tomato, yard long bean and okra was found as 97, 96 and 97 %, respectively. Plant damage was found as 1.6, 2.8 and 1.9 % in tomato, yard long bean and okra, respectively.

Manjunatha *et al.* (2016) developed and evaluated the performance of the tractor operated rotary weeder in

redgram crop. The weeder was designed using computer aided design (CAD) software and prototype was fabricated. The operational parameters selected for the study were, three forward speeds (2.0, 2.5 and 3.0 km/ha), two rotary speeds (210 and 240 rpm) and three types of blades (L-type, C-type and J-type). The field performance of weeder was found to be better at 2.5 km/ha with rotary speed of 210 rpm for L-type blade compared to other types. The maximum weeding efficiency of 92.5 % with a field capacity of 0.42 ha/h and fuel consumption of 5.2 l/ha was recorded with minimum plant damage (3.15 %). The cost of weeding with tractor operated rotary weeder in redgram crop was found to be 1469 ₹/ha, which was 41.25 % less as compared to manual weeding (2500 ₹/ha).

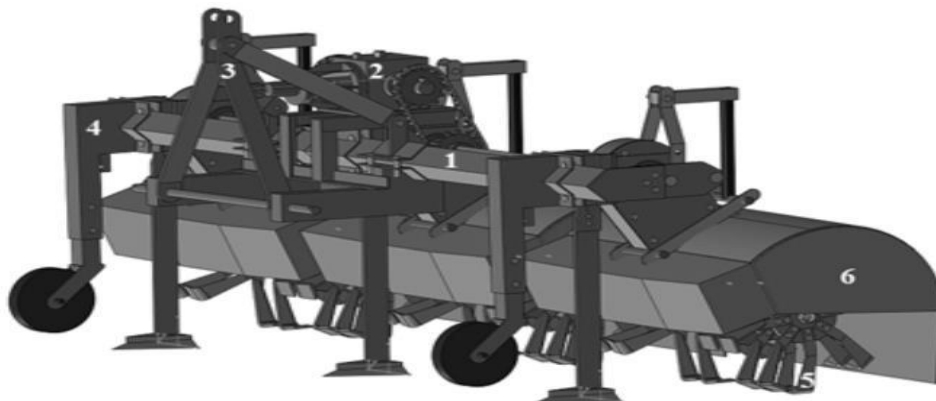


Fig.8 Isometric view of tractor operated rotary weeder.

#### IV. DEVELOPED SPRAYER CUM WEEDER

To perform spraying and weeding simultaneously by a mini tractor a machine was developed considering the required agronomical crop parameters (like crop type , variety, row to row distance, height of crop, weeding

stages, spraying interval, spraying rate etc.). Also considered that the machine should be simple in design and made of locally available materials. The developed machine was mainly consisted of battery powered sprayer and mechanical weeder, run by a mini tractor. To run the spraying unit a tractor battery, diaphragm pumps, water

pipe, nozzles, tanks to store the spraying liquid for spraying were used. For weeding T-type blades were used. The developed machine with different parts is shown in

Fig.9 and specifications of the machine are shown in Table 1.



Fig.9 Developed sprayer cum weeder

Table 1: Specifications of the developed sprayer cum weeder

Sr. No.	Particulars	Specifications
1	<b>Overall Dimensions</b>	
	(L × B × H), mm	2000 × 820 × 690
2	<b>Main frame</b>	
	Material of fabrication	MS pipe (75 mm dia.)
	Dimension (length × width)	2000 mm × 510 mm
3	<b>Blade</b>	
	Material of fabrication	Carbon steel
	Type	Straight (T-Blade)
	No. of blades	5 (Full size blade 3, Half size blade 2)
4	<b>Boom</b>	
	Material of fabrication	GI square pipe (20 mm×20 mm )
	Length	2000 mm
5	<b>Tractor Battery</b>	
	Voltage	12V
	Capacity	75 AH
6	<b>Diaphragm pump</b>	
	Voltage	12 V
	AMPS	3.5 A
	Flow	5.0 LPM

	Pressure	100 psi
7	<b>Nozzle</b>	
	Type	Hollow cone plastic nozzle
	Number of nozzles	4
8	<b>Flexible plastic hose pipe</b>	
	Diameter	8 mm
9	<b>Spraying tank</b>	
	Capacity of each tank	50 lit
	No. of tanks	2

Performance of the developed sprayer cum weeder was evaluated in laboratory as well as in the field. The machine was evaluated for field capacity, field efficiency, weeding efficiency, fuel consumption, plant

damage, spray angle, spray volume, spray application rate, swath width, and cost economics. Observations of all parameters are shown in Table 2.

Table 2: Observations of the developed sprayer cum weeder

Sr. No.	Particular	Observation
1	Field capacity (ha/h)	0.33
2	Field efficiency (%)	85.7
3	Fuel consumption (l/h)	1.69
4	Time required (h/ha)	2.39
5	Spray angle (°)	71
6	Spray volume (l/min)	2.76
7	Spray application rate (l/ha)	360
8	Swath width (mm)	2000
9	Weeding efficiency (%)	84.53
10	Plant damage (%)	4.58
11	Cost (₹ /ha)	584.90
12	Cost (₹/h)	244.73
13	Payback period (Year)	2.71
14	Energy consumption (MJ/h)	54.95

### Comparison of the developed sprayer cum weeder with the existing spraying and weeding methods

Developed machine performs spraying and weeding operations simultaneously, in a single pass. While in the existing methods, spraying and weeding operations are completed by performing each operation separately in a separate pass. The existing spraying methods as stated

above are manually lever operated spraying, animal drawn spraying and power operated spraying and existing methods of weeding are manual weeding, bullock drawn weeding and power operated weeding. The results related to the existing methods are collected/borrowed from some farm and literatures are used to compare with the results of the developed sprayer cum weeder as shown in Table 3.

Table: 3 Comparison of the developed spraying cum weeding machine with the existing methods

Parameter	Existing methods of Spraying			Existing methods of Weeding			Developed Sprayer cum Weeder
	Manually Lever Operated knapsack Sprayer	Animal Drawn Sprayer	Power Operated Sprayer	Manual Weeding ( Hand Weeding)	Animal Drawn Weeder	Power Operated Weeder	
Source of data	Borrowed from farm	Desal <i>et al.</i> (2013)	Padmanathan and Kathirvel (2007)	Borrowed from farm	Karale <i>et al.</i> (2015)	Ambaliya (2022)	
Time required to cover a hectare, h	6.88	9.51	1.55	50	15.45	2.35	2.39
Cost of operation, ₹/ha	319.17	575.83	88.25	6562.50	-	553.42	584.90
Cost of operation, ₹/ha	46.28	60.55	76.45	131.25	-	235.50	244.73
Effective field capacity (ha/h)	0.145	0.66	0.72	0.02	0.064	0.34	0.33
Spray application rate (l/ha)	250	441.80	380	-	-	-	360
Plant damage, %	-	-	-	1.40	-	3.30	4.58
Weeding efficiency, %	-	-	-	96.20	82.37	86.12	84.53

From the above data it is clear that one hectare of land requires only 2.39 hours by using the developed machine which performs both the operations spraying and weeding simultaneously. In existing methods to cover one hectare of field requires 2.35 h, 15.45 h and 50 h by power operated weeder, animal drawn weeder and by manual weeding respectively. Thus, the developed sprayer cum weeder saves 84.53 % and 95 % of time as compared to animal drawn weeder and manually weeding method. As shown in the Table 3 time required (2.35h/ha) by the power weeder is more or less equal to the developed machine (2.39 h/ha) but in the existing method of power weeder only weeding operation is performed. For a spraying one hectare of field requires 6.88 h, 9.51 h and 1.5 h by manually lever operated knapsack sprayer, animal drawn sprayer and by power operated sprayer respectively. Thus, the developed sprayer cum weeder saves 74.86 %

and 65.26 % of time as compared to animal drawn sprayer and lever operated sprayer. As shown in the Table 3 time required (1.55 h/ha) by the power operated sprayer is more or less equal to the developed machine (2.39 h/ha) but in the existing method of mini tractor operated sprayer only spraying operation is performed. Thus, the developed sprayer cum weeder saves 95.79 %, 90.42 % and 38.71 % of time as compared to existing manual methods, animal drawn machine and power operated machines in spraying and weeding operations respectively.

Further, from the above Table 3 it is clear that the operational cost by the developed machine (for combined spraying & weeding) for one hectare of land came ₹ 584.90 and by existing methods it costs ₹ 553.42 and ₹ 6562.5 by power operated weeder and by manual weeding respectively. Thus, the developed sprayer cum weeder saves 91.08 % of cost as compared to manually weeding



method. But the weeding costs ₹ 553.42 per hectare by the power weeder which is more or less equal to the operational cost of developed machine (₹ 584.90 /ha) but in the existing method of power weeder only weeding operation is performed. Spraying of one hectare of field costs ₹ 319.17, 575.83 and 88.25 by manually lever operated knapsack sprayer, animal drawn sprayer and by power operated sprayer respectively. Thus, the developed sprayer cum weeder saves 91.50 and 8.84 % operational cost as compared to existing manual methods and power operated machines in spraying and weeding operations respectively.

The weeding efficiency of the developed sprayer cum weeder for combined operations was found 84.53 % as compared to power operated weeder (86.12 %) which is more or less equal while it is only for weeding operation. However, manually weeding efficiency is always found highest due to inter-row and intra-row weeding.

## V. CONCLUSION

Application of chemical and weeding are the most important operations in farming for high yielding. Present different category of sprayers and weeders available in the market that are tractor mounted, power operated, manual and self-propelled are available. Use of these machines in the Indian Agricultural scenario is difficult as most of the Indian farmers are small and medium and their economic conditions are not sound to adopt advanced machines and increasing the operating cost of labours and bullock power, along with very low efficiency, needs replacement. Therefore, by the matching size of tractor with the equipment not only reduces the operating cost but also maintains the quality of work. Hence, mini tractor operated sprayer cum weeder was found the most suitable for not only the small land holding farmers but also for the light operations like spraying and weeding operations. For profitable agriculture timely operations are the most important. Second point is the cost of operation. Considering these points, the mini tractor operated sprayer cum weeder is well suitable for farmers as compared to the existing methods. By using the developed machine time saving of 95.79 %, 90.42 % and 38.71 % could be achieved as compared to existing manual methods, animal drawn machine and power operated machine of spraying and weeding operations respectively. The developed machine could save 91.50 and 8.84 % operational cost as compared to existing manual methods and power operated machines in spraying and weeding operations respectively. The weeding efficiency of the developed sprayer cum weeder for combined operations was found 84.53 % as compared to power operated weeder

(86.12 %) which is more or less equal while it is only for weeding operation. However, manually weeding efficiency is always found highest due to inter-row and intra-row weeding.

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# Economic efficiency of human urine as fertilizer in the production of NERICA 4 rice in the Sudano-Sahelian zone of Mali

## Efficacité économique de l'urine humaine comme engrais dans la production du riz NERICA 4 en zone soudano-sahélienne du Mali.

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**Abstract**— In West Africa, and particularly in Mali, rice is the staple food of the population. However, its production is faced with various constraints, including soils poor on the one hand and difficult access to inputs on the other. Indeed, waste, especially excreta, contains nutrients that are essential for crop development. It is within this framework that this study was conducted to determine the human urine effect on the production of NERICA 4 rice in the Sudano-Sahelian zone. Different urine doses in combination or not with organic fertilizer were put in competition through an experimental design in Ficher Block with eight repetitions. The results after variance analysis showed statistical differences between treatments. The yield increase was most marked with the 111 kg N/ha urine + 5 t organique fumure (T7), which provided the highest rice yield with 1623 kg/ha compared to the mineral fertilizer control (T9) with 1550 kg/ha. The analysis of economic profitability shows that the dose 111kg N/ha urine alone with a ratio of 3.32 is better than the other doses of fertilizers compared to the FAO standard (RVC > 2).



**Keywords**— human urine, NERICA 4 rice, yield, economic rentability.

**Resume**— En Afrique de l'ouest et particulièrement au Mali, le riz constitue la base de l'alimentation de la population. Cependant, sa production est confrontée à différentes contraintes parmi lesquelles la pauvreté des sols et l'accès difficile aux intrants. C'est dans ce cadre que cette étude a été conduite afin de déterminer l'effet de l'urine humaine sur la production du riz NERICA 4 et d'évaluer son efficacité économique en zone soudano-sahélienne. Différentes doses d'urine en combinaison ou non avec la fumure organique ont été mises en compétition à travers un dispositif expérimental en Bloc de Ficher à huit répétitions. Les résultats après analyse de variance ont montré des différences statistiques entre les traitements. L'augmentation du rendement de riz paddy est plus marquée par l'apport de la dose 111 kg N/ha d'urine + 5 t fumure organique avec 1623kg /ha à la concurrence de la fumure minérale (74kg/ha) avec 1550 kg /ha. L'analyse de la rentabilité économique montre que la dose 111kg N/ha d'urine seule

avec un ratio valeur sur cout (RVC) de 3,32 est meilleure que les autres doses d'apports de fertilisants comparé à la norme FAO (RVC > 2).

**Mots clés**— urine humaine, riz NERICA 4, rendement, rentabilité économique.

## I. INTRODUCTION

La population de l'Afrique subsaharienne devrait doubler d'ici 2050 pour atteindre 2 milliards d'habitants, alors que les besoins alimentaires seront multipliés par quatre (PNUD, 2011). Cette situation d'accroissement de la population et d'urbanisation rapide entraîne une demande de plus en plus croissante des denrées alimentaires parallèlement à une production des déchets par habitant (Useni, 2012). Ainsi la consommation moyenne devrait passer de 20 à 48 millions de tonnes en 2050 (Agrimonde, 2009). Le souci majeur du gouvernement malien est d'intensifier la production agricole afin d'assurer la sécurité alimentaire au profit d'une démographie galopante et faire du Mali le grenier de l'Afrique de l'Ouest (Gouvernement du Mali, 2013). Or, cette production est confrontée à des contraintes majeures dues à la pauvreté caractéristique des sols d'une part et d'autre part à l'accès difficile aux intrants surtout l'azote en particulier (Dicko, 2005). Dans un contexte d'insécurité alimentaire, de réduction de la pauvreté des sols, de fort taux de chômage, causant la pauvreté de la population et de la hausse des prix des engrais sur les marchés, il apparaît nécessaire d'utiliser pour l'agriculture, les fertilisants disponibles et à faible coût (Folefack, 2008). Au regard des potentialités agronomiques énormes qu'offrent les urines humaines, leur traitement et leur réutilisation pourraient, non seulement améliorer la production agricole, mais aussi contribuer à la gestion des quantités importantes de déchets produites en préservant ainsi notre environnement (Martin, 2020). Le présent travail a pour objectif de contribuer à l'amélioration de la productivité du riz NERICA 4 par l'utilisation de l'urine humaine comme engrais en zone soudano sahélienne.

## II. MATERIEL ET METHODES

### 2.1. Matériel

#### 2.1.1. Site de l'essai:

L'essai a été implanté sur les parcelles de recherche du Centre Régional pour l'Eau Potable et Assainissement (CREPA) Mali à N'Djinina, commune rurale de Guégnéka. Ses coordonnées géographiques sont entre 12° 33' 33" et 12° 43' 26" latitude Nord 7° 2' 06" et 6° 52' 12" longitude Ouest. Le climat est de type soudano-sahélien avec une pluviométrie variant entre 600 à 1200 mm (PDESC 2015-2020). Le riz est l'aliment de base de la zone après le maïs, sorgho et le mil.

#### 2.1.2. Matériel végétal :

Le matériel végétal utilisé est le riz NERICA 4 issu du croisement (*Oryza sativa* x *Oryza glaberrima*). C'est une variété de riz de plateau à cycle court (95-100 jours), tolérant aux maladies avec un rendement potentiel de 5t / ha à la récolte (ADRAO-Centre du riz pour l'Afrique, 2006). Cette variété de NERICA est pluviale et peut être cultivée dans les mêmes conditions que les autres cultures sèches c'est-à-dire le maïs, le sorgho (Sokei, 2011), raison pour laquelle elle est appréciée par les producteurs de la zone.

#### 2.1.3. Fertilisants utilisés

- ✓ Urine (5 g/l N ; 0,4g/l P ; 0,3g/l K)
- ✓ Urée 46%N
- ✓ Complexe coton (14-22-12...)
- ✓ Fumure organique Profeba (1,57%N-8,78%P<sub>2</sub>O<sub>5</sub>-1,76%K<sub>2</sub>O)

## 2.2. Méthodes

### 2.2.1. Facteur étudié et traitements :

Le facteur étudié est la fertilisation prise à 9 niveaux de variation, qui constituaient les différents traitements. Les traitements à travers différentes doses d'urine en combinaison ou non avec la fumure organique sont : T1= Sans fertilisant, T2 = 74 kg N/ha, T3= 37 kg N/ha, T4 = 111 kg N/ha, T5 = 74 kg N/ha +5t FO, T6 = 37 kg N/ha +5t FO, T7 = 111 kg N/ha +5t FO, T8 = 37 kg N/ha +1/2 dose fumure minérale (FMV), T9 = FMV (74kgN/ha).

### 2.2.2. Dispositif expérimental

Le dispositif expérimental est un bloc de Fisher avec à 8 répétitions et 72 parcelles élémentaires. Les traitements ont été affectés de façon aléatoire aux parcelles élémentaires. Chaque parcelle élémentaire avait 2mx2m soit 4m<sup>2</sup> de superficie avec 1m entre les parcelles élémentaires et 2m entre les blocs. La longueur totale de la parcelle d'expérimentation est de 30m, et sa largeur de 26m soit une superficie totale de 780m<sup>2</sup>. Le semis du riz a été effectué en ligne en raison de trois à quatre graines par poquet aux écartements de 20cm x 20cm. .

### 2.3. Opérations culturales

Le profeba (fumure organique) et le complexe coton ont été apportés sur les parcelles correspondantes une seule fois juste avant le semis sur toute la surface des parcelles élémentaires et enfouies dans le sol. L'urine et l'urée ont été apportées aussi sur les parcelles correspondantes par

fractionnement en trois apports comme fumure d'entretien et enfouies dans le sol. Les opérations d'entretien ont consisté au démariage, regarnissage, le désherbage.

#### 2.2.4. Paramètres observés

Les observations ont porté sur le rendement et l'analyse économique et agronomique des différentes doses.

- Le rendement du riz a été déterminé après le battage, le vannage des panicules récoltées des traitements et le pesage des graines avec une balance de précision par extrapolation à l'hectare.
- L'efficacité agronomique du fertilisant a été déterminé par le rapport du surplus de rendement obtenu par l'application d'engrais sur la quantité d'engrais appliquée par hectare.

$$\text{Efficience} = \frac{\text{Surplus rdt (kg/ha)}}{\text{Quantité d'engrais (kg/ha)}}$$

-L'efficacité économique est déterminée par le ratio Valeur sur Coût (RVC), c'est à dire le rapport de la valeur du surplus de rendement obtenu sur le coût de l'engrais appliqué pour obtenir ce surplus.

$$\text{RCV} = \frac{\text{Prix du kg grain} \times \text{surplus (kg)}}{\text{Prix du kg} \times \text{kg engrais utilisé}}$$

Pour l'analyse de l'efficacité agronomique, dans le calcul des paramètres de profitabilité de différents traitements les références de prix suivant ont été utilisées :

- Le prix du kg de riz paddy est de 200 F CFA à la récolte, il est obtenu auprès des commerçants de la zone.

- Le coût des engrais minéraux non subventionnés par l'Etat est calculé en considérant le prix du marché pratiqué par les fournisseurs d'intrants de la zone (18 000 F pour le sac de 50 kg de complexe coton et 16 000 F CFA pour l'urée).
- Le prix de cession de la fumure organique PROFEBA non subventionnée est de 10 000 FCFA la tonne.
- L'urine étant un déchet a été considérée comme gratuite donc son prix de session est surtout le coût d'achat des bidons servant de collecte et stockage. Il est estimé à 300 FCFA le bidon de 20 litre.

#### 2.4. Traitement des données :

L'analyse de variance des données a été effectuée avec le logiciel GenStat 12<sup>ème</sup> édition en considérant le taux de signification 5% et le test de Student, Newman et Keuls pour la comparaison des moyennes des traitements.

### III. RESULTATS

#### 3.1. Rendement des plants du riz paddy (kg/ha)

Après analyse statistique des données collectées sur le rendement, il ressort une différence hautement significative entre les différents traitements (Tableau 1). Les traitements T7 et T9 sont statistiquement égaux et donnent les meilleurs rendements avec respectivement 1623 et 1550 kg/ha de paddy. Ces deux traitements ont des gains de rendement les plus élevés, respectifs de 833 et 760 kg/ha par rapport au T1. On constate que l'ensemble des traitements avec les doses d'apport de l'urine donnent des meilleurs rendements comparativement au traitement T1.

Tableau 1 : Rendement et gain de rendement du riz paddy (kg/ha) :

Traitements	T1	T2	T3	T4	T5	T6	T7	T8	T9
Rendement (kg/ha)	790d	1067bcd	945cd	1343ab	1333ab	1247abc	1623a	1257abc	1550a
Gain Rdt kg/ha par rapport au Témoin		277,33	155	553	648	490,66	833,33	490,66	760

Légende : T1, T2, T3... = traitements, T1 = Sans fertilisant, T2 = 74 kg N/ha, T3 = 37 kg N/ha, T4 = 111 kg N/ha, T5 = 74 kg N/ha + 5tFO, T6 = 37 kg N/ha + 5tFO, T7 = 111 kg N/ha + 5tFO, T8 = 37 kg N/ha + 1/2 FMV, T9 = FMV, a, c, d, ab, abc, bc, cd, bcd ... sont les groupes homogènes

#### 3.2. Efficience agronomique et économique des fertilisants utilisés

Les résultats du calcul de l'efficacité agronomique et économique (Tableau 2) montrent que l'efficacité agronomique varie de 10,27 pour le T9 à 3,74 pour le T3. Le Ratio valeur sur coût (RVC) varie de 3,32 pour le

traitement T4 à 1,52 pour le traitement T6. Les données renseignent qu'en terme d'efficacité agronomique le T9 est le plus important cependant le T4 ayant le RVC le plus élevé est celui qui a un seuil de rentabilité économique plus important suivi respectivement des traitements T2, T3, T7. Ils constituent les traitements les plus

économiquement rentables comparé à la norme FAO (RVC > 2) avec ces traitements, le gain de rendement couvre largement les dépenses liées à l'utilisation des

engrais. Les traitements les moins rentables économiquement sont les traitements T5, T8, T9 et T6 avec le ratio inférieur à 2.

Tableau 2: Valeurs des grandeurs économiques obtenues à la récolte

Trait	Qté fert N/kg/ha	Rdt kg/ha	Gain Rdt kg/ha par rapport au Témoin	Valeur surplus en FCFA	Coût intrant FCFA	RVC	EFF (kg de paddy/kg fertilisant)
T2	74	1067	277,33	55 466	22 200	2,49	3,74
T3	37	945	155	31 000	11 100	2,79	4,18
T4	111	1343	553	110 600	33 300	3,32	4,98
T5	152,5	1333	648	129 600	72 500	1,78	4,24
T6	115,5	1247	490,66	93 132	61 100	1,52	4,24
T7	189,5	1623	833,33	166 666	83 300	2,00	4,39
T8	74	1247	466,66	93 332	58 100	1,64	6,30
T9	74	1550	760	152 000	94 000	1,62	10,27

Trait=traitement, Qté= quantité, fert= fertilisant, kg/ha=kilogramme par hectare, Rdt= rendement, RVC= ratio valeur coût, EFF= efficacité

#### IV. DISCUSSION

L'ensemble des traitements avec l'apport de l'urine augmente le rendement de plus que le traitement sans engrais. L'effet bénéfique de l'urine sur l'augmentation du rendement et ses composants a été trouvé par plusieurs autres recherches notamment Johansson et al. (2001) ; Kirchmann & Pettersson (1995) ; Kvarmo (1998) ; Richert Stintzing et al. (2001). Ils ont montré que les nutriments contenus dans les urines sont de forme ionique et leur disponibilité à la plante rivalise bien avec l'engrais chimique. Le réseau Centre Régional pour l'eau potable et l'Assainissement ((CREPA (2005)) a montré une augmentation de rendement de diverses cultures fertilisées à l'urine de plus que le témoin notamment le coton au Mali de 48,57%, le maïs de 32,95% au Bénin, la laitue de 55,17% au Togo, l'aubergine de 82,40% au Burkina Faso. Comoé et al. (2009) ont fait le même constat en Côte d'Ivoire sur l'igname de 50%, de même que Denon (2010) sur le riz au Mali avec 52% et Kinanpara et al. (2020) en Côte d'Ivoire sur le riz. L'ajout de la fumure organique à l'urine a induit une augmentation du rendement de l'ordre de 266 kg/ha par rapport à l'urine seule, similaire à celui trouvé par Coulibaly et al. (2016) et qui montre que la combinaison de l'urine avec le Fumier bovin et avec le Phosphate Naturel de Tilemsi (PNT) a induit en moyenne une augmentation du rendement du coton grain de 238,30 kg rapport à l'apport de l'urine seule. L'augmentation de la dose d'urine a favorisé le rendement au même titre que l'ajout de la fumure organique à l'urine. Le rendement du riz a augmenté en fonction des doses croissantes d'urine, et

mieux en combinaison avec la fumure organique. Ce résultat est similaire à ceux de Brassard (2007) et de Ziadi et al. (2007). Il en est de même que les résultats obtenus par Kpéra et al. (2017) dont la combinaison de bouse de vache et l'urine a donné la meilleure croissance des plants et/ou des fruits de l'ananas. L'apport de la dose d'urine à 74 kg N/ha seule équivalent de la dose de la fumure minérale s'est avéré insuffisante pour une production optimale du riz. Ce résultat est similaire à ceux de Mkeni et al. (2006) et Germer et al. (2006) qui ont montré respectivement que le rendement en grains du maïs et de sorgho augmente avec les doses d'urine hygiénisée et qu'il n'existe pas de différence significative entre la fertilisation à l'urine et l'engrais minérale aux doses presque équivalentes.

L'analyse de l'efficacité agronomique donne le T9 plus élevé cependant il donne un RVC de 1,62 contre 3,32 pour le T4 ce qui montre que l'utilisation de l'urine seule est plus économiquement rentable que la fumure minérale selon la norme FAO. Cependant ce résultat est inférieur à la norme de Koning et al (1998) selon eux le RVC doit atteindre 4 au Sahel pour limiter le risque financier d'application de l'engrais. Plus la dose d'urine augmente plus la rentabilité est importante. Il ressort de cette analyse que le traitement le plus productif n'est pas le plus rentable ce résultat corrobore avec celui de Coulibaly (2016) avec l'utilisation de la fumure organique (tourteau de pourghère) sur le maïs au Mali.

## V. CONCLUSION ET PERSPECTIVES

Les urines humaines sont très riches en nutriments et permettent d'obtenir des rendements compétitifs à ceux obtenus avec la fumure minérale en culture céréalière. L'analyse de rentabilité économique montre que la fertilisation avec l'urine est rentable plus que la fumure minérale ;

En perspective, les nouvelles recherches à entreprendre pourraient s'orienter vers :

- L'exploration d'autres manières d'apport de l'urine enfin de minimiser les pertes d'azote de l'urine et réduire le contact direct de l'utilisateur avec le produit lors de la manipulation.
- L'évaluation des qualités organoleptiques des produits issus de la fertilisation avec les urines

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# Effect of priming treatment and storage containers to enhance the seed quality of tomato seeds

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**Abstract**— A study was undertaken at the Department of Seed Science and Technology, CCS Haryana Agricultural University, to investigate the Effect of priming treatment and storage containers to enhance the seed quality of tomato seeds. The experiment, conducted in 2021-2022, utilized a randomized complete block design to assess various seed priming techniques and their influence on tomato germination and morphological characteristics. Eighteen month-old seeds of tomato seeds were exposed to the following priming treatments T<sub>0</sub>: Control (untreated). T<sub>1</sub>: Priming with GA<sub>3</sub> @ 25, 50 and 75 ppm for 24 hours and drying at room temperature. T<sub>2</sub>: Priming with KNO<sub>3</sub> (Potassium Nitrate) @ 0.5 %, 1 %, and 1.5 % for 24 hours followed by drying at room temperature. T<sub>3</sub>: Priming with Ethanol @ 25, 50 and 75 ppm for 24 h and drying at room temperature. Within the various priming treatments, tomato seeds subjected to GA<sub>3</sub> priming at a concentration of 50 ppm exhibited the highest rates of germination, seedling length, seedling dry weight, seed vigor index, viability percentage, and radicle emergence. Following closely were seeds primed with KNO<sub>3</sub> at 1.5%. Conversely, ethanol at 50 ppm resulted in the lowest values for germination percentage, seedling length, seedling dry weight, seed vigor index, and viability percentage. Notably, GA<sub>3</sub> priming at 50 ppm demonstrated a substantial improvement, enhancing tomato germination by 24.6% compared to unprimed seeds in 18-month-old seed samples.



**Keywords**— Priming, Storage containers, Germination percentage, Seed vigor

## I. INTRODUCTION

In India, vegetables form a significant part of the diet due to the large number of vegetarians in the country. Individuals consume an average of 400 grams of vegetables per day, surpassing the World Health Organization's recommended daily intake of 300 grams (World Health Organization). Among the various vegetables, tomatoes (*Solanum lycopersicon* L) hold great importance as they are consumed both fresh and cooked [1].

Tomatoes are cultivated across more than four million hectares of land worldwide, solidifying their status as one

of the most extensively grown and consumed vegetables on a global scale

[2].

In India, tomatoes are grown across an expanse of 841 thousand hectares, yielding an average annual production of 20.33 lakh million tons. [3]. Tomatoes belong to the Solanaceae family, which includes other well-known species such as potatoes, eggplants (brinjal), tobacco, and peppers. The origin of tomatoes can be traced back to the Americas. They were introduced to Africa in the 16th century and have since become one of the most widely grown vegetables by small-scale farmers [4].

Tomatoes are not only delicious but also highly nutritious. They are rich in vitamins A, C, and E, as well as antioxidants like lycopene [5]. Additionally, tomatoes are a good source of fiber, carbohydrates, essential amino acids, minerals, vitamins, iron, and phosphorus. They can be consumed raw in salads or used in various culinary preparations such as sauces, soups, ketchup, pure juices, and dishes with meat or fish. Due to their commercial appeal and high yield, tomatoes are considered an economically important crop with a relatively short growing season.

To enhance the performance of tomato seeds, particularly in terms of germination rate and uniformity, seed priming is a commonly practiced pre-sowing hydration technique [6-8]. Seed priming encompasses the immersion of seeds in water, osmotic solutions, or a blend of a solid matrix carrier and water at defined concentrations. This is succeeded by drying before the emergence of the radicle. The objective of seed priming is to induce qualitative enhancements in the seeds, with the intent that these improvements endure even after the treatment concludes. This simple, cost-effective, and low-risk strategy has been shown to increase seedling emergence, seedling vigor, and overall crop yields in various field crops [9].

The process of seed priming significantly contributes to aiding plants in mitigating the detrimental impacts of unfavorable environmental conditions. [10]. By enhancing the performance of seeds, priming contributes to improved germination, seedling establishment, and overall crop productivity. This technique has proven to be beneficial in mitigating the negative impacts of suboptimal conditions, thereby ensuring more robust and uniform crop growth.

## II. MATERIAL AND METHODOLOGY

An investigation was conducted at the laboratory of the Department of Seed Science & Technology, CCS Haryana Agricultural University, Hisar, focusing on 18-month-old seeds subjected to various priming treatments. The seed material utilized consisted of Tomato (variety Selection-7) with a germination rate exceeding 70%, meeting the Indian Minimum Seed Certification Standard (IMSCS). These seeds were sourced from the Department of Vegetable Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar.

Two types of containers were employed: seeds stored in a cloth bag (C1) and seeds stored in polythene bags exceeding 700 gauge (C2). These containers were placed under ambient conditions. Observations were made on seed quality parameters, including seed germination

percentage, seedling length (cm), seedling dry weight (mg), vigour index -I, and vigour-II. The standard germination test, seedling length test, and vigour index were determined using established procedures. The results were statistically analyzed and presented in below table.

### Priming treatment details: -

**T0:** Control (untreated).

**T1:** Priming with GA<sub>3</sub> @ 25, 50 and 75 ppm for 24 h at 25°C and drying at room temperature.

**T2:** Priming with KNO<sub>3</sub> (Potassium Nitrate) @ 0.5 %, 1 %, and 1.5 % for 24 h at 25°C followed by drying at room temperature.

**T3:** Priming with Ethanol @ 25, 50 and 75 ppm for 24 h at 25°C and drying at room temperature

### Observation recorded

The different observations recorded were

- I. Germination (%)
- II. Seedling length (cm)
- III. Seedling dry weight (mg)
- IV. Seedling vigour index -I (Germination percentage × Seedling length)
- V. Seedling vigour index -II (Germination percentage × Seedling dry weight)
- VI. Test weight (g)
- VII. Tetrazolium test (%)

### I. Standard germination (%)

Three sets of one hundred seeds for each crop were individually positioned between adequately moistened rolled towel papers (BP) and placed in a seed germinator at 25°C. The initial assessment was conducted on the 5th day, with the conclusive count performed on the 14th day. Only healthy seedlings were taken into account for calculating the percentage of germination, following the guidelines of the International Seed Testing Association [11].

### II. Seedling length (cm)

At the final count, ten typical seedlings were randomly chosen from each replication of all seed lots, and their lengths were measured in centimeters. The average length of these selected seedlings was then computed.

### III. Seedling dry weight (mg)

The ten healthy seedlings, previously utilized for measuring seedling length, were also employed for assessing seedling dry weight. These seedlings underwent a drying process in a hot air oven at 80°C for 48 hours,

after which they were taken out, allowed to cool in desiccators for 30 minutes, and then weighed using an electronic balance. The average weight of the dried seedlings from each replication was calculated and reported in milligrams.

#### Seed vigour indices

Seedling vigour indices were calculated by using the formula suggested by [12] as follows: -

#### IV. Vigour Index-I

Vigour Index-I = Standard germination (%) × Average seedling length (cm)

#### V. Vigour Index-II

Vigour Index-II = Standard germination (%) × Average seedling dry weight (mg)

#### VI. Test weight (g):

A total of one thousand seeds from each variety, distributed across three replications, were meticulously counted and weighed utilizing an electronic balance. The resultant average seed weight for both crops was then expressed in grams.

#### VII. Tetrazolium test (%)

In three separate replicates, fifty seeds from each variety were submerged in 50 ml of water. These seeds were maintained under these conditions for 16 hours at 25°C to activate dehydrogenase enzymes. Following this, a longitudinal incision was made through the mid-section of both the embryo and the endosperm. Subsequently, the seeds were placed in petri plates and stained with a 0.5 percent tetrazolium solution (2, 3, 5-triphenyl tetrazolium chloride) for 4 hours at 38 °C. After draining the solution, the seeds underwent a brief rinse in tap water and were examined under magnification. Seeds displaying a red stain throughout the entire embryo were considered normal and viable, with the results expressed as a percentage.

### III. RESULTS AND DISCUSSION

The priming treatments applied to tomato seeds had a significant positive impact on germination and viability even after 18 months of storage. The results showed that all priming treatments, except for ethanol at 75 ppm, significantly improved the germination percentage. Among the different priming treatments, seeds primed with GA<sub>3</sub> @ 50 ppm exhibited the highest germination percentage, recording 76.33 % and 68.67 % germination rates. Following closely, seeds primed with KNO<sub>3</sub> @ 1.5 % showed a germination percentage of 71.67 % and 66.67 %. On the other hand, the lowest germination percentage was observed in seeds primed with ethanol @

50 ppm, which recorded 61.33 % and 58.33 % germination rates in polythene and cloth bags, respectively. Polythene bags demonstrated a higher germination percentage at 64.50 % compared to cloth bags at 60.00 % (Table 1). However, ethanol @ 75 ppm negatively affected the germination percentage compared to the control.

Similar findings were reported by [13], who observed an increase in germination percentage with the application of GA<sub>3</sub>. [14] found similar results in hot pepper, [15] in sesame seeds, [16] in Indian mustard, and [17] in their study on gibberellic acid treatment in various crops. [18] also reported a significant increase in germination percentage in wheat with GA<sub>3</sub> at 50 ppm. Research has shown that the release of gibberellic acid from the embryo during germination triggers specific genes responsible for α-amylase mRNA transcription [19]. Consequently, the introduction of external gibberellic acid (GA<sub>3</sub>) can activate these genes within the seeds. Additionally, exogenous GA<sub>3</sub> has the capacity to impact cytokinin transport across membranes, playing a crucial role in initiating the biochemical processes essential for successful germination [20].

The favorable effects of priming treatments on both seedling length and seedling dry weight aligned with the patterns observed in germination percentages. Among the diverse priming treatments, seeds treated with GA<sub>3</sub> at 50 ppm displayed the highest seedling lengths, measuring 10.17 cm and 8.77 cm. In close succession, seeds subjected to KNO<sub>3</sub> at 1.5% exhibited seedling lengths of 9.81 cm and 8.53 cm. Conversely, seeds primed with ethanol @ 50 ppm demonstrated the minimum seedling length, recording 8.78 cm and 7.40 cm in polythene and cloth bags, respectively (Table 2). Regarding seedling dry weight, seeds primed with GA<sub>3</sub> @ 50 ppm recorded the highest values, with seedling dry weights of 16.89 mg and 14.36 mg. Seeds primed with KNO<sub>3</sub> @ 1.5 % followed closely with seedling dry weights of 16.29 mg and 14.15 mg. On the other hand, seeds primed with ethanol @ 50 ppm showed the lowest seedling dry weight, measuring 13.60 mg and 11.66 mg in polythene and cloth bags, respectively (Table 3).

Consistent with previous findings, the seed vigor indices, namely Seed Vigor Index-I and Seed Vigor Index-II, exhibited similar trends (Table 4 and 5). All priming treatments, except for ethanol @ 75 ppm, significantly improved the Seed Vigor Index-I. Among the treatments, seeds primed with GA<sub>3</sub> @ 50 ppm achieved the highest Seed Vigor Index-I values, measuring 776.2 and 602.3. Following closely, seeds primed with KNO<sub>3</sub> @ 1.5 % showed Seed Vigor Index-I values of 702.8 and 568.8. On the other hand, seeds primed with ethanol at 50 ppm

exhibited the lowest Seed Vigor Index-I, recording 535.3 and 431.7 in polythene and cloth bags, respectively.

Similar trends were observed in Seed Vigor Index-II, with seeds primed with GA<sub>3</sub> @ 50 ppm displaying the highest values, recording Seed Vigor Index-II of 1288.9 and 985.7. Seeds primed with KNO<sub>3</sub> @ 1.5 % followed closely, exhibiting Seed Vigor Index-II values of 1167.4 and 943.6. Conversely, seeds primed with ethanol at 50 ppm showed the lowest Seed Vigor Index-II, measuring 833.8 and 680.3 in polythene and cloth bags, respectively.

These results further validate the effectiveness of the priming treatments, particularly GA<sub>3</sub> @ 50 ppm, in enhancing seed vigor indices. The increased Seed Vigor Index-I and Seed Vigor Index-II values indicate improved seed quality, germination potential, and overall seedling performance. Similar findings have been reported in studies conducted on hot pepper by [14], on chilli by [22] and on tomato and chilli by [9].

Among the treatments, seeds primed with KNO<sub>3</sub> @ 0.5% exhibited the highest test weight, measuring 3.51 and 3.49 g, followed by seeds primed with GA<sub>3</sub> @ 25 ppm

with test weights of 3.47 and 3.46 g. On the other hand, seeds primed with ethanol @ 75 ppm showed the lowest test weight, registering 3.43 and 3.41 g in polythene and cloth bags, respectively. Nevertheless, no notable impact of the varied priming treatments was observed on the test weight of the seeds. (Table 6). These findings are consistent with the results reported by [14] in hot pepper, which also showed no significant effect of different priming treatments on test weight.

The viability percentage of tomato seeds, even after 18 months of storage, demonstrated a significant positive response to the priming treatments. Notably, seeds subjected to GA<sub>3</sub> priming at 50 ppm exhibited the highest viability percentages, reaching 77.67% and 73.33%. Following closely, seeds primed with KNO<sub>3</sub> @ 1.5% showed viability percentages of 75.67 % and 71.33 %. Conversely, seeds primed with ethanol @ 50 ppm recorded the lowest viability percentages, registering 65.33 % and 61.33 % in polythene and cloth bags, respectively (Table 7).

Table. 1 Effect of priming treatments and storage containers on seed germination (%) of tomato seeds

Treatments (T)	Storage containers (C)		
	Cloth bag	Polythene bag	Mean
Control	55.67 (48.24)	60.67 (51.14)	58.17 (49.69)
GA <sub>3</sub> 25ppm	61.33 (51.53)	67.00 (54.92)	64.17 (53.22)
GA <sub>3</sub> 50ppm	68.67 (55.97)	76.33 (60.87)	72.50 (58.42)
GA <sub>3</sub> 75ppm	57.67 (49.39)	63.67 (52.91)	60.67 (51.15)
KNO <sub>3</sub> 0.5%	55.00 (47.85)	61.33 (51.53)	58.17 (49.69)
KNO <sub>3</sub> 1%	63.00 (52.52)	66.00 (54.32)	64.50 (53.42)
KNO <sub>3</sub> 1.5%	66.67 (54.71)	71.67 (57.82)	69.17 (56.27)
Ethanol 25ppm	61.67 (51.73)	64.00 (53.11)	62.83 (52.42)
Ethanol 50ppm	58.33 (49.78)	61.33 (51.53)	59.83 (50.65)
Ethanol 75ppm	52.00 (46.13)	53.00 (46.70)	52.50 (46.42)
Mean	60.00 (50.78)	64.50 (53.48)	
C.D (P=0.5)	C= 0.797, T= 1.783, CxT= 2.521		
SE(m)	C= 0.278, T= 0.621, CxT= 0.879		

Table. 2 Effect of priming treatments and storage containers on seedling length (cm) of tomato

Treatments (T)	Storage containers (C)		
	Cloth bag	Polythene bag	Mean
Control	7.30	8.77	7.90
GA <sub>3</sub> 25ppm	8.13	9.63	8.88
GA <sub>3</sub> 50ppm	8.77	10.17	9.47
GA <sub>3</sub> 75ppm	7.57	9.46	8.51

<b>KNO<sub>3</sub> 0.5%</b>	7.46	8.70	8.08
<b>KNO<sub>3</sub> 1%</b>	8.10	9.44	8.77
<b>KNO<sub>3</sub> 1.5%</b>	8.53	9.81	9.17
<b>Ethanol 25ppm</b>	7.63	8.87	8.25
<b>Ethanol 50ppm</b>	7.40	8.78	8.09
<b>Ethanol 75ppm</b>	6.80	8.20	7.50
<b>Mean</b>	7.74	9.15	
<b>C.D (P=0.5)</b>	C=0.094, T= 0.211, CxT= 0.298		
<b>SE(m)</b>	C=0.033, T= 0.073 CxT=0.104		

Table. 3 Effect of priming treatments and storage containers on seedling dry weight (mg) of tomato

Treatments (T)	Storage container (C)		
	Cloth bag	Polythene bag	Mean
<b>Control</b>	9.60	11.42	10.51
<b>GA3 25ppm</b>	14.20	16.15	15.10
<b>GA3 50ppm</b>	14.36	16.89	15.62
<b>GA3 75ppm</b>	13.71	15.44	14.58
<b>KNO<sub>3</sub> 0.5%</b>	13.01	15.41	14.21
<b>KNO<sub>3</sub> 1%</b>	13.77	15.66	14.72
<b>KNO<sub>3</sub> 1.5%</b>	14.15	16.29	15.22
<b>Ethanol 25ppm</b>	13.08	15.43	14.26
<b>Ethanol 50ppm</b>	11.66	13.60	12.63
<b>Ethanol 75ppm</b>	9.55	11.24	10.40
<b>Mean</b>	12.69	14.75	
<b>C.D (P=0.5)</b>	C=0.146, T=0.327, CxT=0.436		
<b>SE(m)</b>	C=0.051, T=0.114, CxT=0.161		

Table.4 Effect of priming treatments and storage containers on seed vigour Index-I of tomato

Treatments (T)	Storage containers (C)		
	Cloth bag	Polythene bag	Mean
<b>control</b>	391.8	531.9	461.9
<b>GA3 25ppm</b>	498.8	645.4	572.1
<b>GA3 50ppm</b>	602.3	776.2	689.3
<b>GA3 75ppm</b>	436.3	602.1	519.2
<b>KNO<sub>3</sub> 0.5%</b>	410.7	533.6	472.2
<b>KNO<sub>3</sub> 1%</b>	510.3	622.8	566.6
<b>KNO<sub>3</sub> 1.5%</b>	568.8	702.8	635.8
<b>Ethanol 25ppm</b>	470.7	567.4	519.1
<b>Ethanol 50ppm</b>	431.7	535.3	483.5
<b>Ethanol 75ppm</b>	353.6	434.8	394.2
<b>Mean</b>	467.5	593.6	
<b>C.D (P=0.5)</b>	C=10.24, T=22.90, CxT=32.38		
<b>SE(m)</b>	C=3.57, T=7.98, CxT=11.29		

Table.5 Effect of priming treatments and storage containers on seed vigour index- II of tomato

Treatments (T)	Storage containers (C)		
	Cloth bag	Polythene bag	Mean
Control	534.1	692.8	613.4
GA3 25ppm	862.0	1081.8	971.9
GA3 50ppm	985.7	1288.9	1137.3
GA3 75ppm	790.4	983.4	886.9
KNO <sub>3</sub> 0.5%	715.4	944.9	830.2
KNO <sub>3</sub> 1%	867.7	1033.7	950.7
KNO <sub>3</sub> 1.5%	943.6	1167.4	1055.5
Ethanol 25ppm	806.4	987.7	897.1
Ethanol 50ppm	680.3	833.8	757.0
Ethanol 75ppm	496.7	596.0	546.3
Mean	768.2	961.0	
C.D (P=0.5)	C=14.08, T=31.48, CxT=44.52		
SE(m)	C=4.91, T=10.97, CxT=15.52		

Table.6 Effect of priming treatments and storage containers on test weight (g) of tomato seeds

Treatments (T)	Storage containers (C)		
	Cloth bag	Polythene bag	Mean
Control	3.40	3.43	3.42
GA3 25ppm	3.46	3.47	3.47
GA3 50ppm	3.45	3.45	3.45
GA3 75ppm	3.44	3.44	3.44
KNO <sub>3</sub> 0.5%	3.49	3.51	3.50
KNO <sub>3</sub> 1%	3.47	3.49	3.48
KNO <sub>3</sub> 1.5%	3.45	3.47	3.46
Ethanol 25ppm	3.43	3.45	3.44
Ethanol 50ppm	3.43	3.44	3.44
Ethanol 75ppm	3.40	3.43	3.42
Mean	3.44	3.46	
C.D (P=0.5)	C=0.007, T=0.015 CxT=N.S		
SE(m)	C=0.002, T=0.005, CxT=0.008		

Table. 7 Effect of priming treatments and storage containers on viability (%) of tomato seeds

Treatments (T)	Storage containers (C)		
	Cloth bag	Polythene bag	Mean
Control	61.67 (51.73)	66.67 (54.72)	64.17 (53.22)
GA3 25ppm	70.33 (56.98)	73.67 (59.11)	72.00 (58.04)
GA3 50ppm	73.33 (58.91)	77.67 (61.78)	75.50 (60.35)
GA3 75ppm	66.67 (54.71)	69.00 (56.14)	67.83 (55.43)
KNO <sub>3</sub> 0.5%	65.33 (53.92)	67.67 (55.33)	66.50 (54.62)
KNO <sub>3</sub> 1%	68.67 (55.94)	72.67 (58.47)	70.67 (57.20)

<b>KNO<sub>3</sub> 1.5%</b>	71.33 (57.61)	75.67 (60.42)	73.50 (59.02)
<b>Ethanol 25ppm</b>	67.33 (55.12)	70.00 (56.77)	68.67 (55.95)
<b>Ethanol 50ppm</b>	61.33 (51.53)	65.33 (53.91)	63.33 (52.72)
<b>Ethanol 75ppm</b>	56.00 (48.43)	61.00 (51.34)	58.50 (49.88)
<b>Mean</b>	66.20 (54.49)	69.93 (56.80)	
<b>C.D (P=0.5)</b>	C=0.814, T=1.821, CxT=2.145		
<b>SE(m)</b>	C=0.284, T=0.635, CxT=0.898		

#### IV. CONCLUSION

According to a research study, the priming of seeds with GA<sub>3</sub> @ 50 ppm has shown notable benefits. The germination rate of tomato seeds increased by 24.6 % compared to untreated seeds. Furthermore, GA<sub>3</sub> @ 50 ppm effectively controlled fungal infections and enhanced various seed quality parameters, including seedling length, seedling dry weight, seed vigor indices, viability percentage, test weight, seed density, and radicle emergence.

In summary, the research findings indicate that the application of GA<sub>3</sub> @ 50 ppm during seed priming offers significant advantages. It enhances germination rates for tomato, helps control fungal infections, and improves various seed quality characteristics, thereby contributing to overall seed performance.

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# **A Cost-Benefit Analysis of Building a Dam in Pakistan: To Mitigate Floods, Promote Tourism, Boost Agriculture, and Generate Electricity**

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**Abstract**— Every year, Pakistan is on the verge of a serious water crisis. It is expected that the crisis of water shortages in Pakistan will reach an alarming level in the near future. Water degradation, climate change, particularly recent global warming, overuse, misuse, diminishing water availability, a lack of water management policies, pollution, population growth, and environmental neglect have all contributed to this crisis. This has undermined Pakistan's economy. Floods in Pakistan have adversely affected livestock, infrastructure, lives, and property, as well as agricultural land and crops. Floods negatively impact agricultural production and the country's economy without adequate water management. The travel and tourism sector in Pakistan plays a crucial role in the country's economy and contributes to its growth and development. However, despite its potential, this sector remains unsatisfied and faces several challenges and limitations. Furthermore, Pakistan faces a persistent electricity shortfall that has led to the implementation of load shedding across the country. The shortage in electricity supply has become a significant issue for both businesses and consumers, impacting the economy and overall quality of life. This paper examines the cost-benefit analysis of building a dam to reduce flood damage, promote tourism, and boost agriculture and electricity. It is difficult to provide accurate estimates of dam construction costs due to a lack of precise information. So many assumptions have been made to complete the analysis. There will also be a sensitivity analysis to test some assumptions.



**Keywords**— *Cost-benefit analysis, Dam construction, Analyze assumptions, Floods impact agriculture, Reduce flood damages.*

## **I. METHOD**

The construction of dams is a complex and costly endeavor that requires meticulous planning and careful consideration of various factors such as location, design, materials, and environmental factors. However, accurately estimating dam construction costs poses a significant challenge due to the inherent uncertainties and complexities involved. In order to gather the necessary information, this paper will utilize literature studies, secondary sources, case studies, research papers, surveys, and evaluations of challenges. By combining these methodologies, a comprehensive analysis will be conducted to develop reliable cost estimates, providing valuable insights for dam construction projects.

In addition to the sources mentioned above, this paper also relies on numerous assumptions and considerations in order to conduct a comprehensive analysis. One notable limitation is that the exact data on the construction costs of the dam was not available in the recommendation project. As a result, several assumptions have been made to complete the analysis and provide a balanced assessment. In order to ensure that the recommendations are as accurate as possible, a sensitivity analysis has been conducted to test a number of the assumptions. This analysis allows us to evaluate the impact of varying input values on the overall conclusions and recommendations.

Building dams has emerged as a crucial strategy for addressing various issues related to water resource

management, flood control, and hydroelectric power generation. This paper provides a comprehensive and actionable plan that takes into account the unique challenges and circumstances of the situation the country is facing and explores the potential benefits of building dams as well as provides recommendations and solutions for the government to excellently implement these programs.

## II. INTRODUCTION

Pakistan faces many challenges that urgently require attention, including the severe electricity shortfall, the impact of climate change-induced floods on agriculture, the poor natural, cultural, and non-leisure resource ratings, and the deepest energy and economic crisis in its history. The country's development must address these issues head-on and work towards a more sustainable and resilient future.

Pakistan is currently facing a severe electricity shortfall, resulting in frequent power outages across the country. Even people in industrial areas face heavy electric load shedding for many hours a day. This persistent shortage of electricity not only hampers productivity but also disrupts daily life and hampers economic growth. The electricity shortage in Pakistan can be attributed to various factors, including inadequate infrastructure, energy theft, and inefficient energy production. The lack of investment in power generation and transmission infrastructure has further exacerbated the situation. Pakistan is currently facing a massive energy crisis, with a significant demand and supply shortfall of between 5000 and 8000MW. This power shortage has created severe consequences for the economy and the country as a whole. To address the urgent need for reliable and sustainable power, the government must develop a comprehensive initiative for the future (PEC et al., 2014). The government must take immediate action on several fronts in order to alleviate the energy crisis. As a first step, it is essential to invest in new power plants, upgrade existing ones, and build new dams in order to increase their efficiency and productivity.

The tourism industry in Pakistan is a substantial sector that holds significant importance for the country's economy. It not only provides employment opportunities but also contributes to the generation of foreign exchange revenues. However, despite its significance, this area of research has received relatively little attention in Pakistan. According to a study conducted by the World Forum Report 2019, Pakistan ranked among the lowest in terms of competitiveness for travel and tourism worldwide, especially in comparison to other South Asian countries. This low ranking indicates that Pakistan needs to prioritize the development of tourist points, eco-friendly destinations, and exploring opportunities in order to attract national and

international tourists. The tourism sector has the potential to contribute significantly to Pakistan's economic growth. By attracting tourists from both within the country and abroad, Pakistan can generate foreign currency reserves, boost domestic tourism, and promote cultural exchanges. This, in turn, can lead to the development of infrastructure Pakistan's low ranking in the Travel and Tourism Competitiveness Index can be attributed to the lack of travel and tourism spots, such as ecofriendly environments and parks, fisher spots guesthouses, and hotels. By prioritizing the development of these areas, Pakistan can enhance its attractiveness as a tourist destination and attract a wider range of visitors (Altaf et al., 2021; World Economic Forum, 2019). In order to enhance the tourism sector, Pakistan should focus on developing attractive tourist points. Additionally, investing in hotels and resorts, as well as implementing efficient marketing strategies, can attract more tourists. Developing eco-friendly destinations such as dams is another crucial aspect of attracting tourists.

In rural areas, the agriculture sector is crucial to poverty alleviation, economic development, and food security. This sector contributes 19.2 percent to GDP and employs 38.5 percent. The country produces cotton fifth in the world. The country exports 60% of textiles and cotton products. Due to this, agriculture contributes 0.6 percent to GDP and 2.4 percent to value added (Finance Division, Government of Pakistan, 2022). The Finance Division, Government of Pakistan's report emphasizes the importance of sustainable growth in the agriculture sector for food security and rural development in the country. The report highlights that water interests in Pakistan are often conflicting when it comes to water use in agriculture. It stresses the need to adopt water management techniques to ensure food security in the country (Finance Division, Government of Pakistan., 2022). Climate vulnerability poses significant challenges to Pakistan's agricultural sector, negatively affecting its economy. Investing in dams is crucial for addressing these challenges. Dams support irrigation, increase agricultural production, and contribute to sustainable development. By prioritizing sustainable development and implementing effective water management policies, Pakistan can enhance its resilience to climate-related risks and ensure the well-being of its people.

Pakistan is one of the countries in South Asia which experiences the most floods each year. Floods occur regularly in Pakistan, causing significant destruction and loss of life. These floods often result from heavy rainfall, melting of snow in mountainous regions, or the release of water from dams or reservoirs. The intensity and frequency of these floods have increased significantly in recent years, posing a significant threat to the country's infrastructure and development. The damage caused by floods is extensive. It

not only affects human lives but also disrupts economic activities, destroys infrastructure, and damages agricultural land. Pakistan has not been able to manage water efficiently so far and has not been able to build enough reservoirs and dams to meet the country's needs and protect the country from natural climate change floods (Ali, 2013; Akbar et al., 2021).

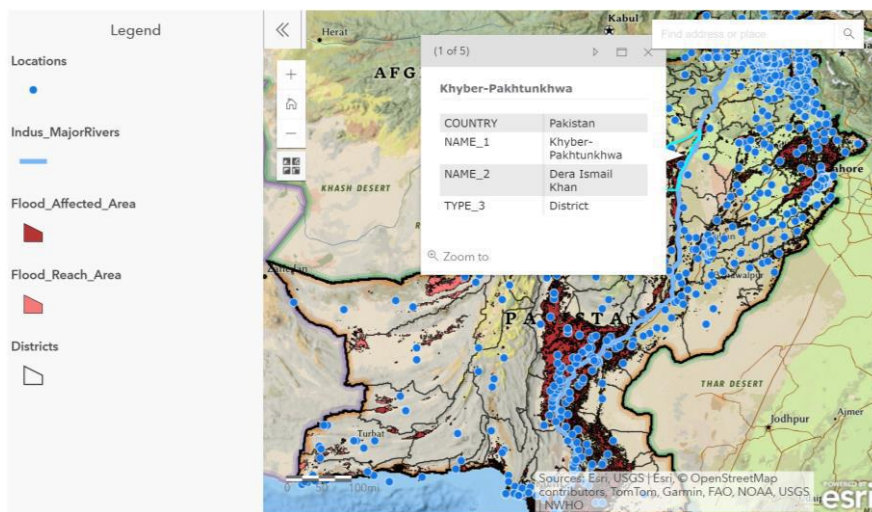
The paper aims to perform a cost-benefit analysis of building a new dam in Pakistan to mitigate floods, promote tourism, boost agriculture, and generate electricity. The cost-benefit analysis of dam construction is a complex and multifaceted exercise. It involves examining the potential benefits and costs associated with constructing a new dam in Pakistan. This analysis will provide insights into the economic feasibility and the potential benefits of dam construction. This analysis involves varying key assumptions and parameters to assess their impact on the project's economic viability. By examining the potential benefits and costs associated with constructing a new dam, we can determine whether it is economically beneficial compared to the status quo. Policymakers and development practitioners will gain valuable insight from this paper regarding the building of new dams in Pakistan.

### III. LITERATURE REVIEW

The literature review has provided an overview of floods and their impacts, the causes of floods in Pakistan, the country's flood protection facilities, the institutional responsibility for managing floods, and the flood management options. The paper concludes by emphasizing the need for sensible water management and flood protection facilities in Pakistan, such as the construction of a dam. This dam would not only facilitate water management but also promote tourism, mitigate floods, and benefit agriculture. Additionally, a cost-benefit analysis will be conducted to assess the viability of constructing a big dam in Pakistan.

#### Pakistan's major floods and their impacts

Pakistan has been identified as one of the most climate-vulnerable countries in many United Nations and government reports. Sectors such as water, agriculture, and food are among them. There have been several major flood disasters hit Pakistan in the last decade, including those of 2003, 2010, 2011, 2012, 2013, and 2014. As a result of the scale and intensity of natural hazards, such as severe thunderstorms, floods, glacial melting, and droughts, all sectors of society are placed at risk (Ismail & Malik, 2020).



Shahzad Ismail's GIS Map Project 2023, Devastating Floods In Pakistan, published at <https://newworldhope.org/environment.html>

The GIS Map Project 2023 highlights the devastating impact of floods on the Indus River in Pakistan. The map provides valuable insights into the potential effects during and after floods when heavy rain hits the country. The Indus River flows for more than 1900 miles (3,060 kilometers) throughout Pakistan, making it the longest river in the country., traversing through Pakistan before reaching the southern province of Sindh and the Indus Delta, where it

meets the Arabian Sea. The river plays a crucial role in Pakistan's environment, economy, and culture. However, due to the absence of a dam system, the Pakistani government struggles to effectively control and mitigate the damage caused by heavy rainfall. The maps provide a comprehensive view of the potential effects caused by floods, particularly with the Indus River. When heavy rain hits Pakistan, the Indus River becomes full, resulting in

extensive flooding in the surrounding areas. This flooding not only damages and destroys the populated areas and agricultural lands located near the river but also poses a significant risk to lives and economic stability. The GIS Map Project 2023 includes data that identifies and highlights the districts and areas in Pakistan that are most vulnerable to flooding. By identifying the high-risk areas, policymakers and disaster management agencies can allocate resources and efforts more efficiently to alleviate the impact of floods. Flash floods in rivers and streams in Pakistan have increased during the rainy season when heavy rains fall in hilly and semi-hilly areas. Over the years, the frequency and intensity of these floods have increased due to the increase of the population near the rivers (Ghumman et al., 2012).

In 2003, the Pakistan floods occurred due to monsoon rains and melting snow causing great damage to many areas of Sind, Khyber Pakhtunkhwa (KPK), and Baluchistan Provinces. 130 people lost their lives as a result of this flood and 4,476 villages were badly affected as a result (Yaqub & Eren, 2015).

As a result of heavy monsoon rains in Sindh province, the flood of 2011 affected many districts of this province severely. The Pakistan floods of 2011 caused a significant loss of crops in terms of both standing and stored crops. Total losses were approximately 1,840 million dollars, with 92 percent of the losses occurring in agriculture and 74 percent of the damage occurring in cotton crops (Pakistan Economic Survey, 2011). There were approximately 434 deaths due to this flood, approximately 5.3 million people were displaced, and approximately 1,524,773 homes were partially or fully damaged (Aon plc, 2010).

Flash floods occurred in KPK, Sindh, and Punjab provinces in 2013 as a result of heavy rains during the monsoon season. Pakistan floods in 2013 have not only resulted in the loss of human lives, property, and infrastructure but also caused severe damage to agriculture. It has been estimated that the floods of 2013 have affected a cropped area of approximately 1.107 million acres, affecting 8,297 communities' areas, claiming 333 lives, fully damaging 33,763 houses and partially damaging 46,180 houses, and affecting a population of approximately 1.489 million people. Over the past 66 years, Pakistan has lost more than US\$ 37.554 billion in financial losses. A total of 603,942 square kilometers were affected by 21 major flood events from 1950 to 2013 causing 11,572 deaths, and 188,531 villages to be damaged/ destroyed (Ministry Of Water and Power, 2013).

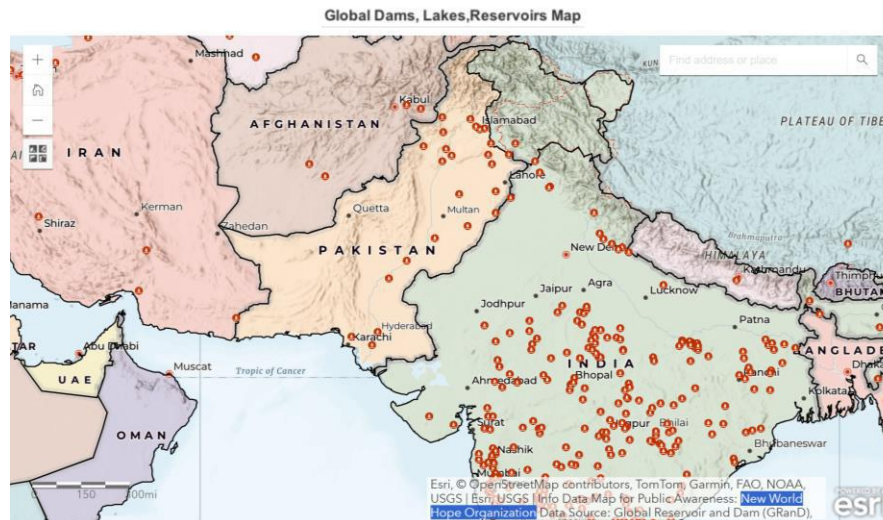
In 2014-15, heavy monsoon rains affected the central region, Punjab, and the eastern regions, Gilgit Baltistan and Azad Jammu and Kashmir, of the country for the fourth consecutive year. This natural disaster resulted in the deaths of approximately 367 people and the displacement of approximately 2.5 million people. A total of one million acres of agricultural land and approximately 2.5 hundred thousand farmers were adversely affected (Yaqub & Eren, 2015).

There were severe floods in Pakistan in June 2022. Three-quarters of the country's landmass was under water, destroying over 3.6 million acres of crops and killing more than 750,000 animals. There have been almost 24,000 schools damaged, and thousands of roads lost. Floods affected infrastructure, crops, and food production, costing US\$30 billion. Floods have affected about 33 million people, displaced over 7 million people, and killed over 1500, including 552 children. 16 million children and 650,000 pregnant women have been affected by the floods (Ministry of Planning Development & Special Initiatives, 2022).

### Causes of Floods in Pakistan

Since Pakistan was established, it has experienced more than 21 major floods, but the intensity and frequency of these floods have increased over the past decade. Around 70 percent of global disasters are caused by hydrometeorological events. Historically, heavy rainfall has been a major contributor to the continuity of devastating floods in Pakistan. Heavy rains raise the level of water in rivers and streams, and when the water level rises due to heavy rain, the riverbanks overflow and the water comes out from the banks, causing damage to surrounding areas and crops. A second main cause of floods is heavy and abnormal rains due to climate change and the melting of glaciers as a result of the changes in the weather. It is estimated that up to 73% of the runoff is generated by snowmelt and glacial melting. This results in an increase in the water ratio of rivers which eventually causes flooding (Taraky et al., 2021).

Another important factor that contributes to the occurrence of floods in Pakistan is the lack of management of water and the lack of big dams to control the levels of water. Flooding is one of the greatest risks to sustainable development in Pakistan. Generally, dams influence flooding frequency, duration, and timing by regulating water flow. Large-scale dams can control the discharge and prevent floods.



Shahzad Ismail's GIS Map Project 2023, Global Dams, Lakes, and Reservoirs Data Map, published at <https://newworldhope.org/index.html>

The Pakistani government relies heavily on its small and medium-sized dams and barrages in order to prevent rivers from flooding and to meet its energy demands. However, unfortunately, these dams and barrages were not able to control and reduce the frequency of recent floods that occurred in Pakistan. Therefore, there is an urgent need for a large dam that can not only fulfill the nation's electricity needs but also control and reduce the level of water in rivers during rainy seasons while providing valuable water storage for agriculture during the rainy season. In Pakistan, the economy is largely based on agriculture, and water resources are crucial to the economy. By constructing the big dam, the country will be able to boost agricultural output, give livelihood opportunities to those in need, and contribute to the improvement of their living conditions. One of the primary goals of dam construction is to control floods and disasters (Nazeer, 2020). Despite this, the dam is not able to meet the country's needs. The increasing population and climate change have a profound impact on water and water management. The increasing water demand, coupled with more frequent flood occurrences, places significant pressure on this vital resource. By implementing sustainable water management practices, such as water reservoirs, water recycling, and rainwater harvesting, we can mitigate the challenges posed by population growth and climate change and ensure long-term availability and access to clean water for all. (Janjua et al., 2021).

### Pakistan's Flood Protection Facilities

There are several policies and institutions in place by the Pakistani government such as Flood Policy and Strategy to ensure effective and sustainable flood management, Flood Institutions to implement structural and nonstructural

interventions, Flood Risk Managing Institutions (FRMI) to promote sustainable and long-term flood resilience, and Flood Crisis Management Institutions to supervise and direct rescue at local and national levels. (Ministry Of Water and Power, 2013).

Even though there are currently many flood management and preparedness measures in place in Pakistan to ensure effective and sustainable flood management, Pakistan has made significant strides in improving flood management through various measures. However, the country still faces challenges in implementing a comprehensive and sound flood management system that can adequately mitigate the devastating impacts of these natural disasters. Based on the current situation, there is an urgent need to create an effective flood management system that can deal with floods in a responsible manner (Department of Civil Engineering, The University of Lahore, 2018; Ministry Of Water and Power, 2013). There is still one crucial element lacking in Pakistan despite the implementation of flood policies, flood laws, flood think tanks, flood management institutions, and flood crisis management organizations - the construction of large dams that can control and manage flood water both during and after floods.

### Economic Benefits of Dam Construction

Dam construction offers several key economic advantages, including increased agricultural productivity, tourism development, and enhanced electricity production ability. These advantages contribute to economic growth, job creation, and sustainable development, making them important features for the overall development and prosperity of a country.

### Increased Tourism and Fisheries

The tourism sector is a significant generator of economic activity on a rapid and broad scale. Before the COVID-19 pandemic, the World Travel & Tourism Council (WTTC) report revealed that the employment in the travel and tourism sector of Pakistan in 2019 was 3,888 thousand. Pakistan's neighboring countries such as India generated employment through travel and tourism in 2019, reaching about 40,104 thousand, which is almost 10 times higher than Pakistan. Additionally, China's travel and tourism employment rate for 2019 was approximately 82, 240 thousand, which is 21 times higher than Pakistan. In terms of contribution to GDP, China's travel and tourism sector generated \$1,665.6 billion in 2019, while the sector contributed \$191.3 billion to India's economy in the same year. However, it is important to note that Pakistan is not on the list of G20 countries list so in the WTTC report, so there are no GDP data available for Pakistan (WTTC, 2022).

Pakistan's ranking in the Travel and Tourism Competitiveness Index for 2019 was 121 out of 140 countries, while India was placed at 34 and China at 13. In the Asia Pacific region, Pakistan is ranked at the bottom of the list (World Economic Forum, 2019). There is a significant need to improve Pakistan's travel and tourism sector in order to generate an environmentally friendly environment and enhance employment opportunities in the country. Recognizing the importance of improving the Travel and Tourism sector in Pakistan, a number of measures can be taken to enhance the eco-environment and generate employment opportunities in the country. Building dams is considered an ideal option in this regard. By investing in dams, Pakistan can generate hydroelectric power, improve water resources, and create opportunities for recreational activities such as boating and fishing. The presence of a dam in any area can contribute to the development of infrastructure such as guesthouses, restaurants, and parks, further enhancing the overall tourism experience for national and international tourists. This, in turn, can boost tourism in the country and generate new job opportunities.

### **Boosted Agriculture**

The (Finance Division, Government of Pakistan, 2022) report emphasizes the importance of sustainable growth in the agriculture sector for food security and rural development in the country. With a contribution of 22.7 percent to the Gross Domestic Product (GDP), agriculture plays a significant role in the economy of Pakistan. Additionally, it employs around 37.4 percent of the labor force, highlighting its significance in terms of job creation and poverty reduction.

There are frequent floods and droughts in the river basin. During a flood, infrastructures are destroyed, urban and

rural areas are inundated, and agricultural land is damaged. The Pakistan Council of Research in Water Resources (PCRWR) report highlights that water interests in the country are often conflicting when it comes to water use in agriculture. There is a need to improve the productivity of the agricultural sector in the report. Agricultural activities consume a significant amount of freshwater and play a crucial role in food security. The report highlights the construction of dams is essential for several reasons. They enable the transfer of flows from the high-flow rainy season to the dry seasons, ensuring a consistent water supply throughout the year. Dams play a vital role in ensuring an adequate and reliable water supply for agriculture, especially during drought periods. By capturing and storing water, dams help mitigate the impact of water shortages on agricultural productivity, ensuring food security for the population (Fatima et al., 2021).

Increased agricultural production can have several significant benefits, both in terms of economic returns and food security for the country. Dams play a crucial role in harnessing the stored water, regulating its release, and ensuring a steady water supply for agricultural purposes. Dams contribute to the overall economic development of a nation by increasing crop yields and providing a reliable water supply. When farmers have access to sufficient water, they can cultivate a wider range of crops, leading to increased production. Dams not only increase the amount of food available but also create job opportunities in the agricultural sector.

### **Increased Electricity Production**

The energy area is one of the most important components of any country's economic development. Over the last two decades, there has been a substantial increase in the energy demand. However, the energy sector in Pakistan has been facing challenges for quite a long time. One such challenge that persists is the long-standing issue of circular debt in the energy sector. Circular debt in the energy sector refers to the accumulation of overdue payments and pending liabilities, leading to a financial imbalance (Finance Division, Government of Pakistan., 2022). Despite facing these challenges, Pakistan's energy needs continue to grow, with the demand for energy expected to rise significantly in the coming years. This increasing energy demand, driven by population growth, will put increasing pressure on the country's existing energy resources and distribution systems. However, the generation capacity of electricity has not kept pace with the power demand, leading to a persistent electricity demand shortfall of about 5000 megawatts (MW) since 2007, leading to long hours of load shedding in both rural and urban areas. In 2022, the shortfall stood at 5,944

MW, highlighting the need for urgent measures to address this problem. (PEC et al., 2014; Bokhari, 2023).

To address this issue, Pakistan can consider harnessing hydroelectric power generation. Building dams can provide a reliable and affordable source of power, contributing to the overall development of the energy sector. By investing in dam and hydroelectric power generation, Pakistan can overcome the electricity demand shortfall and contribute to its economic development. The dam is not just a structure used to generate hydroelectric power; it also plays a key role in providing a clean and renewable energy source for both domestic and industrial use. The process of eco-friendly electricity generation can result in changes to the ecosystem, potentially impacting fish populations and habitats and the tourism sector.

### Features of the Proposed Dam - The Construction of a Dam and Its Assumption

Consider that floods in Pakistan cause an average annual loss of \$3.6 billion. These floods cause immense damage to infrastructures and the economy, resulting in billions of dollars of losses. It is imperative to explore ways to reduce the total damage caused by these floods.

#### Reducing Damages by 5% with the Dam

One potential solution that can help decrease the damage caused by floods is the construction of a dam. A dam is designed to store and control the flow of water, decreasing the risk of floods and the resulting destruction. However, it is critical to note that preventing all damage caused by floods is virtually impossible. The dam will contribute to the generation of electricity through its 2 MW electricity turbines. This paper aims to reduce the overall damage caused by floods by 5%. To achieve this, it is estimated the average cost of floods per year is \$3.6 billion. By reducing this cost by 5%, we can save approximately \$188 million in damages annually.

#### The Dam Location

In the northeastern region of the country, where mountains are abundant, the construction of a dam will be undertaken. The mountain geography of the region presents several advantages that will make the construction process easier than in other parts of the country. Firstly, the presence of mountains will provide a natural barrier against erosion. This will lessen the risk of landslides and other natural hazards that can hinder dam construction. Additionally, the mountains can act as a natural source of construction materials, such as rocks and gravel, which can be harvested for the dam's construction. The northeastern region is known for its vulnerability to floods. Its location, surrounded by mountains and rivers, makes it more susceptible to flooding events. However, the construction of

a dam in the region can help mitigate the flood risks. By storing water during the wet season and releasing it in a controlled manner during the dry season, the dam can help regulate the water flow and reduce the likelihood of devastating floods.

#### Tourism and Fisheries

The creation of a larger reservoir through the construction of the dam will bring about several opportunities for growth in tourism and fisheries. The scenic view of the reservoir will attract tourists, who can enjoy recreational activities such as boating, fishing, and hiking. Furthermore, the dam will stabilize the water conditions and provide a conducive environment for the growth of fish and other aquatic species, resulting in increased fishing activities. A tourism and travel impact of 0.075 percent is expected on the country's GDP due to the dam construction.

#### Electricity Generation

In addition to its benefits for flood control and irrigation, the dam will also contribute to the generation of electricity. Equipped with 2 megawatts (MW) hydropower turbines, the dam can generate sufficient power to meet local demand or supply excess electricity to the grid. This will not only contribute to the sustainable development of the country but also provide opportunities for economic growth through the sale of electricity to neighboring areas. It is estimated that the dam will generate sufficient power and generate \$2.5 billion in revenue.

#### Life Span Assessment of the Dam

The life span of the dam is a crucial factor that requires careful consideration. Due to the high sediment load carried by the river and the severe floods and heavy rains that occur during the rainy season, it is expected that the dam may experience wear and tear over time. However, the assumption that the life span of the dam will be 50 years is based on the absence of any upstream dams. The presence of an upstream dam could potentially affect the sediment flow patterns and mitigate the detrimental effects on the dam's structural integrity. Additionally, the effectiveness of flood control measures can also be influenced by the presence of other dams along the river. These factors need to be thoroughly evaluated when determining the life span of the proposed dam.

#### Costs

Building a dam is a complex and costly endeavor. When considering the construction of a dam, it is important to take into account several costs associated with the project. These costs include not only the initial construction costs but also ongoing expenses.

#### The Cost of the Land

This analysis indicates that the government of Pakistan is expected to pay approximately \$20 million for the land where the dam will be built. It is important to note that this amount is subject to change, as it is possible that the actual cost could be less than the expected amount due to the nature of the area.

In addition to the land cost, the government will also incur an additional cost of approximately \$2 million before the dam construction begins. This cost is considered an incremental cost, as it represents the additional expenses incurred at the beginning of the project.

Overall, the total cost of acquiring the land and commencing the project is estimated to be around \$22 million. However, it is important to note that this estimate is subject to change and may be modified based on further analysis and evaluation.

### **The Cost of the Construction.**

Based on the estimates derived from similar dam projects, it is anticipated that the construction of the dam will cost approximately \$1 billion. This estimation takes into account factors such as the complexity and scale of the project.

The construction of the dam is expected to take six years, starting from the initial planning stages to completion. During this period, the project will incur various costs, including labor, materials, and equipment.

In the first year of construction, the annual cost of the project is estimated to be \$166.7 million. This figure takes into account the initial setup, procurement of materials, and initial labor expenses. It does not include ongoing maintenance costs or any additional costs associated with unforeseen challenges or delays.

It is significant to note that the cost of the program may increase or decrease throughout the construction phase. Factors such as changes in market conditions, variations in material prices, and delays due to unforeseen circumstances can all impact the final cost.

To ensure a smooth and efficient construction process, it is crucial to manage costs effectively and allocate resources efficiently. Regular monitoring of project progress, adherence to timelines, and proactive management of potential risks can help mitigate any cost deviations.

### **The Cost of the Main Maintenance**

The cost of the main maintenance for dams is estimated to be 25% of the total project cost. This percentage was determined based on information from other projects and aligns with the recommendation that dams undergo major maintenance every 25 years.

The main maintenance project will involve various areas of the dam, including the walls and reservoir areas. It is crucial

to ensure that these structures are in good condition to prevent leaks and potential hazards. The cost of the main maintenance is estimated to be \$250 million, which is equivalent to 25% of the total project cost.

It is planned to conduct the main maintenance during the 25th and 26th years after the construction is completed. By scheduling the maintenance at these specific time points, the dam can be effectively maintained, and any potential issues can be addressed promptly. Regular maintenance will not only extend the life of the dam but also ensure its safety and functionality.

### **The Cost of the Regular Maintenance**

As part of the regular maintenance, it will be carried out every 7th to 50th year. For a dam of this size to function correctly, it is recommended that such large constructions require regular and permanent maintenance to ensure they are working properly. According to our estimates, the annual cost of regular maintenance is estimated at \$50 million. Regular maintenance consists of the cost of the machinery and equipment that needs to be maintained. As part of the regular maintenance program, the electricity turbines and other equipment related to the operation of the electricity turbines will also have to be maintained.

### **The Cost of the Electricity Turbines**

The dam will be equipped with 2 megawatts (MW) hydropower turbines for electricity generation. These turbines will have a capacity of 180 MW each, and the total cost of installing them is estimated to be approximately \$10 million for both units. Each turbine will produce 180 MW of power, and both turbines together will have a maximum nameplate capacity of 360 MW.

The turbines are scheduled to enter the construction phase in the 7th year of the project, which coincides with the anticipated completion of the dam. This staggered construction schedule allows for a seamless transition from the dam's construction phase to its operational phase. By having the turbines installed in the 7th year of construction, the dam will be able to meet its power demands efficiently and sustainably. This arrangement ensures a consistent and reliable power supply for the surrounding communities, while also minimizing the environmental impact associated with energy production.

Overall, the installation of these hydropower turbines at the dam represents an important step towards harnessing renewable energy and providing a reliable power source for the local community. The projected capacity and estimated cost of the turbines indicate their suitability and efficiency in delivering the required power output. During the life cycle of these turbines, it is anticipated that they will need to be replaced every ten years. This regular maintenance is



necessary to ensure the efficiency and reliability of the turbines. By replacing the turbines periodically, the dam can ensure a consistent supply of electricity to the surrounding area.

Overall, the cost of installing the electricity turbines for the dam is a significant investment, but the benefits in terms of power generation and reliability make it a worthwhile project.

**The Cost of the Labor**

The cost of labor for the construction phase of a dam is estimated at \$127.8 million for six years. This calculation assumes of employing 250 engineers, 500 foremen, 2500 construction workers, and 500 support workers during the construction phase (years 1 to 7).

The minimum wage set by the government of Pakistan is 55,068 Pak Rupees, which is equivalent to \$194. Therefore, the total cost of labor on an annual basis is estimated at \$21.3 million.

It is important to note that there will be a total labor cost of \$127.8 million during the construction phase of the project.

This figure takes into account the annual salary and benefits of all the aforementioned employees.

In addition to the construction phase, the main maintenance work will be conducted during the 25th and 26th years. For this maintenance work, an estimated \$42.6 million will be spent on labor. This investment is necessary to ensure that the dam remains operational and meets the necessary safety standards.

In summary, the cost of workers for the construction of a dam is approximately \$127.8 million, which includes both construction and maintenance expenses. This amount takes into account the cost of employing various personnel, including engineers, foremen, construction workers, and support workers, as well as the minimum wage set by the government of Pakistan.

The following analysis assumes that the wages below mentioned are paid on a monthly basis;

- Supporter workers; \$450
- Construction workers; \$500
- Foremen; \$650
- Engineers; \$800

Table. Labor costs during the project cycle



The cost of labor for the construction of a dam is a crucial consideration in the overall cost of the project. To ensure the success of the construction, a sufficient number of engineers and foremen will be employed to oversee and guide the workers. Once the dam is completed, regular maintenance will be essential for its continued functionality. This involves employing a team of engineers, foremen, workers, and support workers to carry out the necessary inspections, repairs, and replacements.

On the other side, after the construction of the dam, regular maintenance will take place. During this period, the following number of individuals will be employed, which may be increased depending on the circumstances.

- 10 engineers,
- 20 foremen,
- 100 workers,
- 45 support workers

The yearly labor cost for regular maintenance is estimated to be \$850,000, and the total cost for the entire project is estimated to be \$37.4 million over 43 years.

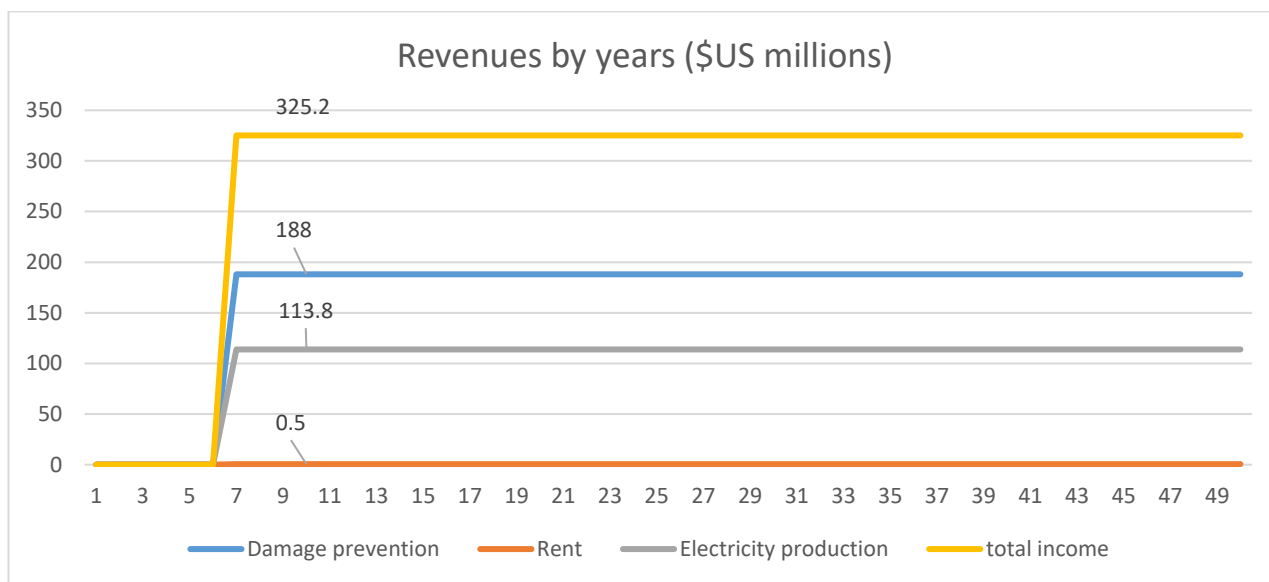
The total labor cost for the entire project is estimated to be \$207.8 million.

**Revenues of the Dam**

By constructing a dam, several kinds of revenues can be generated according to similar projects that have already

been completed. This analysis is going to be divided into two groups based on the main benefits: direct revenues and indirect gains. The direct revenues are generated by electricity revenues rents, and tourism while the indirect revenues are generated as a result of increased agricultural production and damage prevention.

Table. Revenues by years



**Electricity revenues**

The plan to develop a hydroelectric power plant in the dam involves the installation of two turbines with a capacity of 360 megawatts (MW). This ambitious project aims to capitalize on the vast water resources available in the dam and harness them for electricity generation. By harnessing the power of water, the plant will not only ensure a reliable power supply but also contribute to reducing carbon emissions.

Hydropower is currently the largest renewable power generation source, accounting for a significant portion of the US energy mix in 2021. With its significant potential to reduce carbon emissions, hydroelectric power continues to be an attractive option for sustainable energy development. One of the key advantages of hydroelectric power is its low cost compared to other power generation technologies. Research has shown that hydropower offers some of the lowest-cost electricity available. This affordability makes it an attractive choice for both developed and developing countries, particularly in the context of energy security and affordability concerns (Musa et al., 2023).

In addition to cost-effectiveness, hydropower generates electricity without burning fossil fuels, making it a greener alternative to traditional power sources. By displacing other forms of power generation, hydroelectric power can contribute to reducing greenhouse gas emissions and mitigating the impacts of climate change. It is important to

note that all power plants have capacity factors, which vary depending on resources, technology, and purpose. While the average capacity factor for hydroelectric power plants around the world is 44%, it is important to note that this value can vary depending on factors such as the availability and intensity of water flow, as well as maintenance and operational efficiency. (Kumar et al., 2018).

The dam will be able to produce 182648 kWh of electricity within the hour and 4383562 kWh per day by setting up the 02 hydropower turbines. This capability will enable the dam to meet the electricity demand of approximately 2.5 million residents in Pakistan.

The dam is expected to generate some 1.6 billion kWh of clean electricity per year. This substantial amount will contribute to meeting the country's growing demand for electricity. By generating electricity through hydropower, the dam will provide stable and cheap energy to the country.

The cost of 1 kWh of electricity in Pakistan is currently around 10.06 Pak Rupees under 101 - 200 Units, dated in Jan 2024. This is equivalent to \$0.036 per kWh. This pricing structure indicates the potential revenue generated by the electricity generated by the dam.

Once the dam is completed, its revenue is expected to amount to about \$57.3 million per year. In Pak Rupees, this translates to approximately 16.2 billion Pak Rupees per year. This revenue generation will contribute to the financial stability and sustainability of the project.

### **Creating Local Employment Opportunities**

The construction of the Hydropower dam, a highly anticipated project, is expected to have a significant impact on the local job market. It is estimated that the dam will generate approximately 2000 local job opportunities on an annual basis. These jobs will not only provide employment but also contribute to the economic development of the region.

### **Tourism Development**

Many dams have become popular tourist attractions, attracting visitors for their scenic beauty and recreational options. The revenues generated from tourism activities, such as guided tours, recreational facilities, eco-park, and visitor fees, can be a significant source of income for the dam's owner. Dams can provide opportunities for fishing and recreation, attracting anglers and other outdoor enthusiasts. The revenues generated from these activities, such as fishing licenses and facility rentals, can further enhance the economic impact of the dam. The dam is expected to have an economic impact on the country's GDP of 0.075 percent.

### **Rent**

It is assumed that the government will receive an income of \$0.5 million from the rental of its properties. Considering the recreational effects of the reservoir of the dam, it is likely that tourists will be attracted to it. It is anticipated that a variety of fisheries will be available in the area, as well as tourism facilities such as hotels, restaurants, and boat tours that may be leased out to private companies by the nature of the area. Receiving rent revenues will be possible after the sixth year.

### **Damage Prevention and Increased Agricultural Production.**

Pakistan, being a developing country, relies heavily on agriculture for its economic growth and sustenance. However, the agriculture sector faces numerous challenges, one of which is the negative impact caused by floods. Floods pose a significant threat to both human lives and agricultural infrastructure, resulting in significant losses and disruptions to agricultural activities.

Over the years, floods have inflicted significant damage on Pakistan's agricultural sector. These natural calamities not only destroy crops and agricultural land but also disrupt the entire agricultural value chain. For example, as a result of the Pakistan floods of 2010, nearly 17 million acres of cropland were flooded. Pakistan's economy and crops were severely affected by the floods of 2010. The floods affected sugarcane crops for approximately \$600 million; rice crops of \$247 million; maize crops of \$259 million; wheat stocks of \$200 million; and fruit crops in the amount of \$518,000.

(Aon plc, 2010). A total of 2.9 million households were damaged, of which 1.9 were severely affected or completely destroyed, and 80% of food reserves were destroyed (Kirsch et al., 2012). To mitigate the adverse effects of floods and ensure sustainable water management practices, the construction of dams has become indispensable. Dams serve as vital infrastructure for water storage, regulation, and distribution. By capturing and storing excess rainwater, dams provide a consistent water supply throughout the year, enabling farmers to cultivate their fields even during dry spells. Agriculture is Pakistan's core industry, accounting for 25% of the country's GDP and 60% of its export earnings. Additionally, the industry employs about 60% of the population (Ismail & Malikh, 2020).

The implementation of dams has revolutionized Pakistan's agricultural sector in several ways. Firstly, dams enable farmers to cultivate crops throughout the year, leading to increased productivity and food security. By providing a reliable source of water for irrigation, farmers can cultivate off-season crops, diversifying their agricultural portfolios and enhancing the production cycle. Secondly, dams help mitigate the negative impacts of floods. By storing excess water during flood seasons, dams act as a buffer, preventing excessive flooding and ensuring the stability and sustainability of water resources for agriculture. This not only safeguards crops but also protects agricultural lands, infrastructure, and human lives. Furthermore, The dam will provide employment opportunities in the agricultural sector. The construction and maintenance of the dam will increase the country's economy contribute to overall development and contribute decent input to the country's economy.

### **Flood Damage in Pakistan: Economic Losses and Reconstruction Needs**

Pakistan is a country prone to devastating floods, which cause significant economic losses and impact the country's GDP. Floods in Pakistan cause losses of about 10 billion dollars each year. These floods have a detrimental impact on various sectors, including agriculture, infrastructure, and public services. The average annual flood damage from 1960 -2011 has been estimated to be in the range of 1-5% of the mean annual GDP. However, the 2010 flood stands out as the most severe in terms of economic costs, exceeding \$10 billion. This flood caused immense destruction, affecting millions of people and causing major disruptions to economic activities(The Asian Development Bank, 2013)

The Ministry of Planning Development & Special Initiatives Report Pakistan Flood 2022 estimates the damage and economic losses caused by the floods in 2022 to be more than \$30 billion. The report also highlights the

need for reconstruction efforts, which will cost around \$16 billion.

To mitigate the impact of flooding, there is a great need of the construction of a dam. This dam has the potential to significantly reduce the damage caused by floods. As indicated above, floods in Pakistan result in an average annual loss of \$3.6 billion. The proposed dam is expected to reduce flood damage by 5%, which is equivalent to approximately \$188 million per year.

While the negative effects of climate change on floods are expected to increase in the future, we assume that the total amount of damage to the agriculture sector, livelihoods, communities, and infrastructure will remain constant for the next five years. However, after the sixth year of the proposed dam project, prevention will be available, offering protection to these important sectors.

**Cost Benefit Analysis**

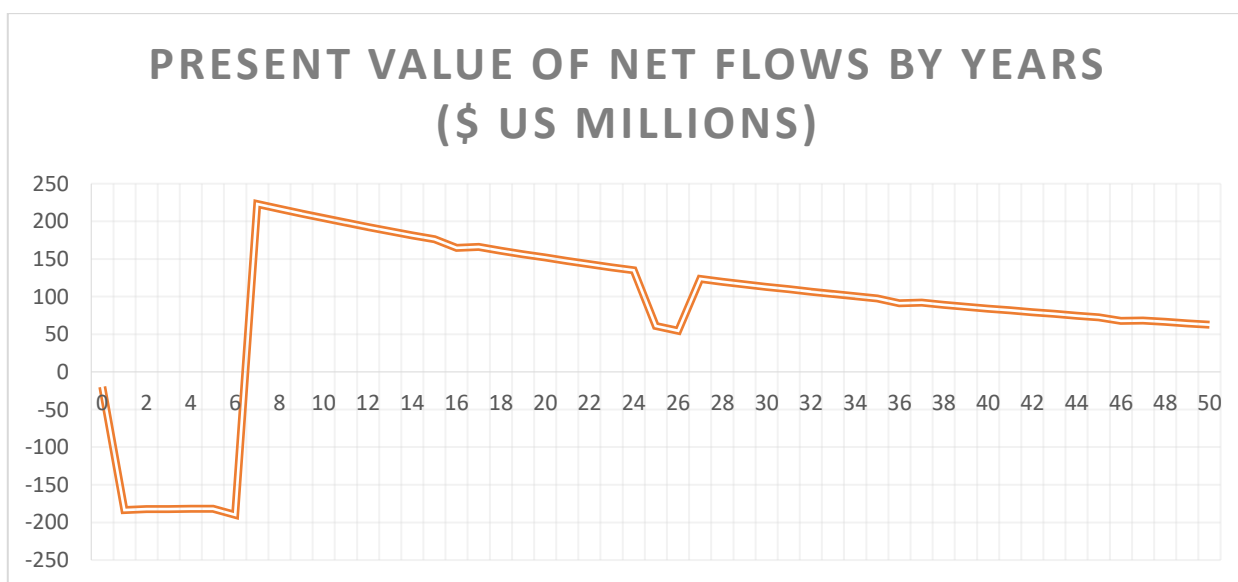
The cost-benefit analysis (CBA) is used to evaluate the benefits and costs of an intervention measured in monetary terms. This analysis is used to assess the feasibility and economic viability of the proposed dam project. By comparing the benefits generated by the proposed project with its costs, this analysis will help to determine whether constructing the dam is the best option available. The formula for calculating the cost-benefit ratio is Benefits/Costs. This ratio provides a snapshot of the financial viability and sustainability of the proposed dam project.

In the case of the proposed dam in Pakistan, the estimated life span of the project is 50 years due to the significant number of sediments carried by the river, frequent floods, and heavy rainfall. The total cost of the project is estimated to be \$3.8 billion, encompassing the construction costs, transmission lines, and a reasonable estimate of potential cost overruns.

The total revenue estimated to be generated by the dam is estimated to be \$22.5 billion. This revenue includes income from electricity, rent, tourism development, fisheries and recreation, agricultural production increase, and the prevention of damages. These sources of income provide a comprehensive picture of the impact of the dam on the local and regional economy.

The discount rate used in the analysis is 3%. This discount rate reflects the time value of money, considering the present value of future cash flows. Based on the assumptions made, the net present value of the net flow is equal to \$8.1 billion. This value represents the present value of the economic benefits generated by the project. The cost-benefit ratio for the proposed dam project is estimated to be 5.9. This means that the benefits outweigh the costs by a factor of 5.9. In other words, the construction and subsequent operation of the dam will have positive effects on the Pakistani economy. However, it is important to note that the assumptions made regarding factors like river flow, efficiency of the dam, and access to markets can significantly impact the analysis of the investment.

Table. Present value of net flows by years



**Sensitivity Analysis**

In order to assess the robustness of the analysis, conducting a sensitivity analysis is a crucial step. This paper presents two sensitivity analyses conducted on the proposed dam project.

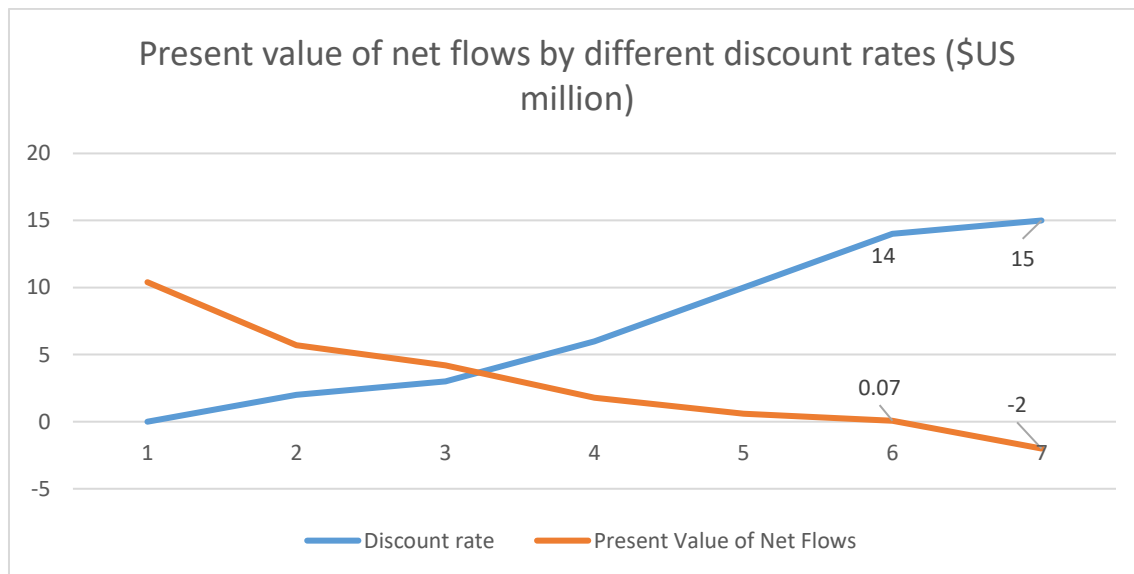
The first sensitivity analysis focuses on damage prevention. As mentioned above, the analysis foresees 5% damage prevention. However, it is crucial to explore the impact of varying this percentage. By reducing the damage prevention

rate to 2%, the impact on the cost-benefit ratio of the proposed dam can be observed.

According to the calculations, the cost-benefit ratio decreases to 2%. Despite this reduction, the present value of net flows remains positive, standing at \$2 billion. This outcome signifies that the project would still yield economic benefits even at a lower damage prevention rate.

The second sensitivity analysis examines the sensitivity of the results to the discount rate used. The calculations assume a discount rate of 3%, but it is relevant to explore how changes in this rate may affect the financial viability of the proposed dam. If the discount rate does not exceed 14%, the present value of net flows remains positive. This signifies that the proposed dam is financially viable under a range of discount rates, providing confidence in the results.

Table. Present value of net flows by different discount rates.



**IV. LIMITATIONS TO THE STUDY**

As mentioned in this paper, the cost-benefit analysis employed several assumptions. This can be attributed to several factors, one of which is a scarcity of reliable and readily available data. Additionally, the presence of policy recommendations also plays a role in the reliance on assumptions. The construction costs, regular maintenance expenses, and electricity turbine costs are all estimated using assumptions, enabling the calculation of the total cost. As determining the cost of a similar project was not feasible, revenue was estimated based on assumptions as well.

One of the challenges encountered during the analysis is obtaining accurate cost estimates, as precise information is unavailable. As a result, numerous assumptions have been made to complete the analysis. To validate these assumptions, a sensitivity analysis will be conducted to examine their impact on the overall findings.

One reason for this assumption is the inability to calculate the actual capacity factor of an electricity turbine before it has been put into operation. This factor can only be determined after the turbine has been put into operation. Similarly, predicting the impact of future floods is challenging. The amount of rainfall can vary significantly, leading to higher or lower flooding levels compared to

previous occurrences. Consequently, the impact of floods cannot be accurately estimated until it occurs.

**V. CONCLUSION**

A dam serves a variety of purposes, including storing water for irrigation, agriculture, tourism promotion, providing drinking water, and protecting against flooding. However, Pakistan, a country facing severe water scarcity, has become handicapped in terms of development and economic growth. The demand for water in Pakistan is expected to multiply five times by 2050 due to population growth. The agriculture sector heavily relies on water resources, which is decreasing at an alarming rate. The harmful effects of climate change on the country's limited water resources further exacerbate the situation. To address these issues and ensure the protection, management, and sustainability of water in Pakistan, the construction of small a dam and the implementation of water conservation measures in both rural and urban areas is crucial. These measures have the potential to alleviate water scarcity, promote agricultural development, and ensure a more sustainable future for the country (Ismail & Malik, 2020). Pakistan has made a lot of efforts to establish various flood management systems, policies and institutions, but the absence of a large dam

continues to be a pressing issue. Instead of investing heavily in manpower to establish institutions, systems, and policies, Pakistan must prioritize the construction of a large dam. This dam would serve as a significant barrier against flood waters, protecting lives and infrastructure from the devastation caused by floods. Additionally, it would enable the country to better manage and store excess water, preventing future floods from reaching such devastating levels. The construction of such a dam would not only alleviate the damage caused by floods but also contribute to the overall development and prosperity of the country.

The paper aims to analyze the cost-benefit analysis of building a big dam to mitigate floods, promote tourism, boost agriculture, and generate electricity. The cost-benefit analysis conducted in the paper has demonstrated the significant benefits of constructing such a dam. Firstly, it will provide significant relief to Pakistan's agriculture sector by reducing the negative effects of floods. By effectively managing floodwaters, the dam will help safeguard crops and prevent significant financial losses caused by crop destruction. Furthermore, the proposed dam can bring significant economic benefits for both the government and communities nearby. From travel and tourism opportunities to revenue generation through tourism taxes, fishery licenses, eco-friendly parks, guest houses, and recreation activities, the construction of this dam offers opportunities for sustainable growth and economic progress. Pakistan is a country with a rich cultural heritage and a diverse range of attractions for visitors. However, despite its potential, the travel and tourism sector in Pakistan is not meeting its full potential. The travel and tourism sector can play a vital role in Pakistan's economy. It can contribute to more job creation, foreign exchange earnings, and infrastructure development and can serve as a recreational destination, attracting visitors and promoting tourism-related activities. This could provide an additional source of revenue for the government and help boost the local economy.

Moreover, the project offers an opportunity to harness clean energy for Pakistan. By utilizing the power generated by the dam, the government can significantly reduce its reliance on external sources of power generation and promote sustainability. This will not only benefit the country economically but also contribute to the fight against climate change.

The paper's findings clearly indicate that building a big dam is a highly beneficial project for Pakistan. The cost-benefit analysis clearly demonstrates the numerous benefits of constructing a dam. By mitigating flood damage, preventing human losses and damages, boosting agriculture, and promoting clean energy generation and the tourism sector, the dam provides significant contributions to the

government and society as a whole. It is an investment that not only addresses immediate challenges but also lays the foundation for a sustainable future. Moreover, it has the potential to create a positive impact on the tourism sector. Given the aforementioned advantages, this paper strongly recommends Pakistan Government consider constructing such a dam.

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# In Vitro Efficacy of Entomopathogenic Nematodes (EPNS) against Economically Important Insect-Pests of Cauliflower

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**Abstract**— Cabbage butterfly, *Pieris brassicae* (Linnaeus), Tobacco caterpillar, *Spodoptera litura* (Fabricius) and *Plusia orichalcea* (Fabricius) causes considerable yield loss in economically important crops such as cabbage, cauliflower, cotton, tobacco, castor, and pulses etc. The nation has been using more pesticides to combat these insects, which has increased environmental pollution, pesticide resistance, pest resurgence, and residue in food, soil, and water. The present study was assessed to susceptibility of *P. brassicae*, *S. litura* and *P. orichalcea* to entomopathogenic nematodes (EPNs), *Metarhabditis amsactae* and mass multiplication of infective juveniles (IJs) in all three insects. Two strains, HAR-St-II and HAR-Ht-III of *M. amsactae* were tested against all three insects, at four inoculum levels i.e. 5, 10, 20 and 40 IJs/insect larva, under laboratory conditions at Department of Nematology, CCS Haryana Agricultural University, Hisar during 2021-2022. Results revealed that in both the strains of *M. amsactae*, as the observation time and level of IJs increased, there was a significant increase in per cent mortality of all three insects. Observation on recovery of *M. amsactae* was less from cadaver of *P. orichalcea* than *P. brassicae* and *S. litura*.

**Keywords**— *Metarhabditis amsactae*, *Spodoptera litura*, *Pieris brassicae*, *Plusia orichalcea*, inoculum level, strain, mortality



## I. INTRODUCTION

Entomopathogenic nematodes (EPNs) are those nematodes that kill insects within 24 to 72 hours by inducing septicemia. So, these are important component of biological control of several insect- pests in agricultural crops. They are members of genus *Steinernema*, *Heterorhabditis* and *Rhabditis* (*Oscheius*) comes under the families Steinernematidae, Heterorhabditidae and Rhabditidae. These nematodes have a symbiotic association with insect pathogenic bacteria belonging to the genera *Photorhabdus*, *Xenorhabdus* and *Serratia* respectively. The third stage juveniles called as infective juveniles (IJs) are stunted, non-feeding, and possess characteristics of both predators and insect parasitoids. They have strong tendency to find

their host (insect) and enter its body cavity mostly through places with weak cuticles or natural body apertures. Temperature affects the life cycle and development of EPNs, which varies between species and strains and lasts from the time IJs enter the host until new IJs appear (Hazir *et al.*, 2001). In *Galleria mellonella*, it typically takes 6–18 days at a temperature of 18–28 °C (Nguyen and Smart, 1992).

Over the past few decades, the use of nematodes as biological pest control agents has grown considerably due to their excellent virulence, high reproduction rate, and long-lasting benefits without destroying non-targets organisms (Georgis *et al.*, 1991). EPNs are effective and favored biological control agents because of their wide

range of insect hosts, superior seeking capabilities, ease of production in large quantities and application, quick host death, and safety for plants, animals, and other morphological, physiological, and behavioral abnormalities, (Gaugler, 1988; Laznik and Trdan, 2012). When it comes to controlling soil-dwelling insects and plant-boring insects, EPNs are not in conflict with other biocontrol agents. Because they are suited to the local soil and atmospheric conditions, native nematodes are thought to be superior for local biological control efforts. It is crucial to look for regional strains of EPNs, and scientists from different nations are still identifying regional strains from national surveys (Hazir et al., 2003; Malan et al., 2006; Noosidum et al., 2010). It is suggested that novel biological control agents be created using these native nematodes (Ehlers, 2005; Lewis et al., 2006).

## II. MATERIALS AND METHODOLOGY

**Culture of EPNs:** *Metarhabditis amsactae* strain HAR-St-II and HAR-Ht-III were mass cultured under laboratory conditions and pure culture of individual strain was maintained on late instar larvae of *Galleria mellonella*

(Plate 1-a and b). These strains were multiplied using the method (Plate 1-c) of Woodring and Kaya (1988). Nematodes were extracted with white traps and stored in a thin layer of distilled water in tissue culture flasks (Plate 1-d) which were kept in BOD at 16 °C that served as inoculum for further experiments.

**Rearing of insects:** First and second instar larvae and eggmasses of Tobacco caterpillar, *S. litura* along with leaves were collected from the castor crop, Department of Genetics and Plant Breeding. Larvae of semilooper, *P. orichalcea* and cabbage butterfly, *P. brassicae* were collected from the cucumber and cauliflower crop, Department of Nematology. These larvae were reared on cucumber and castor leaves in glass jars under laboratory at room temperature in month of August-September at Department of Nematology (Plate 1-e). Fresh leaves of cucumber and castor were provided to larvae daily in hygienic conditions. The glass jars were covered with muslin cloth and larvae were carefully handled. Sufficient larvae (fourth instar) of cabbage butterfly, *P. brassicae* were available in field area, Department of Nematology, were collected. These larvae were used for check the pathogenicity of both strains of *M. amsactae*.

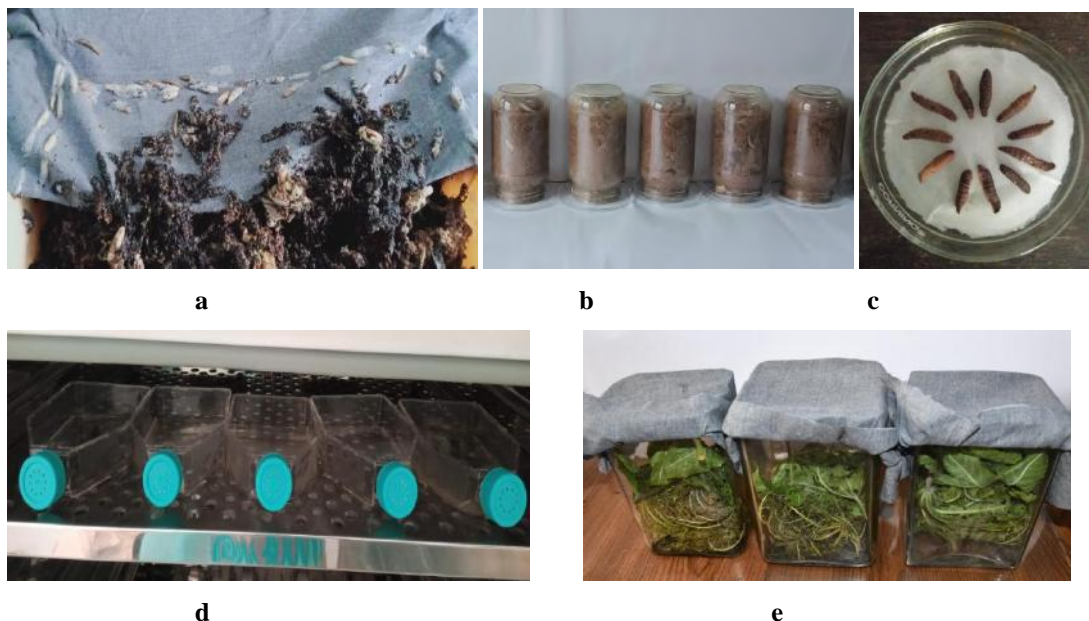


Plate 1. Larvae of *Galleria mellonella* cultured in wax comb (a), white trap method (b), soil baiting technique (c), EPNs stored in tissue culture flask (d) and rearing of *Spodoptera litura* larvae (e).

### Efficacy of EPNs against insect- pests of cauliflower:

The present study was conducted in Petri plates under laboratory condition. The isolated strains of EPNs were tested for virulence on *S. litura*, *P. orichalcea* and *P. brassicae*. To test the pathogenicity of *M. amsactae* on these insects, filter paper was spread into Petri plates and 10 fourth instar larvae of the test insect per Petri plate were

released. The EPN suspension consisting of IJs, stored in tissue culture flasks was diluted with a known quantity of sterile distilled water for making the suspension to get the required number of IJs. Insect larva, inoculated with inoculums levels @ 5, 10, 20 and 40 IJs per larvae in 5 ml distilled water. Distilled water without nematodes were taken as untreated check. Each treatment was replicated

four times and kept at room temperature. Observations on the mortality of larvae were recorded at 24 hours interval up to 4 days after inoculation and the per cent mortality was calculated.

**Mortality (%)** = Number of dead larvae x 100 / Total number of larvae

The recovery of EPNs were recorded from the dead larvae of test insects. EPNs infected dead larvae of *P. brassicae*, *P. orichalcea* and *S. litura* were removed from the Petri plate and kept on white trap for the emergence of EPNs from the body of insect larvae. IJs were collected at three days intervals, up to 21 days, till the emergence of IJs were stopped. From this collection, the total emerged populations of EPNs were counted thrice under a microscope and mean values were worked out.

### Statistical Analysis

The statistical analysis of data obtained in experiments was done based on completely randomized design using OPSTAT software available online at CCS HAU website ([www.hau.ernet.in](http://www.hau.ernet.in)). Comparison of treatments was made

at 5 % level of significance. Necessary transformation of data was done as per requirement.

### III. RESULTS AND DISCUSSION

Two selected isolates of *Metarhabditis amsactae* viz., HAR-St-II and HAR-Ht-III were tested in lab for their virulence in causing larval mortality of *P. brassicae*, *P. orichalcea* and *S. litura*. Maximum and significantly higher mean mortality of *P. brassicae* was observed at 40 IJs of strain HAR-Ht-III followed by 20, 10 and 5 IJs resulting in a mortality of insect larvae i.e. 89.2, 81.1, 69.9 and 38.1 per cent, respectively (Table -1). Irrespective of inoculum level, maximum mean mortality of *P. brassicae* larvae was 69.9 per cent on 4<sup>th</sup> day which was 37.0 per cent on 1<sup>st</sup> day. Nearly 90.0 per cent mortality was caused by 10 IJs on 4<sup>th</sup> day and by 40 IJs on 2<sup>nd</sup> day. On 4<sup>th</sup> day, 20 and 40 IJs caused 97.3 and 99.7 per cent death of *P. brassicae* larvae which were statistically at par. Per cent mortality on 4<sup>th</sup> day at 10 IJs were significantly higher than at 5 IJs but significantly lower than 20 and 40 IJs.

Table 1. *In vitro* per cent larval mortality of *Pieris brassicae* caused by *Metarhabditis amsactae*

Treatments (IJs/Petri plate)	Strain HAR-Ht-III at different time intervals					Strain HAR-St-II at different time intervals				
	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	3 <sup>rd</sup> Day	4 <sup>th</sup> Day	Mean	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	3 <sup>rd</sup> Day	4 <sup>th</sup> Day	Mean
<b>5 IJs</b>	12.5 (20.4)	30.0 (33.0)	47.5 (43.5)	62.5 (52.3)	38.1 (37.3)	27.5 (31.5)	35 (36.2)	52.5 (46.4)	72.5 (58.6)	46.8 (43.2)
<b>10 IJs</b>	42.5 (40.5)	62.5 (52.2)	85.0 (67.4)	89.9 (73.3)	69.9 (58.4)	37.5 (37.7)	52.5 (46.4)	77.5 (62.1)	87.4 (71.4)	63.7 (54.4)
<b>20 IJs</b>	57.5 (49.3)	77.5 (61.7)	92.3 (77.2)	97.3 (83.2)	81.1 (67.9)	62.5 (52.3)	77.5 (62.1)	89.9 (73.4)	97.3 (83.2)	81.8 (67.7)
<b>40 IJs</b>	72.5 (58.4)	89.9 (73.3)	94.8 (79.3)	99.7 (87.0)	89.2 (74.5)	72.5 (58.4)	85 (67.5)	94.8 (79.3)	99.7 (87.1)	88.0 (73.1)
<b>Untreated check</b>	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
<b>Mean</b>	37.0 (34.3)	52.0 (44.6)	64.0 (54.0)	69.9 (59.7)	-	40.0 (36.6)	50.0 (43.0)	63.0 (52.8)	71.4 (60.6)	-
<b>C. D. at 5 %</b>	treatment:(4.1), time: (3.7), treatment x time: (8.4)					treatment: (3.9), time: (3.4), treatment x time: (7.8)				

Values in parentheses are angular transformations

As the observation time and level of IJs increased in both strain of *M. amsactae*, HAR-Ht-III and HAR-St-II, there was a significant increase in per cent mortality of *P. brassicae* larvae. In strain HAR-St-II, at minimum inoculum level of 5 IJs/insect larva, mean mortality of 46.8 per cent was recorded followed by 63.7 per cent at 10 IJs, 81.8 per cent at 20 IJs and 88.0 per cent mortality at 40 IJs

per larva of *P. brassicae*. Highest inoculum level of 40 IJs per larva resulted in 72.5 per cent death of *P. brassicae* larvae on 1<sup>st</sup> day. Similarly, at the lowest inoculum level of 5 IJs, a maximum of 72.5 per cent mortality were recorded on 4<sup>th</sup> day of inoculation. At inoculum level of 20 and 40 IJs/insect larva could result in causing highest mortality upto an extent of 97.3 to 99.7 per cent on 4<sup>th</sup> day, which

were statistically at par. Larval mortality recorded on 2<sup>nd</sup> day, at 10 and 20 IJs /insect larva differed significantly from each other. Yadav and Lalramliana (2012) showed that susceptibility of *Athalia lugens proxima* varied in response to the infection caused by *Steinernema glaseri*, *S. thermophilum* and *Heterorhabditis indica*. The difference in the pathogenicity level may be due to different insect pest species. Differences in the susceptibility among insect life-cycle stages have also been observed in the family Pyralidae, with the pupae being less susceptible than the larvae. Similar study was conducted by Walia et al. (2006) and reported that *S. pakistanense* @ 50 IJs per larva caused 100 per cent mortality of *Agrotis ipsilon* and *P. brassicae* after 48 h while it was only 75 per cent in *Helicoverpa armigera* even after 72 h.

Data in Table 2 show that maximum (97.3 %) larval mortality of *P. orichalcea* was recorded on 4<sup>th</sup> day, at 40 IJs of strain HAR-Ht-III. It was followed by 20, 10 and 5 IJs resulting in mortality of insect larvae of 97.3, 89.9, 82.5 and 72.5 per cent, respectively. On 4<sup>th</sup> day, 72.5 per cent larval mortality of *P. orichalcea* was obtained at 5 IJs. Similarly, on 2<sup>nd</sup> day, 72.5 per cent mortality was observed at inoculum level of 40 IJs/ insect larva. As the period of observation increased in both strains, larval mortality increased significantly. When comparing effect of observation timing irrespective of IJs levels, mean mortality of *P. orichalcea* larvae was maximum (68.5 %) on 4<sup>th</sup> day and minimum (36.5 %) on 1<sup>st</sup> day.

Table 2. *In vitro* per cent larval mortality of *Plusia orichalcea* caused by *Metarhabditis amsactae*

Treatments (IJs/Petri plate)	Strain HAR-Ht-III at different time intervals					Strain HAR-St-II at different time intervals				
	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	3 <sup>rd</sup> Day	4 <sup>th</sup> Day	Mean	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	3 <sup>rd</sup> Day	4 <sup>th</sup> Day	Mean
<b>5 IJs</b>	22.5 (27.7)	42.5 (40.7)	60.0 (50.8)	72.5 (58.4)	49.3 (44.4)	17.6 (22.2)	32.5 (34.5)	47.5 (43.5)	60.0 (50.8)	39.4 (37.7)
<b>10 IJs</b>	45.0 (42.1)	55.0 (47.9)	70.0 (56.8)	82.5 (65.4)	63.1 (53.0)	35.0 (36.2)	52.5 (46.4)	72.5 (58.9)	82.5 (65.4)	60.6 (51.7)
<b>20 IJs</b>	52.5 (46.4)	67.5 (55.3)	77.5 (61.7)	89.9 (73.4)	71.8 (59.2)	47.5 (43.5)	67.5 (55.4)	82.5 (65.4)	94.9 (79.3)	73.1 (60.9)
<b>40 IJs</b>	62.5 (52.3)	72.5 (58.4)	85.0 (67.5)	97.3 (83.2)	79.3 (65.3)	62.5 (52.2)	75.0 (60.0)	89.9 (73.3)	97.3 (83.2)	81.2 (67.2)
<b>Untreated check</b>	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
<b>Mean</b>	36.5 (34.3)	47.5 (41.0)	58.5 (47.9)	68.5 (56.7)	-	32.6 (31.4)	45.6 (39.8)	58.5 (48.8)	67.0 (56.3)	-
<b>C. D. at 5 %</b>	treatment: (3.1), time: (2.8), treatment x time: (6.2)					treatment: (4.1), time: (3.8), treatment x time: (8.3)				

Values in parentheses are angular transformations

Results on effect of strain HAR-St-II showed that as the level of IJs increased, there was significant increase in mean per cent mortality of *P. orichalcea*. It was 39.4, 60.6, 73.1 and 81.2 per cent at 05, 10, 20 and 40 IJs, respectively. At inoculum level of 5 IJs per insect larva, 47.5 per cent mortality was recorded on 3<sup>rd</sup> day whereas same larval mortality was obtained on 1<sup>st</sup> day at 20 inoculum level. On 4<sup>th</sup> day, 20 and 40 IJs caused 94.9 and 97.3 per cent mortality of *P. orichalcea* larvae which were statistically at par. Mean larval mortality was significantly increased with time. Maximum mean per cent mortality of *P. orichalcea* larvae was 67.0 per cent on 4<sup>th</sup> day which was 32.6 per cent on 1<sup>st</sup> day. Gulcu et al. (2014) found that

five native EPN strains caused 100 per cent mortality of *Spodoptera ciliun* larvae. Abbas et al. (2021) were also revealed that *H. bacteriophora* and *S. glaseri* at 1500 IJ/ml concentration resulted 100 per cent mortality of all larval instars of *P. brassicae* after 48 h, under *in vitro* conditions. Kalia et al. (2014) who reported that after 36 h treatment, *G. mellonella* (LC<sub>50</sub> = 16.28 IJ/larva) was found to be more susceptible than *S. litura* (LC<sub>50</sub> = 85 IJ/larva), whereas neither host was found to be significantly different from *H. armigera* (LC<sub>50</sub> = 54.68 IJ/larva). In addition to virulence to the larval stages, ovicidal activity up to 84 per cent was observed at 200 IJ/50 and 100 eggs of *H. armigera* and *S. litura*, respectively.

Table 3. In vitro per cent larval mortality of *Spodoptera litura* caused by *Metarhabsditis amsactae*

Treatments (IJs/Petri plate)	Strain HAR-Ht-III at different time intervals					Strain HAR-St-II at different time intervals				
	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	3 <sup>rd</sup> Day	4 <sup>th</sup> Day	Mean	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	3 <sup>rd</sup> Day	4 <sup>th</sup> Day	Mean
5 IJs	10.1 (16.6)	32.5 (34.7)	55.0 (47.9)	75.0 (60.1)	43.1 (39.8)	17.6 (22.3)	27.5 (31.0)	50.0 (44.9)	62.5 (52.5)	39.4 (37.7)
10 IJs	42.5 (40.6)	72.5 (58.4)	80.0 (63.8)	87.4 (71.4)	70.6 (58.5)	52.5 (46.4)	62.5 (52.3)	84.9 (69.3)	87.4 (71.4)	71.8 (59.8)
20 IJs	57.5 (49.3)	75.0 (60.1)	89.9 (73.4)	94.9 (79.3)	79.3 (65.5)	67.5 (55.3)	82.5 (65.4)	92.4 (75.4)	94.9 (79.3)	84.3 (68.9)
40 IJs	77.5 (61.7)	89.9 (73.4)	97.3 (83.2)	99.8 (87.1)	91.1 (76.4)	77.5 (61.7)	92.4 (77.3)	97.3 (83.2)	99.8 (87.1)	91.7 (77.3)
Untreated check	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)	0 (2.9)
Mean	37.6 (34.2)	54.0 (45.9)	64.5 (54.2)	71.5 (60.1)	-	43.1 (37.7)	53.0 (45.8)	65.0 (55.2)	69.0 (58.6)	-
C.D. at 5 %	treatment:(4.2), time: (3.8), treatment x time: (8.4)					treatment: (5.2), time: (4.4), treatment x time : (10.4)				

Values in parentheses are angular transformations

It can be inferred from data in Table 3 that significant higher mean per cent mortality (91.1 %) of *S. litura* larvae at 40 inoculum level of strain HAR-Ht-III, followed by 20, 10 and 05 inoculum level, respectively. As the period of observation increased, mean per cent mortality of *S. litura*, 37.6 per cent on 1<sup>st</sup> day significantly increased to 71.5 per cent on 4<sup>th</sup> day. Nearly 92.0 per cent mortality of *S. litura* larvae was achieved on 3<sup>rd</sup> day at 20 inoculum level which was similar to mortality obtained on 2<sup>nd</sup> day at 40 inoculum level. At inoculum level of 40 IJs, mortality of *S. litura* larvae was 97.3 and 99.8 per cent which were statistically at par. There was no mortality of *S. litura* larvae in untreated check. Per cent mortality on 4<sup>th</sup> day at 10 IJs were significantly higher than at 5 IJs but statistically at par with inoculum level of 20 IJs. Maximum and significantly higher mean per cent mortality was observed at 40 IJs followed by 20, 10 and 05 IJs resulted in mortality of *S. litura* larvae i.e. 91.7, 84.3, 71.8 and 39.4 per cent, respectively.

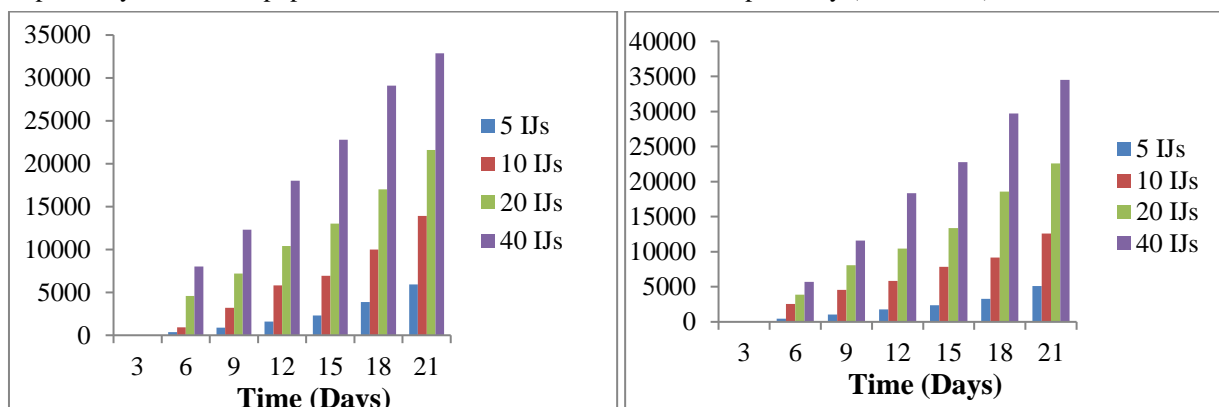
Irrespective of inoculum level of strain HAR-St-II, maximum mean per cent mortality of *S. litura* was 69.0 per cent on 4<sup>th</sup> day which was 43.1 per cent on 1<sup>st</sup> day. On

2<sup>nd</sup> day, 92.5 per cent mortality of *S. litura* larvae was observed at an inoculum level of 40 IJs per larva whereas same larval mortality was obtained on 3<sup>rd</sup> day at 20 inoculum level. On 3<sup>rd</sup> and 4<sup>th</sup> day, 10 IJs caused 84.9 and 87.4 per cent death of *S. litura* larvae which were statistically at par. On 4<sup>th</sup> day, 10, 20 and 40 IJs per insect larva, it resulted in 87.4, 94.9 and 99.8 per cent mortality of *S. litura* larvae which were also statistically at par. Sooraj *et al.* (2019) revealed among the three strains used, strain 2<sup>nd</sup> of *Metarhabsditis rainai* at inoculum level of 300 IJs showed highest mortality of *S. litura* which was 29.99 per cent and maximum emergence of IJs ( $3.5 \times 10^5$ ) at 24 h after treatment. This strain at 200 inoculum level caused 80.52 and 99.35 per cent at 60 and 72 h after treatment. Results of Thakur *et al.* (2022) showed that LC 50 values of 3<sup>rd</sup> and 4<sup>th</sup> instar of *H. armigera* were 60.14 and 57.90 IJs of *H. bacteriophora*. *S. litura* were 59.95 and 50.91 IJs/larvae and *A. segetum* were 54.86 and 57.90 IJs/larvae at inoculum level of 50, 100, 150, and 200 IJs, after 120 hours.

The data in Figure 1 (a) show that highest population of *M. amsactae* strain HAR-St-II (32852

IJs/cadaver) was recovered at 40 inoculum level on 21<sup>st</sup> day followed by 18<sup>th</sup>, 15<sup>th</sup>, 12<sup>th</sup>, 9<sup>th</sup> and 6<sup>th</sup> day which were 29118, 22808, 18000, 12306 and 8000 IJs /cadaver, respectively. Minimum population of IJs was recovered at

5 inoculum level, on 6<sup>th</sup> day which was 364 IJs/cadaver of *P. brassicae* followed by 878, 1600, 2312, 3874 and 5920 IJs/ cadaver up to 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup>, 18<sup>th</sup> and 21 day, respectively (Plate-2, left).



(a) *Metarhabditis amsactae* strain HAR-St-II

(b) *Metarhabditis amsactae* strain HAR-Ht-III

Fig.1. Recovery of IJs of *Metarhabditis amsactae* strain HAR-St-II (a) and *Metarhabditis amsactae* strain HAR-Ht-III (b) from dead larvae of *Pieris brassicae*



Plate 2. Recovery of *Metarhabditis amsactae* strains HAR-St-II (left) and HAR-Ht-III (right) on *Pieris brassicae*

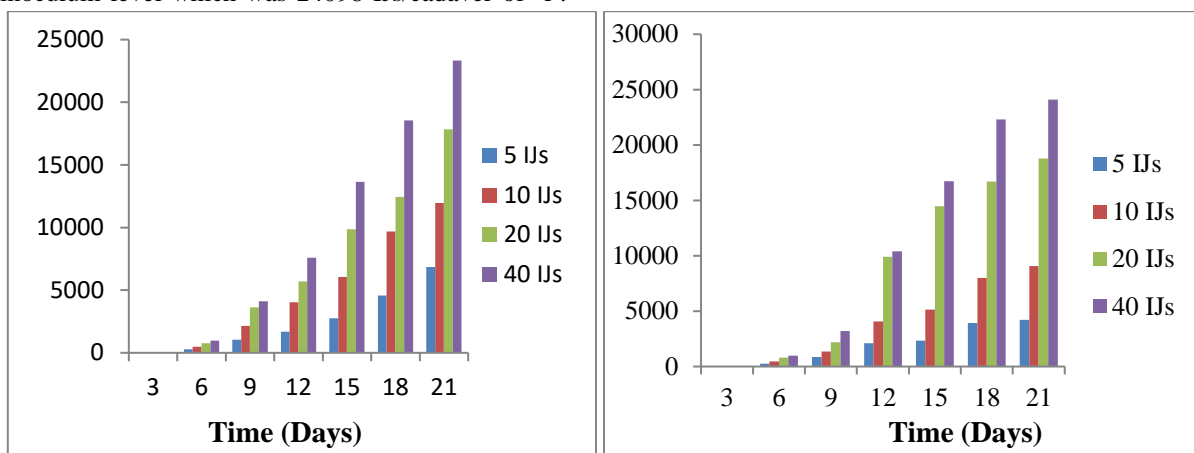
Maximum IJs of *M. amsactae*, strain HAR-Ht-III emerged from cadavers of *P. brassicae* (Plate-2, right) on 21<sup>st</sup> day which was 34516 IJs/cadaver, at 40 IJs per insect larva followed by 20, 10 and 05 inoculum levels showed in Figure 1 (b). For recovery of EPNs, (Figure-1-3), almost all *Metarhabditis amsactae* strains multiplied on the tested insects, but multiplication level varied within *M. amsactae* strains. Population of IJs increased with increase of time at all inoculum level *i.e.* 05, 10, 20 and 40 IJs/ insect larva. No recovery of EPNs were recorded on 3<sup>rd</sup> day in all strains. The present studies, revealed that recovery of IJs in both strains at 40 inoculum level was observed maximum *i.e.* 44615 IJs/cadaver in strain HAR-Ht-III and 38710 IJs/cadaver in strain HAR-St-II from dead larvae of *S. litura*. In *P. brassicae*, recovery obtained 34516 IJs/cadaver in strain HAR-Ht-III and 32852 IJs/cadaver in strain HAR-St-II up to 21<sup>st</sup> day at 40 inoculum level. In *P. orichalcea*,

recovery was 23330 IJs/cadaver in strain HAR-Ht-III and 22308 IJs/cadaver in strain HAR-St-II up to 21<sup>st</sup> day at 40 inoculum level. Pervez (2017) found that eight native EPNs were virulent on semi-looper and caused 100 per cent mortality within 72 h. Maximum multiplication of 9,324 IJs per larva was observed with *O. gingeri* (IISR-EPN 07) within 15 days, followed by 8,638 and 8,236 IJs per larva with *Oscheius* sp. (IISR-EPN 04) and *Oscheius* sp. (IISR-EPN 08) respectively.

In Figure 2 (a), as is evident less recovery of IJs (268) was observed on 6<sup>th</sup> day at 05 inoculum level. Highest yield of *M. amsactae* HAR-St- II, which was 23330 IJs/cadaver was obtained on 21<sup>st</sup> day, emerged from the body of the *P. orichalcea* at 40 inoculum level followed by 18562, 13655, 7585,4126 and 977 IJs/ cadaver of *P. orichalcea* on 18<sup>th</sup>, 15<sup>th</sup>, 12<sup>th</sup>, 9<sup>th</sup> and 6<sup>th</sup> day, respectively. Recovery of IJs of *M. amsactae* strain HAR-

Ht-III on 6<sup>th</sup> day at 5, 10, 20 and 40 inoculum level which was 265, 470, 795 and 982 IJs/cadaver showed in Figure 2 (b). Maximum population of IJs was recovered on 21<sup>st</sup> day at 40 inoculum level which was 24098 IJs/cadaver of *P.*

*orichalcea* followed by 22308, 16732, 10401, 3200 and 982 IJs/cadaver on 18<sup>th</sup>, 15<sup>th</sup>, 12<sup>th</sup>, 9<sup>th</sup> and 6<sup>th</sup> day, respectively.



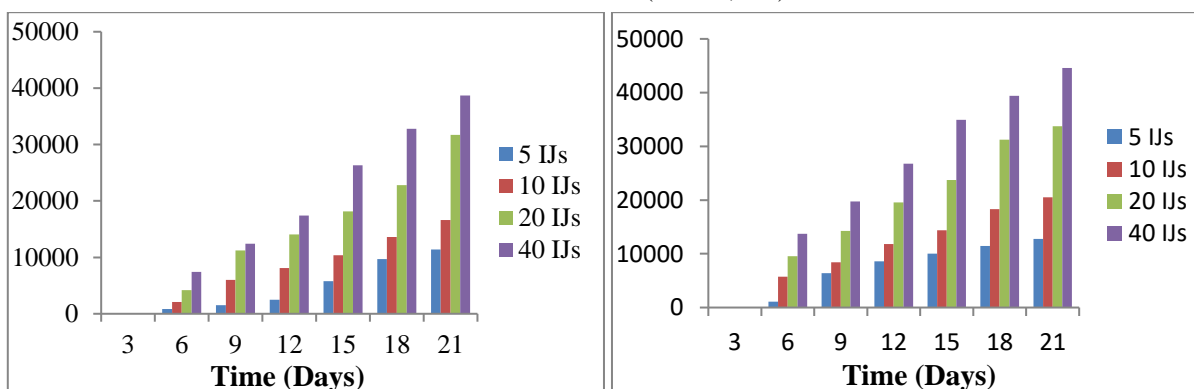
(a) *Metarhabditis amsactae* strain HAR-St- II

(b) *Metarhabditis amsactae* strain HAR-Ht-III

Fig.2. Recovery of IJs of *Metarhabditis amsactae* strain HAR-St- II (a) and HAR-Ht-III (b) from dead larva of *Plusia orichalcea*

Data presented in Figure 3 (a) clearly show that highest recovery (38,710 IJs/ cadaver) obtained from cadavers of *S. litura*, at 40 IJs inoculum level of *M. amsactae* strain HAR-St-II /larva were used. At inoculum level of 5 IJs

/larva, yield of IJs was very low i.e. 818 on 6<sup>th</sup> day followed by 1514, 2478, 5798, 9716 and 11412 IJs/larva of *S. litura* upto 9<sup>th</sup>, 12<sup>th</sup> 15<sup>th</sup>, 18<sup>th</sup> and 21 day, respectively (Plate-3, left).



(a) *Metarhabditis amsactae* strain HAR-St-II

(b) *Metarhabditis amsactae* strain HAR-Ht-III

Fig.3. Recovery of IJs of *Metarhabditis amsactae* strain HAR-St-II (a) and *Metarhabditis amsactae* strain HAR-Ht-III (b) from dead larvae of *Spodoptera litura*

Highest population of *M. amsactae* strain HAR-Ht-III (44615 IJs/cadaver) was recorded at 40 inoculum level, on 21<sup>st</sup> day followed by 18<sup>th</sup>, 15<sup>th</sup>, 12<sup>th</sup>, 9<sup>th</sup>, and 6<sup>th</sup> day which were 39408, 34960, 26800, 19765 and 13695 IJs/cadaver (Figure 3.b). Less recovery obtained at 5 inoculum level,

on 6<sup>th</sup> day which was 1092 IJs/cadaver of *S. litura* followed by 6412, 8602, 10036, 11446 and 12796 IJs/cadaver upto 9<sup>th</sup>, 12<sup>th</sup>, 15<sup>th</sup>, 18<sup>th</sup> and 21<sup>th</sup> day, respectively (Plate-3, right).



Plate. 3 Recovery of *Metarhabditis amsactae* strains HAR-St-II (left) and HAR-Ht-III (right) on *Spodoptera litura*

Similarly, Banu *et al.* (2003) suggested that EPNs multiplied well on the adult of red weevil, *Rhynchophorus ferrugineus* and highest nematode multiplication was observed in *Steinernema* sp. ( $12.01 \times 10^3$  IJs/weevil) followed by *H. indica* ( $8.99 \times 10^3$  IJs/weevil) and *S. glaseri* ( $2.4 \times 10^3$  IJs/weevil). Results of Lalramliana and Yadav (2010) showed that the progeny production of EPNs by larvae of *P. brassicae* was noted to be highest in case of *H. indica*. It was considerably low in *S. thermophilum* and *S. glaseri*. The production increased along the concentrations till the highest concentration for both *H. indica* and *S. thermophilum* but declined from 50 IJs/larva onwards in case of *S. glaseri*. In my study, *Metarhabditis* strains were found more virulent against *S. litura*, *Pieris brassicae* and *Plusia orichalcea*. Similar results were reported by Gaugler and Kaya (1990) and Ali *et al.* (2008), which stated that EPNs were considered potential biopesticides and used on IPM field of these insect pests under field conditions. The larvae of *S. litura* were more suitable host for multiplication of IJs and these insects could be selected as the alternate host for production of IJs of EPN under laboratory conditions.

#### IV. CONCLUSION

The efficacy of these local strains, HAR-St-II and HAR-Ht-III of EPNs were tested against *Pieris brassicae*, *Spodoptera litura* and *Plusia orichalcea* under laboratory condition, at four inoculum levels i.e. 05, 10, 20 and 40 IJs /insect larva. In both strains, as the observation time and level of IJs increased, there was a significant increase in per cent mortality in all the three insects. In *P. brassicae*, mean mortality at 10 IJ/larva in HAR-St-II and HAR-Ht-III was 63.7 and 69.9 per cent, respectively. In *P. orichalcea*, mean mortality at 10 IJ/larva in HAR-St-II and HAR-Ht-III was 60.6 and 63.1 per cent, respectively. In *S. litura*, mean mortality at 10 IJ/larva in HAR-St-II and HAR-Ht-III was 71.8 and 70.6 per cent, respectively. Larvae of *S. litura* and *P. brassicae*, were found to be more susceptible to EPNs than *P. orichalcea*. Although all the *Metarhabditis amsactae*

strains tested caused 100 per cent mortality at 40 inoculum level on 4<sup>th</sup> day, their infectivity levels varied at different IJs in different insect species. For recovery of EPNs, both strains of *Metarhabditis amsactae* multiplied on the tested insects, but multiplication level varied within strains. Further this experiment was continued at field level, no doubt there was comparative difference in result of laboratory experiment and on field application which was due to several environmental and edaphic factors. From this experiment we have concluded that application of EPNs is as much effective method of control of insect pest as chemical control.

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# Multi-criteria analysis of the environmental vulnerability of the cotton zone of Mali: Case of the northeast subsidiary of Koutiala

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**Abstract**— *The northeast subsidiary of Koutiala is a very ancient and important cotton production zone in Mali. Commonly called old cotton basin of Mali, this subsidiary counting ten sectors divided between two divisions, is today finds confronted to environmental problems. However, it is difficult to locate the essential reasons of this problem so much the factors are numerous. To assess the impact of different factors on environment, this study devoted itself as objective to analyze the spatiotemporal dynamics of the environmental vulnerability of the northeast subsidiary of Koutiala between 2003 and 2017. It used several types of data for this purpose (climatic, satellite, socioeconomic and demographic, geographical). The used methodology was based on the Principal Component Analysis (PCA) and the Agglomerative Hierarchical Clustering (AHC), after standardizing the data using the empirical normalization method. The study reveals that the main factors of environmental vulnerability are mainly composed of indicators of occupation of the soil (NDVI and Occupancy rate of the soil by cultures which are present in 100.0% of the factors), socioeconomic (in 83.3%), climatic (in 66.7%) and socio-demographic (in 58.3%). It also reveals that the sector of Konséguéla in the southwest (division of Koutiala) is the least vulnerable contrary to that from Kimparana to the north (division of San) which is the most vulnerable. Globally between 2003 and 2017, there is a downward trend of the environmental vulnerability of the northeast subsidiary of Koutiala.*



**Keywords**— *Multicriteria analysis, environmental vulnerability, cotton zone, Mali.*

## I. INTRODUCTION

Mali is a big cotton-producing country in Africa. During countryside 2017/2018 and 2021/2022, the country ranked first African producer with a record production of 725 000 tons of cotton seed (Maïga, 2019 ; Westerberg et al., 2020 ; WTO, 2021). The cotton is a strategical product for Mali (Soumaré et al., 2020) for reasons which follow: 1. the cotton is the main cash crop and export crop of the country contributing to about 15% in its Gross Domestic Product (GDP), but especially in 40% incomes of the rural population; 2. The cotton cultivation occupies

nearly 70% of the active population in the areas of its production (cotton zones) and gives direct and indirect incomes to more than 15 million persons; 3. finally the cotton zones are areas of production of dry cereals (millet, corn and sorghum) par excellence with a production of more than 2.1 million tons in 2017/2018 (Maïga, 2019), in particular thanks to the access to inputs and agricultural equipment facilitated by cotton cultivation, which contribute substantially to the food security of the small producers. This activity is therefore very important in Mali as well in economic terms as in security food.

The Malian cotton is produced on hundreds of thousand hectares (703 652 hectares in 2017/2018) in the cotton zone, or on about 6% of the national territories (Cissé, 2016). It extends over the southern and central regions of the country, and has five (05) subsidiaries including the northeast subsidiary of Koutiala.

However, the increasingly growing production of cotton in Mali raises questions about its environmental impacts. Indeed, since the 1980s, we have seen more and more land clearing to increase the areas cultivated with cotton and cereals, more pollution of soil and water due to the abusive use of pesticides, land degradation, climate change, etc. (Bidou et al., 2013 ; Camara, 2015 ; Ballo et al., 2016). Yet, this constitutes a major constraint for the achievement of certain strategic objectives which Mali aims to achieve in the short term (MEF/CSLP, 2019), in touch with the Objectives 1, 2, 3 and 12 of the Sustainable Development (OSD). So, the environmental vulnerability of the cotton zone of Mali is multi factor.

Studies showed that the northeast subsidiary of Koutiala of the cotton zone of Mali is confronted with several environmental problems because of the enhancement of agriculture and of population growth (Soumaré, 2008). To clear tracks of alleviation of the effect combined by the different factors of this vulnerability, it is therefore primordial to identify them and to classify them objectively in order of importance. That is why this research settles as main objective to analyze the spatiotemporal dynamics of the environmental vulnerability of the northeast cotton subsidiary zone of Koutiala from 2003 till 2017.

To achieve this objective, three essential tasks will be carried out: (1) evaluate the indicators of environmental vulnerability, (2) identify and classify the most determining vulnerability factors and finally, (3) analyze the spatial-temporal evolution of environmental vulnerability factors of the study area.

## II. PRESENTATION OF THE SITE

The study area is the northeastern subsidiary of the Malian Textile Development Company (CMDT), commonly called old cotton basin of Mali. It stretches over the southeast and the center of Mali (Soumaré et al., 2020), between 6°08' and 4°46' of west degree of longitude and, 12°16' and 12°88' of north degree of latitude, including the regions of Sikasso and of Ségou (Fig. 1). The northeast subsidiary counts ten (10) sectors divided between two (2) divisions, divisions of Koutiala and of San. The zone counts 841 villages retorted

between 77 municipalities and covers a complete area of 20585 km<sup>2</sup>. It shelters a complete population of 1 429 746 inhabitants, that is a density of 56 hbs/km<sup>2</sup>.

The agriculture is the main activity of the population, which is grouped into 2 122 peasant organizations. The cotton is the dominant speculation in the area. However, cereal cultivation (corn, millet, sorghum) and livestock also occupy a significant place.

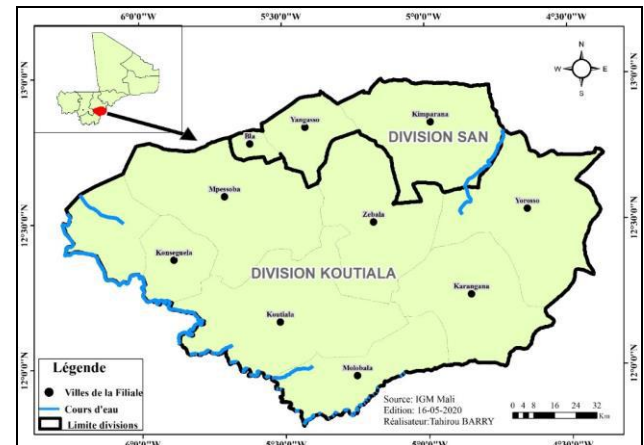


Fig.1: Location map of the study area.

The climate of the area is dry tropical characterized by the alternation of two seasons (rainy season and dry season). The annual cumulative rainfall decreases from south to north with a zonal average of up to 900 mm. For the temperature, it evolves in a bimodal regime with the maximums in April-May (main) and September-October (secondary).

The relief is not very rugged and the soils are of tropical ferruginous types. The vegetation is mainly composed of Shea "*Vitellaria paradoxa*" and Néré "*Parkia biglobosa*" (Soumaré, 2008). The area is crossed by the permanent river the « Banifing » and seasonal rivers including the « Kifa » and the « Kimparana ».

## III. MATERIALS AND METHODS

### 3.1. Data and processing tools

Several types of data were used in this research. The characteristics of these are detailed in the Table 1.

Table 1: Characteristics of the used data

Nº	Types	Variables	Spatial-temporal scales	Descriptions	Sources
1	Climatic	Rain	Sector 2003, 2007, 2012, 2017	Monthly data gathered on 8 stations in the zone	CMDT
		Temperature			
2	Satellite	NDVI image	Sector 2003, 2007, 2012, 2017	Low resolution of 250 m, MODIS Terra sensor, taken every 16 days	USGS Appears- NDVI - Modis
		Landsat 7 (ETM+) and 8 (OLI) images	Division November 2000 and October 2017	- High resolution of 30m by 30m. ETM+ and TM sensor, panchromatic band covers an area of 185 km by 185 km. - 2 sensors, Operational Land Imager (OLI) and thermal infrared. It covers an area of 170 km by 185 km.	USGS, Earth Explorer
3	Socio-economic and demographic	Population	Sector 1998 and 2009	The enrolments of the population of the different sectors	CMDT
		Agricultural statistics	Sector <b>2003, 2007, 2012,</b> 2017	Areas, production and average yields of crops (cotton, corn, millet and sorghum)	INSTAT-Mali
4	Geographical	Limits and attributes	-	These are geographical boundaries and elements (watercourses, roads and villages) in .shp format elements (watercourses, roads and villages) in .shp format	IER/CRRA

The main tools used are ENVI 4.7 for processing satellite images (Landsat 7 and 8) in order to obtain the land occupation rate, the XLSTAT software which is an extension of Microsoft Excel, for carrying out the **3.2. Methods of data analysis**

The analysis of the collected data was carried out in three successive stages.

3.2.1. Preliminary processing (or aggregation) of data and evaluation of indicators

The climatic data (rain and temperature) were processed according to practical climatological standards (WMO, 2018) in order to extract climatic indices, in this case the annual cumulative rainfall (Pan) and the average annual temperatures (Tan), which will then be used in the development of environmental vulnerability indicators.

The satellite images allowed, thanks to the methods of treatment of Remote sensing (Elhadj, 2016), for every geographical unit (sector) and study year, to assess first

Analysis in Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC), and finally ArcGIS 10.3 for producing thematic maps.

from the images of the sensor MODIS Terra minimal, medium and maximum values of the Normalized Difference Vegetation Index (NDVI). Then the rate of the cultivated complete areas of cereals and of cotton, and the rate of annual occupation of cultures by sector were estimated (Formula 1).

$$T_o = \frac{A_a}{A_t} \times 100 \quad (1)$$

where  $T_o$ ,  $A_a$  and  $A_t$  are respectively the occupancy rate of the soil by cultures, the agricultural complete area in the sector and the complete area of the sector.

Disposing only data of the population by sector of 1998 and of 2009 (years of the two last general censuses of the population and habitat (GCPH)), it was undertaken the

extrapolation of these for the study years (2003, 2007, 2012 and 2017). For this, the following population projection equation (Blanchet and Le Gallo, 2008) was used:

$$P_i = (P_o + t)^n \quad (2)$$

where  $P_i$  – population for year  $i$ ;  $P_o$  – population for the reference year (1998 or 2009);  $t$  – average annual natural increase rate of the population (the rate of regional natural increase is taken as reference rate) and  $n$  – the number of years between the years of projection and of reference.

Then, the density of the population ( $D_p$ ), that is to say the report of the population by the area for every sector, was calculated for different years.

Concerning the agricultural data, from the areas and yields per speculation (cotton, corn, millet and sorghum), the average agricultural yield (ton/ha) was evaluated in a weighted manner using Formula 3.

$$R_{moy} = \frac{\sum(As_i \times Rs_i)}{\sum As_i} \quad (3)$$

where  $R_{moy}$  is the average agricultural yield,  $As_i$  and  $Rs_i$  are the area and yield of speculation  $i$ .

Finally, agricultural production per capita ( $P_{hbt}$ ) by sector was also evaluated as the ratio of the total quantity of agricultural production ( $P_t$ ) in the sector by its population ( $P_{op}$ ) for each year of the study.

### 3.2.2. Training of vulnerability components and standardization of indicators

To form the components of environmental vulnerability by sector of the study area, the climatic, environmental, economic and social indices previously assessed, summarized in Table 2, were crossed.

Table 2: Summary of the vulnerability indicators

Nº	Components	Indicators	Units
1	Climate (Cl)	Annual accumulation of the rain ( $P_{an}$ )	mm
		Average of annual temperature ( $T_{an}$ )	°C
2	Environment (En)	NDVI <sub>Min</sub> , NDVI <sub>Moy</sub> , NDVI <sub>Max</sub>	-
		Soil occupation rate by crops ( $T_o$ )	%
3	Society (So)	Population for year $i$ ( $P_i$ )	hbts
		Population density ( $D_p$ )	hbts/km <sup>2</sup>
4	Economy (Ec)	Agricultural production ( $P_t$ )	ton
		Average agricultural yield ( $R_{moy}$ )	ton/ha
		Agricultural production per capita ( $P_{hbt}$ )	kg/hbt

The indicators of vulnerability above in the Table 2 are of natures and very different units, that is why before passing to their classification and organization into a hierarchy, all data were standardized (normalized) and aggregated. In order to bring these multivariate indicators to the same unit of magnitude and maintain an acceptable gap between them, the empirical normalization method was applied (Boulanger et al., 2004). The principle of this method is presented in formula 4 below.

$$y_i = \frac{(x_i - x_{min})}{(x_{max} - x_{min})} \quad (4)$$

where  $y_i$  are the normalized indices,  $x_i$  are the modalities of the variable  $X$ ,  $x_{max}$  and  $x_{min}$  are the minimum and maximum values of the data series.

### 3.2.3. Principal Component Analysis (PCA) and Agglomerative Hierarchical Clustering (AHC)

To the normalized and aggregated data, the PCA algorithm was applied, in order to obtain the best possible combination of variables (climatic, environmental and socio-economic/demographic). This made it possible to determine the factor axes to be retained, where each component constitutes a vulnerability criterion.

A AHC was applied to the factorial axes obtained through the PCA, using the Ward method (Von Storch and Zwiers, 1999). This made it possible to classify the sectors according to their degree of vulnerability, according to the years (2003, 2007, 2012 and 2017), and to produce vulnerability maps.

### 3.2.4. Spatial-temporal evolution of environmental vulnerability factors in the study area

At the end of the AHC, the degree of vulnerability of the different sectors of the subsidiary was assessed, for the years 2003, 2007, 2012 and 2017. It varies strongly from

a sector to other one, from one year to other one and according to the elements. The classification of sectors by level of vulnerability was made according to the weight of the variables of every indicator, varying between 0 and 3. This is how the three levels of vulnerability were defined (Low, Medium and High), with the weight intervals [0; 1], ]1 ; 2] and ]2; 3] respectively.

#### IV. RESULTS AND DISCUSSION

Three main results were obtained at the end of this study, namely the estimates of vulnerability indicators, the classes of vulnerability factors, and the spatial-temporal dynamics of the environmental vulnerability factors of the study area.

##### 4.1. Vulnerability indicators of the study area

The normalized and aggregated estimates, at the scale of the northeast Koutiala subsidiary, of vulnerability indicators are summarized in the Table 3 below.

Table 3: Standardized estimates of vulnerability indicators at the subsidiary level

№	Components	Standardized indicators	Year			
			2003	2007	2012	2017
1	Climate (Cl)	$P_{an}$	0.53	0.57	0.62	0.48
		$T_{an}$	0.41	0.39	0.45	0.45
2	Environment (En)	$NDVI_{Min}$	0.55	0.54	0.56	0.63
		$NDVI_{Moy}$	0.47	0.52	0.43	0.47
		$NDVI_{Max}$	0.34	0.47	0.49	0.58
		$T_o$	0.37	0.37	0.34	0.47
3	Society (So)	$P_i$	0.24	0.22	0.23	0.23
		$D_P$	0.52	0.50	0.44	0.44
4	Economy (Ec)	$P_t$	0.25	0.50	0.44	0.52
		$R_{moy}$	0.55	0.48	0.20	0.56
		$P_{hbt}$	0.18	0.24	0.27	0.24
Total			4,41	4,80	4.47	5.07

Individually taken, normalized estimates by some indicators of vulnerability on the scale of the subsidiary vary considerably from one year to other one. The most unstable indicator is the average return ( $R_{moy}$ ) with an amplitude of 0.36. Its lowest value was obtained for the year 2012 despite a clear increase in rain that year. As for the accumulation of the values of all the indicators at the scale of the study area, it evolves in sawtooth from 2003 to 2017.

##### 4.2. Classes of vulnerability factors of the study area

Table 4: Characteristics of the three main vulnerability factors of the study area

Year	Factor	Own value	Variability (%)	Cumulative (%)
2003	F1	4.51	37.57	37.57
	F2	2.45	20.46	58.03
	F3	1.87	15.54	73.57

Year	Factor	Own value	Variability (%)	Cumulative (%)
2007	F1	3.99	33.22	33.22
	F2	2.77	23.08	56.30
	F3	2.36	19.68	75.97
2012	F1	4.30	35.81	35.81
	F2	2.45	20.44	56.24
	F3	1.91	15.91	72.16
2017	F1	4.17	34.74	34.74
	F2	3.15	26.21	60.94
	F3	2.06	17.18	78.12

Across these results, the official report is that the three main factors (elements) have an individual weight representing more than 30% for the first, between 20 and 30% for the second, and between 15 and 20% for the third. Their combined weight is more than 70% for every

year. What is sufficient to analyze environmental vulnerability across these (Guerrien, 2003).

The details on the composition of the three vulnerability factors, based on climatic (Cl), environmental (En), social (So) and economic (Ec) indicators are given in the Table 5.

Table 5: Summary of the factors explaining vulnerability over the four years

Factors	Year							
	2003		2007		2012		2017	
	Composition	Rate (%)	Composition	Rate (%)	Composition	Rate (%)	Composition	Rate (%)
F1	So-Ec-En	37.57	So-En-Ec	33.22	En-So-Ec	35.81	So-En-Ec	34.74
F2	En-Cl-So	20.46	En-Ec-Cl	23.08	En-Cl-Ec-So	20.44	En-Cl-So	26.21
F3	En-Ec-Cl	15.54	En-Cl-Ec	16.68	En-Cl-Ec	15.91	En-Ec-Cl	17.18
Total	-	73.57	-	75.97	-	72.16	-	78.12

It is visible that the all factors of every year are made up of three indicators, except for the factor F2 of 2012 who in count four. The environmental indicators (En), namely NDVI are present in all factors (100.0%). The other indicators Ec, Cl and So are respectively present in 83.3%, 66.7% and 58.3% of the factors.

Thus, in this study, the different factors So-Ec-En, En-Cl-So and En-Ec-Cl were objectively established on the basis of the PCA, when the variance rate is high. This same principle was used by Mara (2010) in his thesis work on the vulnerability of populations in the Sirba valley in Burkina Faso.

#### 4.3. Spatial-temporal dynamics of environmental vulnerability factors in the study area

In order to understand the spatial-temporal dynamics of the vulnerability factors, maps of the study area composed of sectors were developed for the years of the study on the basis of the factors So-Ec-En, En-Cl- So and En-Ec-Cl (Fig. 2 to 5).

By observing the maps in the Fig. 2, there is an overall increase in environmental vulnerability in the subsidiary from 2003 to 2017, according to the So-En-Ec factor. Indeed, the number of sectors with a low level of vulnerability in 2003 decreased in 2017, unlike the number of sectors with medium and high levels of vulnerability.

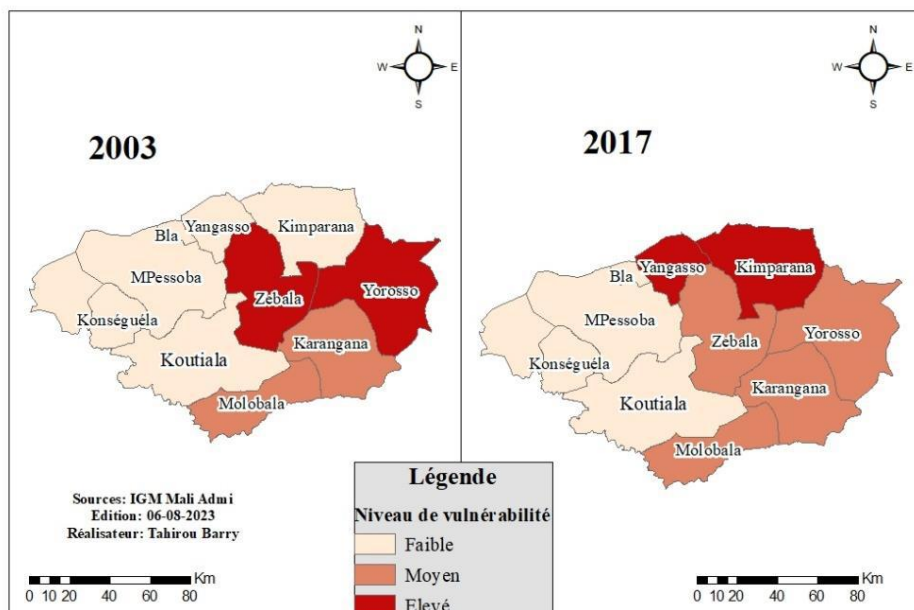


Fig.2: Spatial-temporal distribution of vulnerability according to the So-En-Ec factor.

All sectors with a medium and high level of vulnerability over the entire study period are located in the eastern half of the subsidiary. The Yangasso and Kimparana sectors saw their level of vulnerability go from low to high, while that of the Zébala and Yorosso sectors declined from high to medium.

and as many with an average level, which respectively moved to the numbers of one and three. Thus, the overall level of vulnerability in the subsidiary has fallen significantly (Fig. 3) because, with the exception of the Bla sector, all other sectors with a medium and high level of vulnerability have fallen by a notch.

According to the En-CI-So factor, the situation has on the contrary improved a lot between 2003 and 2017. In fact, there were four sectors with a high level of vulnerability

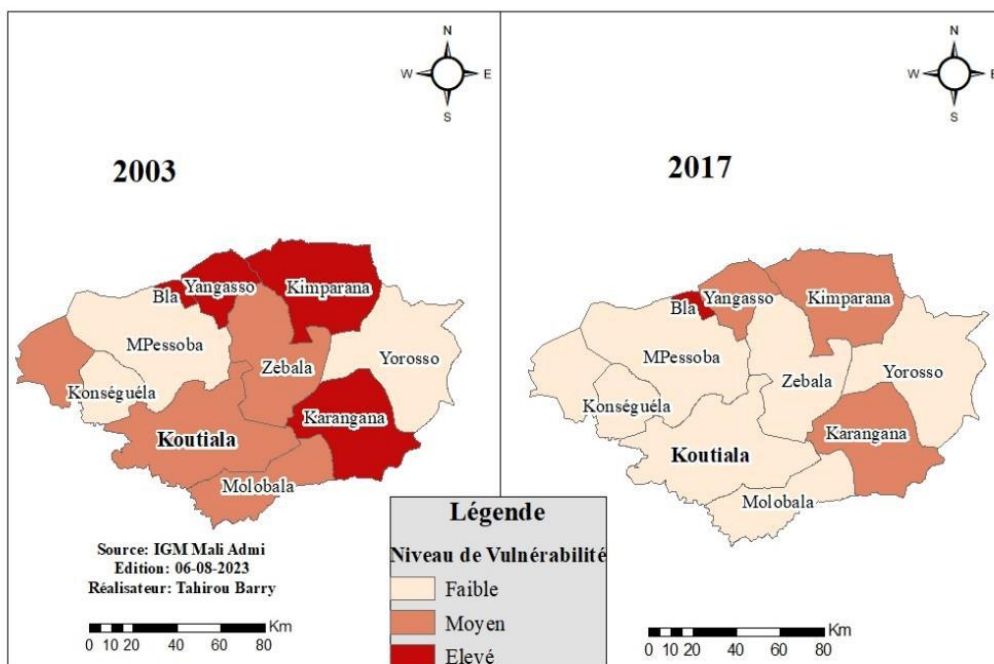


Fig.3: Spatial-temporal distribution of vulnerability according to the En-CI-So factor.



Finally, the spatial-temporal dynamics of the vulnerability in the subsidiary, according to the En-Ec-CI factor, shows a trend towards the average level because the numbers of low and high level sectors decreased at the profile of the average level (Fig. 4). In particular, the Zébala sector went from high level in 2003 to low level in 2017. The

only sectors of the subsidiary which became more vulnerable in 2017 compared to 2003, are that of M'Péssoba (from medium level to high level) and that of Karangana (from low level to high level).

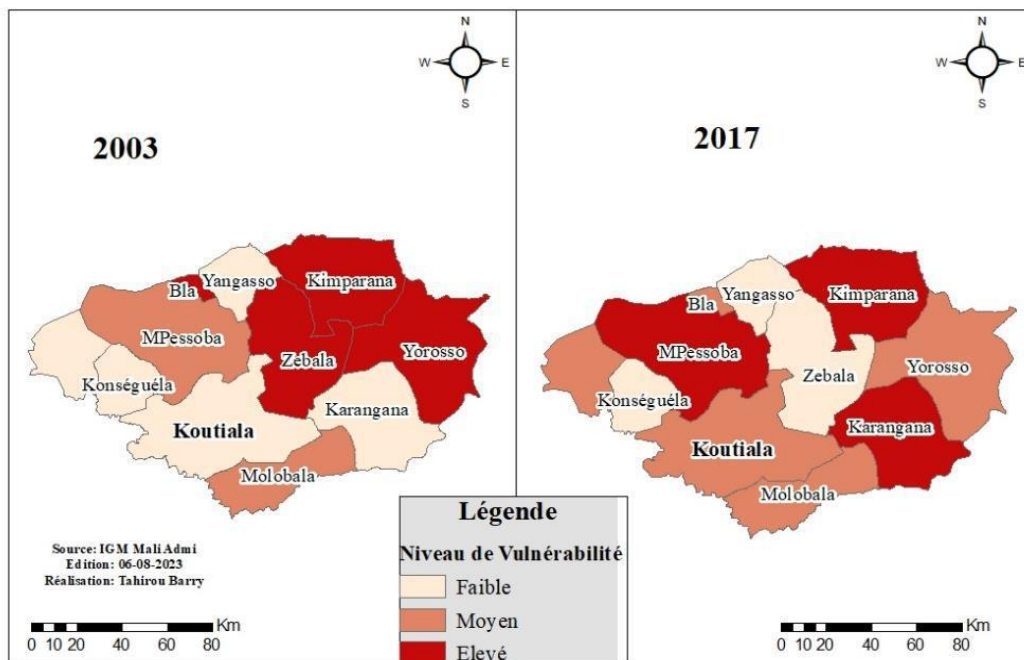


Fig. 4: Spatial-temporal distribution of vulnerability according to the En-Ec-CI factor.

The crossing of the three vulnerability factors (Fig. 5) shows an increase in the general level of vulnerability in the subsidiary until 2007, with only two low level sectors (in 2017) compared to four (in 2003). In 2012, all sectors except Konséguéla had a medium level of vulnerability. Finally, in 2017, heterogeneity is again observable in the spatial distribution of the level of vulnerability in the subsidiary, but with a downward trend except for the Kimparana sector which increased.

The evolution of the proportions of surface areas according to the level of vulnerability, between 2003 and 2017 is presented in Fig. 6.

It shows that the dominant level of vulnerability in terms of surface area is the average level with almost 50% of the total surface area of the subsidiary in 2003, and up to

94.9% in 2012. The high level of vulnerability is as it is the least representative level with 0 to 9.4% of the surface area of the subsidiary, depending on the year.

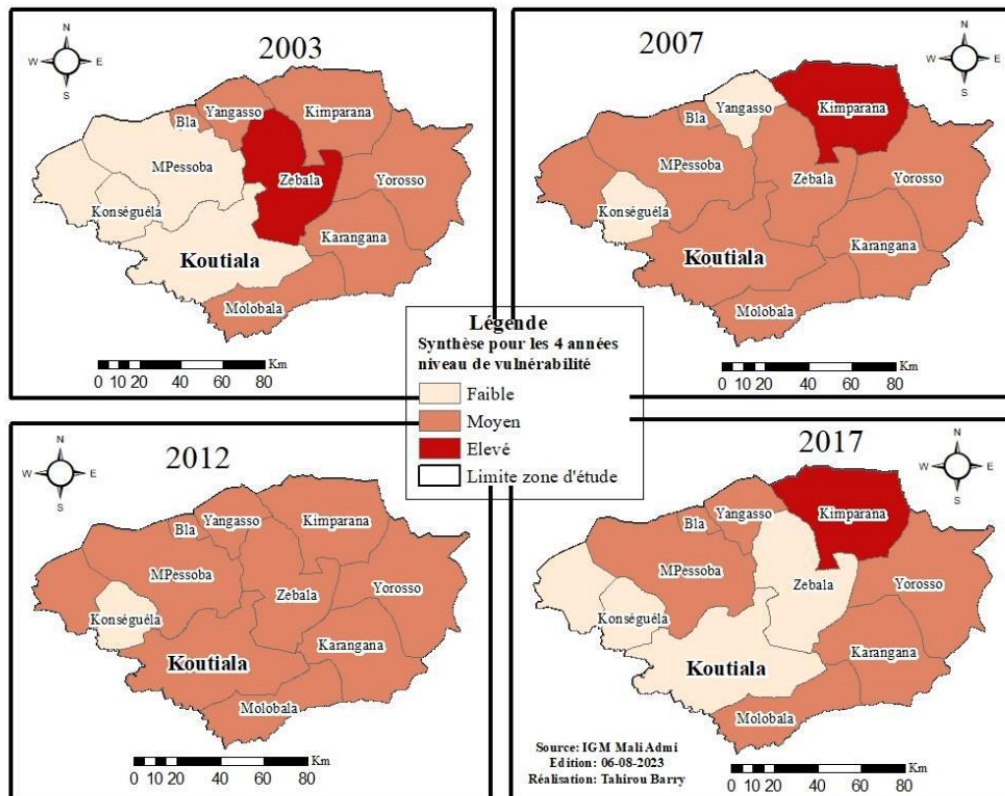


Fig. 5: Spatial-temporal distribution of the vulnerability according to the intersection of the three factors.

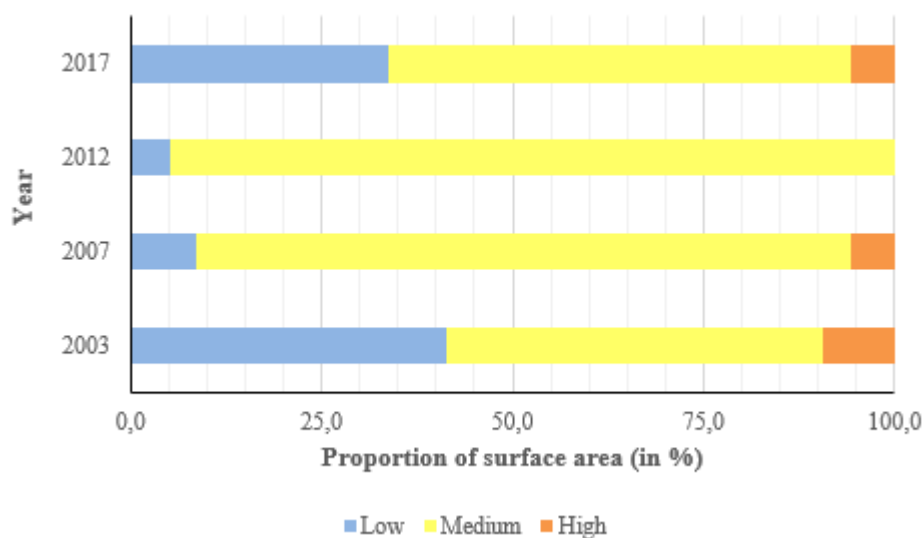


Fig. 6: Evolution of the proportions of surface areas according to the level of vulnerability.

Thus, contrary to what was observed by Mara (2010), it can be assumed that the increase in rain in Sahelian West Africa (Nouaceur, 2020) and in Mali in particular from 2010 (Diawara et al., 2021) probably led to a reduction in the environmental vulnerability of the study area. Indeed, on the maps, it is visible that in 2012 all sectors of the subsidiary, except Konséguéla, had an average

vulnerability and that the downward trend continued until 2017 except for the Kimparana sector.

## V. CONCLUSION

At the end of this work, the application of multicriteria analysis first made it possible to develop indicators representative of the environmental vulnerability of the

cotton zone (northeast subsidiary of Koutiala), and to identify the determining factors of this vulnerability. Then, these environmental vulnerability factors were spatialized and analyzed for the years 2003, 2007, 2012 and 2017. As a result, it turns out that it is the Konséguéla (Koutiala) sector which is the least vulnerable while that of Kimparana (San) is the most vulnerable of the subsidiary.

The study also made it possible to classify the factors influencing environmental vulnerability, namely from the most influential to the least influential: the Environment, the Economy, the Climate and finally the Social factors.

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# Green Synthesis of Gold Nanoparticles Using *Acacia Modesta*

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**Abstract**— Nanotechnology means any technology on a nanoscale that has application in the real world. Nanotechnology involves the production of materials with exceptional precision and dimensions on a scale as small as one billionth of a meter and implies the ability to generate, utilize structure, components and devices. Nanotechnology is the science of building very small particles. Visualizing the scale of nanotechnology can be challenging, but it is essential to understand that 'nano' refers to particles that are incredibly tiny. The ongoing exploration aimed to synthesize stable, environmentally friendly, and biocompatible gold nanoparticles (AuNPs) using *Acacia modesta* leaves and assess their biological activities. Prior research has underscored the effectiveness of nanotechnology in facilitating the production of faster, smaller, and more portable products and systems that are notably more efficient. Utilizing plant extracts for nanoparticle synthesis represents an alternative and more environmentally conscious approach. The green synthesis of nanoparticles aims to reduce waste generation and advocate for sustainable methodologies. In recent years, the focus has shifted towards green processes utilizing mild reaction conditions and non-toxic precursors to advance nanotechnology and foster environmental sustainability. The X-ray diffraction measurements revealed that all AuNPs possessed a polycrystalline structure, evident from the intense graphical peaks within the complete spectrum of 20 values, ranging from 10–80°, supported by data from scanning electron microscopy. Leaves of *Acacia modesta* were gathered, dried, and powdered, resulting in a net weight of the powdered leaves material of 25 grams. Phytochemical screening of various *Acacia modesta* extracts preceded the purification of gold nanoparticles. The antibacterial and antifungal activity of AuNPs and crude aqueous *Acacia modesta* leaves were assessed using the well diffusion method and Slant agar dilution method. The dried powder was mixed with distilled water in a 1:10 ratio and boiled for 30 minutes. Transmission electron microscopy confirmed the nano-particles' size to be within the range of 30–150 nanometers. *Acacia modesta* AuNPs exhibited substantial efficacy against Methicillin-resistant *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Strep.pyogenes*, and *Klebsiella Pneumoniae*. In addition to these microorganisms, *Acacia modesta* AuNPs also demonstrated significant activity against *Trichoderma*, *Aspergillus furfur*, *Penicilium* and *Candida albicans*. Based on the findings of this current research study, it can be concluded that *Acacia modesta* has the potential to inhibit the growth of various pathogenic microorganisms, which could be harnessed by the medical sector for the development of effective drugs to address a range of acute to chronic infections.



**Keywords—** *Acacia modesta* leaves, Antibacterial and antifungal activity, Green synthesis, Gold nanoparticles (AuNPs), Nanotechnology, Nanoscale, Phytochemical screening.

## I. INTRODUCTION

Nanoparticles (NPs), characterized by their dimensions falling within the range of 1–100 nm, when we use the term 'nano' to describe something, we are referring to its incredibly minute size. The magnitude of this smallness is evident when considering that one nanometer is equivalent to one billionth of a meter, which translates to roughly 100,000 times smaller than the width of a human hair. Exploring the intricate realm of innovation at this remarkably small scale defines the scope of nanotechnology (Khan *et al.*, 2019). These structures find diverse applications in fields such as medicine, engineering, manufacturing, and the food industry, as well as in various consumer goods, including food storage solutions, healthcare items, and personal care products. The evolving utilization of nanoparticles has precipitated an urgent need to comprehensively assess their impact on both the environment and human health, necessitating a multidisciplinary approach to ensure the safe and sustainable integration of these materials into various industries (Mateo *et al.*, 2017).

Nanotechnology is one of the most exciting and fast-moving areas of science today. Nanoparticles (NPs) are considered to exhibit heightened biological reactivity compared to their bulk counterparts, primarily attributable to their diminutive size and the resulting larger surface area to volume ratio. Several nanomaterials are naturally present, ubiquitous in volcanic ash, ocean spray, and dust particles. Additionally, natural nanostructures can be found within various plants. This heightened reactivity leads to the production of oxidative effects at the cellular level (Ijaz *et al.*, 2020). Furthermore, NPs demonstrate a remarkable ability to traverse the body, accumulate in specific organs, infiltrate cell membranes, and instigate deleterious responses such as perturbations in calcium homeostasis and gene expression, inflammatory reactions, and DNA damage. The intricate interplay between nanoparticles and biological systems underscores the critical need for a thorough understanding of their biological interactions and potential toxicity, necessitating the implementation of stringent safety assessments to ensure their responsible and sustainable integration across various applications (Zuo *et al.*, 2017).

Gold ions can be transformed, through the use of various reducing and stabilizing agents, into minute assemblies of gold atoms commonly referred to as nanoparticles. Gold nanoparticles (AuNPs) are compact structures at the nanometer scale that can be synthesized in

various shapes and sizes (1–100 nm). In Nano medicine, gold nanoparticles have minimal toxicity and another highly valued property is the targeting ability, which refers to the capacity of specific nanomaterials to target particular cells with precision. Gold nano particles have various clinical applications (Boisselier *et al.*, 2009). Scientists are currently working on developing tiny gold particles that can be loaded with drugs. These nanoparticles can be modified to specifically bind to cancer cells, allowing them to be transported inside. Importantly, drug-loaded gold nanoparticles are engineered to exclusively target infected cells without affecting neighboring healthy cells. Conditions such as malignant brain tumors (glioblastoma and high-grade astrocytoma), *Alzheimer's disease*, *multiple sclerosis*, *Parkinson's disease*, and other neurological disorders necessitate prolonged-acting formulations and the controlled release of drugs in specific brain regions (Eduardo *et al.*, 2021).

Nanotechnology can be used to reshape the world around us literally. Scientist can be creating nanostructure themselves by rearranging the atom of an object. They can make new nanomaterials with new property that can be more effective and used everywhere in future. In historical contexts, individuals during the 16th century were known to utilize exquisitely gold-coated materials for various applications, particularly within the medical domain (Pomerantseva *et al.*, 2019). Historical evidence indicates the use of gold particle-coated materials for oral medicine, pharmaceuticals, and the implantation of tissues and organs. Plants harbor an array of bioactive constituents, including flavonoids, alkaloids, terpenoids, phenolics, amino acids, and steroids, which serve as effective reducing agents for the synthesis of nanoparticles. Extracts derived from plants have demonstrated notable efficacy in the reduction of Au<sup>+++</sup> ions to Au NPs (Wang *et al.*, 2019).

## II. METHODOLOGY

### 2.1. Study Area

The research investigation was performed at Institute of Allied Health Sciences of Sarhad University of Science and Information Technology Microbiology lab.

### 2.2. Plant Collection

*Acacia modesta* (leaves) were taken from an area of the Shamshato forest, Peshawar and then shades dried and packed them in bags.

### 2.3. Aqueous Extract Preparation

The leaves of *Acacia modesta* were procured and subjected to desiccation. Subsequently, the desiccated leaves were pulverized, resulting in a net weight of the powdered leaf material amounting to 25 grams. The dried powder was combined with distilled water in a 1:10 ratio and subjected to boiling for duration of 30 minutes. The resulting infusion was then meticulously filtered using ten pieces of Whatman filter paper, with the purpose of eliminating insoluble components from the extract.

#### 2.3.1. Ferric Chloride Test

The extract, quantifying 50 milligrams, was solubilized in 5 milliliters of distilled water. Following this, a small quantity of neutral 5% ferric chloride solution was introduced into the solution. The manifestation of a dark green hue signifies the existence of a phenolic compound.

#### 2.3.2. Mayer's Test

To assess the presence of alkaloids, 5 milliliters of the plant sample extract were transferred into a test tube, followed by the addition of two drops of Mayer's reagent along the inner wall of the tube. The absence of the formation of a whitish precipitate indicates the absence of alkaloids in the sample.

#### 2.3.3. Flavonoids Test

The addition of 3 milliliters of a 1% aluminum chloride solution to 5 milliliters of each extract led to the observation of a yellow coloration, which serves as an indicator of the presence of flavonoids.

#### 2.3.4. Saponin Test

A 50-milligram portion of the extract was diluted with distilled water to achieve a total volume of 20 milliliters. Subsequently, the resulting suspension was vigorously agitated in a graduated cylinder for duration of 15 minutes. The formation of a 2-centimeter layer of froth indicates the presence of Saponin.

#### 2.3.5. Salkowski Test for Steroid

Transfer 5 milliliters of the aqueous extract solution into a test tube, followed by the addition of a chloroform solution. Subsequently, introduce a few drops of concentrated sulfuric acid into the test tube. The appearance of a reddish-brown color serves as evidence of the presence of steroids.

#### 2.3.6. Benedict Test

To 0.5 milliliters of the aqueous extract, 0.5 milliliters of Benedict's reagent were added. The mixture was thoroughly shaken and subsequently boiled for duration of 1 to 2 minutes in a water bath. A color change

from blue to green denotes the presence of reducing sugars.

### 2.4. Synthesis of Gold Nanoparticles

The synthesis procedure for gold nanoparticles entailed the combination of a measured quantity of the aqueous extract of the *Acacia modesta* plant with water. Specifically, 25 grams of the aqueous extract were introduced into 500 milliliters of distilled water and subjected to a boiling temperature for 25 minutes. The occurrence of nanoparticle formation was discerned through a noticeable alteration in color, transitioning from a light yellow to a reddish wine hue. Additionally, conventional citrate-capped gold nanoparticles were synthesized using the standard procedure for the purpose of comparative analysis.

### 2.5. Purification of Gold Nanoparticles

The purification of water-soluble gold nanoparticles poses a significant challenge due to the closely matched solubility of the nanoparticles and impurities. Consequently, standard purification methods, such as centrifugation, often prove insufficient or ineffective. To address this, the solution containing the nanoparticles was dried in an oven maintained at 50°C. Subsequently, the dried particles were gently dislodged and transferred into Eppendorf tubes with a capacity of 1.5 milliliters, each containing distilled water. The tubes were subjected to centrifugation at a rate of 12000 revolutions per minute for duration of 15 minutes. Post-centrifugation, the resulting pellet was carefully collected and subsequently dried.

### 2.6. Anti-Bacterial assay

The antibacterial efficacy of AuNPs and the crude aqueous leaves of *Acacia modesta* were examined utilizing the well diffusion method. Initially, a stock solution of 3 mg/mL was prepared using sterile DMSO. Subsequently, 100 µL of the working solution was introduced into 6 mm wells carefully arranged on sterile nutrient agar plates. Following this, the test bacterial broth cultures were inoculated onto the Petri plates. The culture plates were then left undisturbed in a laminar flow hood for a minimum of 30 minutes, allowing the test samples to diffuse into the media. Subsequently, all culture plates were incubated in an upright position at 37°C for 24 hours. Sterile DMSO served as the negative control, whereas the standard drug Amoxicillin was used as the positive control. Post-incubation for 24 hours, the percentage of inhibition was determined by calculating the zone of inhibition using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Zone of Inhibition of test Sample (mm)}}{\text{Zone of Inhibition of Standard (mm)}} \times 100$$

## 2.7. Antifungal Assay

The evaluation of the antifungal potential of the crude aqueous extract of *Acacia modesta* and AuNPs was conducted via the Slant agar dilution method. Initially, a stock solution of 24 mg/mL was prepared for the test samples in sterile DMSO. Subsequently, the Sabouraud Dextrose Agar (SDA) medium was augmented with a 70  $\mu$ L working solution of AuNPs and crude aqueous extracts. The media-containing test tubes were positioned in a slanted manner to facilitate solidification. The chosen pathogenic fungal species were then introduced onto the

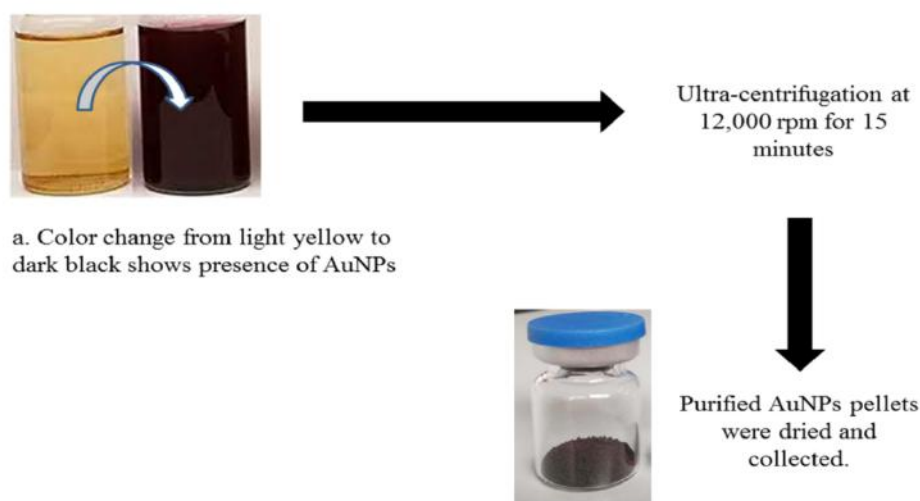
SDA-supplemented media. All test tubes were securely sealed and placed in a fungal incubator set at 28°C for duration of 1 week. Sterile DMSO served as the standard negative control, while the standard antifungal drug Miconazole was used as the positive control. At the culmination of the incubation period, the percentage of inhibition was computed using the provided formula below:

$$\% \text{ Inhibition} = \frac{\text{Linear growth of fungi in test sample (mm)}}{\text{Linear growth of fungi in Standard (mm)}} \times 100$$

## PHYTOSYNTHESIS OF AuNPs



## PURIFICATION OF AuNPs



## 2.8. PHARMACOLOGICAL INVESTIGATION OF AuNPs

### ❖ Antibacterial Activity

The study explored the antibacterial effects of gold nanoparticles (AuNPs) and an aqueous leaf extract using the well diffusion method. The research focused on evaluating the impact of these substances on various pathogenic bacterial species reported by Ahmad *et al.*, (2017).

### ❖ Antifungal Activity

The study conducted thorough investigation utilizing the tube dilution method to examine the antifungal potential of both gold nanoparticles (AuNPs) and an aqueous leaf extract against various pathogenic fungal species Ahmad *et al.*, (2017).

## III. RESULTS

### 3.1. PHYTOCHEMICAL SCREENING

Green biocompatible AuNPs were fabricated with *Acacia modesta* leaves. In order to identify the presence of bioactive phytoconstituents responsible for the reduction of Au<sup>+</sup> to Au<sup>0</sup> in a cost-effective and environmentally friendly manner, an initial phytochemical analysis of the plants was conducted. Various components within the plants acted as potent bioreducers and capping ligands, facilitating the synthesis of monodispersed, stable AuNPs. These nanoparticles were subsequently purified, characterized, and optimized using established protocols from previous literature. Furthermore, the biological and pharmacological properties of the synthesized AuNPs were meticulously evaluated and compared with those of crude ethanolic, methanolic, acetonetic, and aqueous leaf extracts derived from the selected plants.

Table 1. Phytochemical analysis of *Acacia modesta* leaves extract

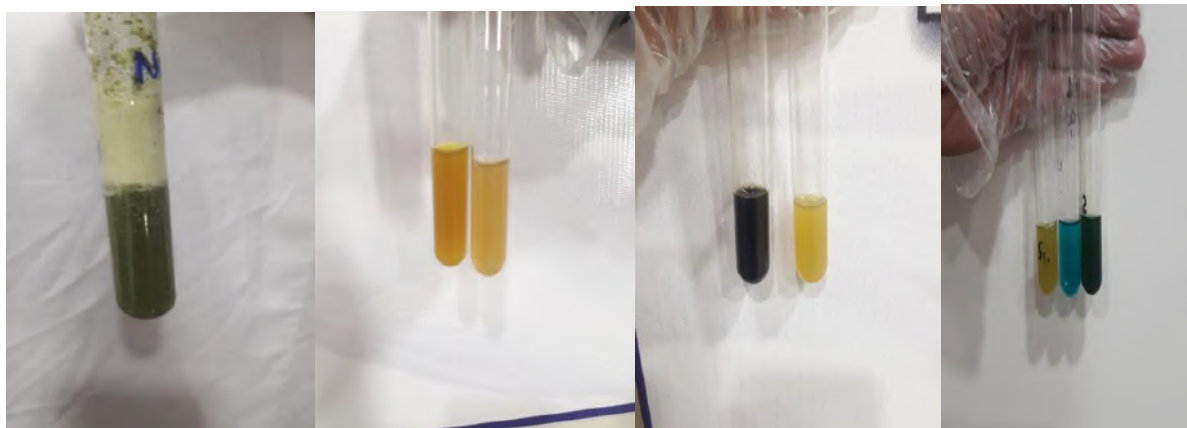
Phytochemical compounds present in plant extract	Presence of compound in <i>Acacia modesta</i> leaves
Alkaloids	+++
Tannins	+++
Phenolic compounds	++
Saponins	+
Steroids	+++
Flavonoids	+++
Reduce in sugar	-

**Note:** (+) less quantity of bioactive phytochemical

(++) moderate quantity of bioactive phytochemical

(+++) high quantity of bioactive phytochemical

(-) absence of bioactive phytochemical



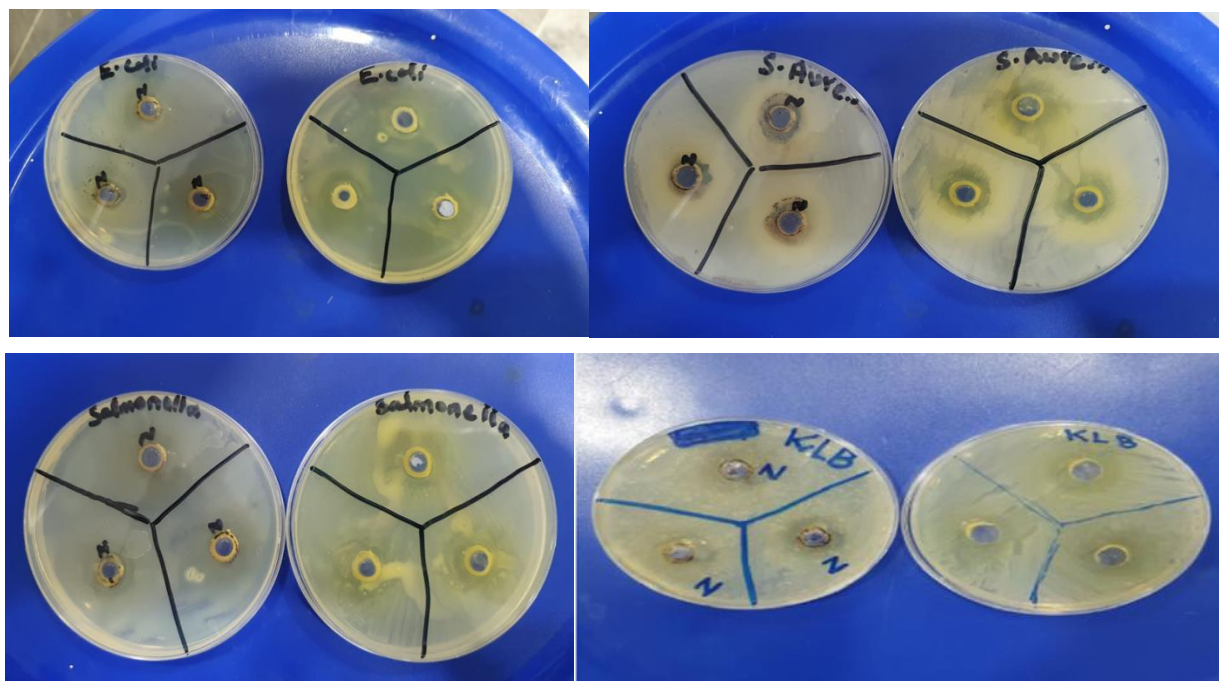
PHYTOCHEMICAL SCREENING OF ACACIA MODESTA LEAVES



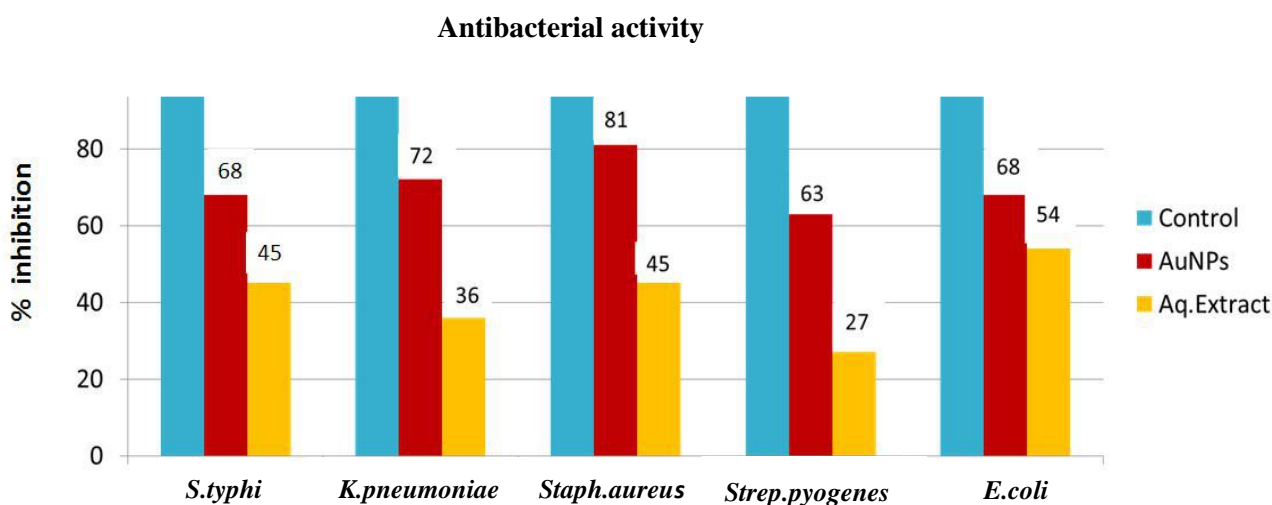
3.2. ANTIBACTERIAL ACTIVITY

Table 2. Antibacterial analysis

Extract	<i>Salmonella typhi</i>	<i>Klebsiella Pnuemoniae</i>	<i>Staphyococcus aureus</i>	<i>Strep. Pyogenes</i>	<i>E. coli</i>
Ethanolic extract of <i>A. modesta</i>	6mm	5mm	6mm	4mm	7mm
Gold nanoparticles	8mm	9mm	11mm	7mm	8mm



Antibacterial analysis of Ethanolic extracts of *Acacia modesta* and gold nanoparticles



The histogram depicted above illustrates the comparative antibacterial analysis of gold nanoparticles (AuNPs) and extracts from the leaves of *Acacia modesta*. The blue line represents the positive control, specifically Amoxicillin. The red

line displays the impact of AuNPs on various bacterial species, while the yellow line demonstrates the effect of *Acacia modesta* leaf extracts on different bacterial species.

**3.3. Antifungal activity**

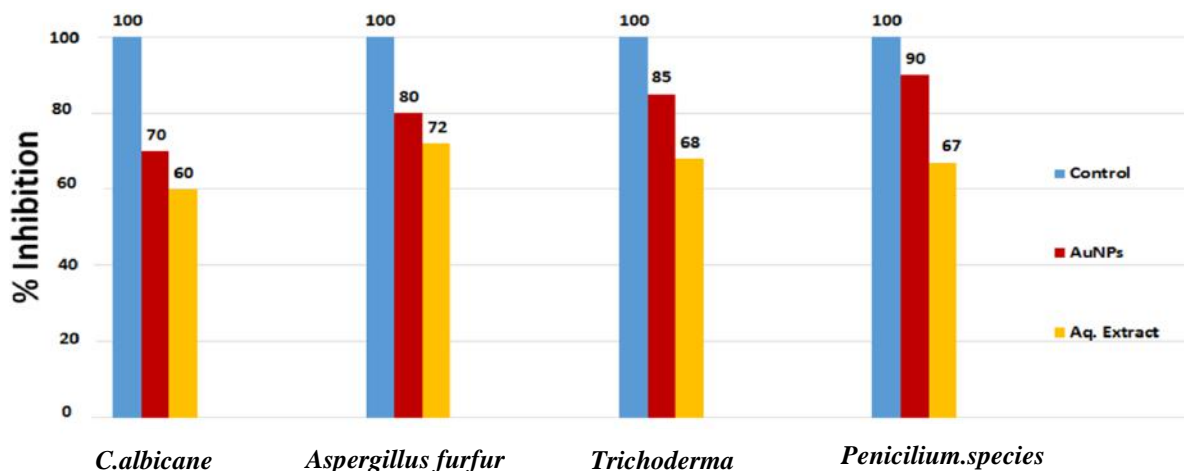
Table 3. Antifungal analysis

Extract	<i>Candida.albicans</i>	<i>Aspergillus furfur</i>	<i>Trichoderma</i>	<i>Penicilium.species</i>
Ethanollic extract of <i>A. modesta</i>	5mm	7 mm	8mm	6mm
Gold nanoparticles	8mm	10mm	11mm	12mm



Antifungal analysis Ethanollic extracts of *Acacia modesta* and gold nanoparticles

**Antifungal Activity**



The histogram presented above delineates the comparative antifungal evaluation of gold nanoparticles (AuNPs) and the bioactive constituents extracted from the leaves of *Acacia modesta*. The blue line is indicative of the positive control, Miconazole. Meanwhile, the red line denotes the impact of AuNPs on diverse fungal species, elucidating their potential antifungal properties. Similarly, the yellow line signifies the effect of the extract obtained from *Acacia modesta* leaves on a spectrum of fungal species, thereby highlighting the potential antifungal attributes of the plant extract.

#### IV. DISCUSSION

Nano-biotechnology offers an eco-friendly way to make tiny gold nanoparticles using *Acacia modesta* plant extract. This extract contains natural compounds from the plant's leaves that help both in making the gold particles and keeping them stable. The study aimed to explore the presence of potential phytochemicals, specifically polyphenols, in the leaves of *Acacia modesta*, and to employ a green synthesis approach to create gold nanoparticles (AuNPs) for subsequent investigation of their pharmacological activities (Timoszyk *et al.*, 2022). The research study outlined the following specific objectives. The objective involved utilizing the aqueous extract of *Acacia modesta* to synthesize gold nanoparticles via a green synthesis approach. The second objective centered on the purification of the synthesized gold nanoparticles. The third objective emphasized the biological assessment of the synthesized gold nanoparticles (Rodriguez-Luis *et al.*, 2016).

In recent studies, scientists are finding easier, cheaper, and eco-friendly ways to make tiny particles. They use things like *Bacteria*, *Fungi*, and plant extracts to do this. These ways are popular because they work well with living things. They help turn metals into tiny particles (Lee *et al.*, 2020). In one study, gold particles made with *Acacia modesta* leaves stopped the growth of some fungi and bacteria, including *E. coli* and *Staphylococcus aureus*. Another study showed that these gold particles also worked against other *Bacteria* like *Staphylococcus aureus*, *Klebsiella pneumonia*, and *E. coli* (Nadeem *et al.*, 2017)

Past studies have shown that making gold particles using natural methods is good for the environment and easy for people to do. made with *Acacia nilotica* and *Olea europaea* leaves didn't harm normal cells, even at higher amounts. From the *Acacia modesta* antimicrobial assay, it is estimated that crude extracts It's also been found to have great potential for medicine with very few side effects (Latif *et al.*, 2020) Found that gold particles of *Acacia modesta* and gold nanoparticles extract

inhibits the growth of various types bacteria at different levels of inhibition, such as *klebsiella*, *salmonella*, *Staphylococcus auerus* and *Escheria coli*. In addition, the results showed that *Acacia modesta* ethanolic extract inhibits the growth of selected *Fungi* at different percentages of inhibition, including *Trichodermas*, *Aspergillus furfur*, *Candida albicane* (Awad *et al.*, 2019).

#### V. CONCLUSIONS

The leaves of *Acacia modesta* harbor phytochemicals with the capacity to facilitate the active synthesis of gold nanoparticles (AuNPs) through their natural functions as reducing and capping agents. Examination of the resulting AuNPs revealed their crystalline nano-spherical structure, with a size distribution of less than 100 nanometers. When contrasted with the crude aqueous leaf extract of *Acacia modesta*, the AuNPs demonstrated notable efficacy in combating microbial and leishmanial infections. They exhibited strong activity against various tested bacterial and fungal strains, while also displaying anti-leishmanial properties on par with the effectiveness of Miltefosine. Remarkably, the cytotoxic effects were minimal, even at high concentrations of 1000 µg/ml. These findings suggest potential applications in the medical domain for the development of innovative therapeutic strategies.

Based on the outcomes of the present research investigation, it can be inferred that *Acacia modesta* possesses the ability to hinder the proliferation of diverse pathogenic microorganisms. This attribute could be harnessed by the medical sector to develop a range of effective drugs for mitigating various forms of acute and chronic infections. Moreover, these extracts hold promise for effective integration into medicinal formulations targeting a wide spectrum of microbial and neurological ailments.

#### RECOMMENDATION

Investigation into a diverse array of phytochemicals suitable for precise utilization in the production of gold nanoparticles (AuNPs) is warranted. *Acacia modesta* holds promise for future exploitation as a source of valuable antimicrobial compounds for the pharmaceutical industry. The potent AuNPs generated can serve as a foundation for the development of novel antimicrobial agents, with potential applications as community medicine. Furthermore, the exploration of AuNPs for application in agricultural and engineering domains is crucial for the development of biosensors, biocatalysts, and nano-devices. Finally, assessment of the

toxicity on human and animal cell lines is essential to ascertain the safety and potential risks associated with these nanoparticles.

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# Wildlife migration corridors from the Community Forest of Nafadji to the Boucle du Baoulé Biosphere Reserve

## Les corridors de migration de la faune sauvage de la forêt communautaire de Nafadji à la réserve de biosphère de la boucle du Baoulé

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**Abstract**— Mali still has natural relics rich in fauna such as that of the Nafadji forest. The study aims to analyze the link between wildlife species and their habitat and to identify the migration corridors they follow. Kings' method was used to inventory the wildlife. The layout of the corridors was carried out both by visual observation of the wildlife and their signs of presence. A survey was carried out among the populations and the technical services to understand the movements of the fauna and the difficulties of management. A total of 366 individuals divided between 14 species, 10 families and 5 orders have been identified. The indirect gradient analysis applied to the matrix of 224 observations x 14 faunal species to understand the relationship between species and their habitats discriminated 4 groups: G1 consisting of *Mellivora capensis*, *Hystrix cristata*, *Papio anubis*, *Orycteropus afer*, *Tragelaphus scriptus*, G2 including *Erythrocebus patas*, *Redunca redunca*, *Sylvicapra grimmia*, *Phacochoerus aethiopicus*, G3 represented *Canis adustus*, *Gazella dorcas*, *Hippotragus equinus* and G4 with *Hyena hyena*, *Viverra civetta*, *Papio anubis*. The analysis reveals that habitat and food factors condition the distribution of wildlife and their migration along the corridors. Controlling these factors is therefore essential for the effective management of wildlife and their habitat. The involvement of the Nafadji alone is not enough to ensure the protection of wildlife, but it will require close collaboration with the communities and all stakeholders in the forest and along the corridors.



**Keywords**— wildlife, diversity, Species group, habitat, Nafadji forest.

**Résumé**— Le Mali dispose encore de reliques naturelles riches en faune comme celle de la forêt de Nafadji. L'étude se propose d'analyser le lien entre les espèces de faune et leur habitat et d'identifier les corridors de migration qu'elles suivent. La méthode de Kings a été utilisée pour inventorier la faune. Le tracé des corridors a été réalisé à la fois par l'observation visuelle de la faune et de leurs indices de présence. Une enquête a été menée auprès des populations et des services techniques pour comprendre les mouvements de la faune et les difficultés de gestion. Au total, 366 individus repartis entre 14 espèces, 10 familles et 5 ordres ont été recensés. L'analyse indirecte de gradient appliquée sur la matrice 224 observations x 14 espèces fauniques pour comprendre la relation entre les espèces et leurs habitats a

discriminé 4 groupes : G1 constitué par *Mellivora capensis*, *Hystrix cristata*, *Papio anubis*, *Orycteropus afer*, *Tragelaphus scriptus*, G2 comprenant *Erythrocebus patas*, *Redunca redunca*, *Sylvicapra grimmia*, *Phacochoerus aethiopicus*, G3 représenté *Canis adustus*, *Gazella dorcas*, *Hippotragus equinus* et G4 avec *Hyena hyena*, *Viverra civetta*, *Papio anubis*. L'analyse révèle que les facteurs habitats et nourriture conditionnent la distribution de la faune et leur migration. Le contrôle de ces facteurs est donc primordial pour la gestion efficace de la faune et de son habitat. L'implication seule de la population de Nafadji ne suffit pas pour protéger cette faune, mais, il faudra une étroite collaboration de tous les intervenants dans la forêt et le long des corridors.

**Mots clés**— Faune, diversité, groupes d'espèces, habitat, forêt de Nafadji.

## I. INTRODUCTION

Mali once had a rich and varied faunal diversity made up of: 136 species of mammals, 647 birds, 106 reptiles including one endemic, 30 amphibians/amphibians with 2 endemics, and 160 fish with 24 endemics and insects/invertebrates including 6 Sphinx Butterflies. The IUCN (1989) listed 70 species of terrestrial mammals. To protect this diversity, it has focused its policy on the creation and implementation of protected areas, currently 26 in number, totaling an area of 9 172 757 ha, or 8% of the territory (MEA, 2012) compared to 12% required by IUCN (2008).

These areas, despite their protection regime, are not immune, like other forest areas, to the combined effect of man and climate (DIALLO et al., 2011). Many studies in Mali carried out in these natural areas have shown that natural resources (flora, terrestrial and aquatic fauna) have declined in Mali, in particular due to poaching and the long drought of the 1970s and 1980s.

Indeed, the assessment to establish the situation of wild livestock by the DNEF (1986), IUCN (1989) reveals that after eight years of hunting closure, large game is still struggling to develop only birds and smaller mammals have seen their numbers increase slightly. Also, the results of research and inventories in Mali on the degradation factors of wildlife and its habitat carried out within the framework of RURGS (1982), AGRER SA (1993); Albignae (1995) highlighted the regression or even the disappearance of a large number of wild animals and the vulnerability of certain large spectacular mammals such as the Elephant, the Derby Elk, the Buffalo, the Giraffe, the Chimpanzee. On the other hand, primates and sedentary birds showed growth. This situation of regression of fauna has been confirmed by studies carried out by the IUCN (2008) and which continues to grow with the extension of crops, transhumant livestock and illegal logging. (IUCN, 2009) ; AID SA (2010); OPNBB (2012); ERSAP (2014). These studies have attributed declines in animal population densities, high mortality rates caused by overexploitation by Malian and Moorish poachers in Mauritania, fragmentation and destruction of natural wildlife habitats

for crop cultivation and obstruction of their migration corridor. The resulting ecological changes have always engendered ecological changes in the distribution and habits of wild animal species, the disruption of their reproductive capacities. To ensure their survival, certain species of fauna, especially mammals such as herbivores, move in groups and most often with their predator from one environment with difficult conditions (lack of food and water) to another in search of suitable conditions (better (plenty of food and water). Others migrate passing from one home range to another along the natural movement corridors commonly called corridors (Buard, 2013), then make the reverse path depending on the seasons which condition the availability and/or the insufficient fodder (Spaccapietra *et al.*, 2008) at the same rate as transhumant herding (PRODESO, 1997).

Today, it must be recognized that the progressive obstruction of these mobility corridors has a negative impact on wildlife potential. This potential is seriously reduced and some species are on the verge of extinction, and the current method of managing forest resources does little to promote the conservation and enhancement of wildlife resources that are hard hit in their diversity (IUCN, 2009 ; OPNBB, 2012).

However, despite the various threats, Mali still has some natural areas rich in wildlife that can still be saved. These are difficult-to-access relics of classified forests and protected areas located in the south-west of the territory and which are of particular importance from a national and global point of view (DNEF, 2014). These isolated and difficult to access areas such as the Nafadji forest still contain most of the diversity of mammals (DNEF, 2014).

This study is part of the perspective of deepening knowledge of the wildlife resources of the Nafadji ecosystem and their pendular migration corridors followed to ensure their survival. This specifically involves: (1) analyzing the link between the fauna species recorded and their habitat, (2) identifying the seasonal migration corridors used by the fauna species.

## II. MATERIALS AND METHOD

### 1.1. Environment Study

Located in the Senegal River basin between the Sahelian zone in the North and the Sudanian zone in the South, the Nafadji forest bearing the name of the village of Nafadji (Figure 1) is located in the rural commune of Séfeto ouest, Kita cercle, region of Kayes covering an area of approximately 10,000 ha. According to the PIRT studies

(1982), Nafadji is located in the agro-ecological zone of upper Kaarta, the mountain range in particular: the Sangarou, the Kouroufing and the extension of the Galla Kourou. Distant about 360 km northwest of the capital Bamako, it is limited between latitude 13.54° to 14.24° North and longitude 9.97° to 10.17° West. The mountains also form the administrative boundary between the Diallan rural commune (Bafoulabé circle) to the north and Séfeto Ouest (Kita circle) to the south.

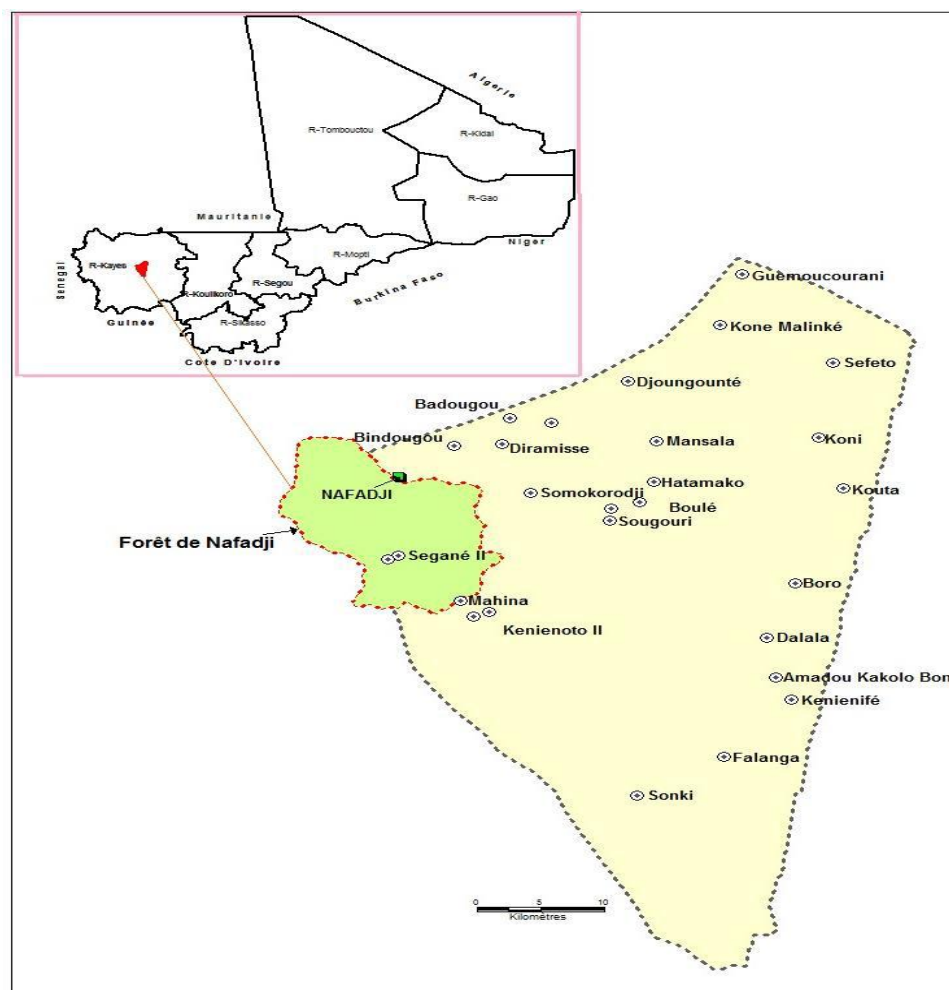


Fig.1. Geographic location of area study.

The villages and hamlets bordering the study area are: Nafadji, Seramissé (commune of Sefeto Ouest), Samine and Sobé (Commune of Diallan). The climate is of the northern Sudanese type with alternating dry and rainy seasons with a general downward trend. The rainy season begins in the area from the month of May to reach its peak in August where the heights of rain can be greater than or equal to 250 mm. This period of heavy rain corresponds to the high humidity period and mild temperature (25°C). The hot weather rages during the dry season between March and May and gradually fades from June. It is also during this period that evapotranspiration is high and

would correspond to a sunshine duration of around 2969 hours (SLA Kita, 2010).

The relief is rugged, made up of a set of plateaus with an altitude of 200 to 500 m dotted with residual reliefs such as the "Kita Kourou" which culminates at 500 m from the central plain and serving as refuges for wildlife. It is flat on the south side and very uneven towards the north and west part of the forest and the soil is of type Tc4 (Armoured Earth) whose soils are characterized by a moderate depth before reaching the armor according to PIRT (1982) which corresponds to armored terrain whose soils are characterized by a moderate depth before

reaching the armor. The Nafadji forest is entirely located on this type of soil. The surface texture is loamy, often sandy with fine silt. Below the surface, it becomes clay silty with the presence of gravels. The structure is weak and subangular blocky or granular. The soil is friable when moist throughout the profile. The pH ranges from moderately to strongly acidic at depth.

The hydrographic network is marked by small streams that originate at the top of the hills of Kouroufing, Sagarou, Galla kourou and Naliokourou. The most important is the Kouaga river with its tributaries which flow into the Bagoue at the level of Badoumbé.

The forest contains at least six (6) permanent water points Malandji, Gnonketo, Dialanikorodji, N'Gagnadji, Kitora, Wontimé which are very important for wildlife and livestock resources in the area. The area also contains large streams that dry up as soon as the rains end. However, it is influenced by the course of the Bakoye River which constitutes the natural limit to the north.

The population of the study area is 4,084 inhabitants for the two major villages in the area, including 3,000 inhabitants for the village of Nafadji and 1,084 inhabitants for that of Siramissé with a number of women (50.4%) slightly higher than that of men (49.6%). In addition to this permanent workforce, the village of Nafadji includes five cultivation hamlets (Kéniénoto I and II, Mahina, Segané I and II) which are only inhabited during the rainy season to set up crop fields there.

Agriculture is the main activity of the people. It is extensive with basic equipment and revolves around cereal crops (sorghum, maize, pearl millet, cowpea, lowland rice) mainly intended for local consumption and cash crops (peanuts and cotton) and production. market gardening (onion, shallot, sweet potato, papaya, okra, carrots, pepper, tomato, cucumber, and cabbage) which contribute significantly to the local and regional economy.

Livestock is developing gradually (1,371 cattle, 1,375 sheep/goats, 256 donkeys and 12 horses) and constitutes a more or less secure means of saving, especially for women.

## 1.2. Methods

Satellite images and national topographic maps were used to identify the topography of the study area, the link with the Baoulé loop reserve. The survey sheets (interview guides) and wildlife inventories were used to report the information collected. Two tapes of 50 m and 100 m were used to measure the viewing distance of wildlife. A SUUNTO brand compass was used to measure the angles of view of the animals and two (2) binoculars for distant visions, a high resolution Nikon digital camera was used

for shooting to illustrate the thesis. The global positioning system (GPS) was used for the georeferencing of the forest and all phases of wildlife inventories.



Photo n°1: Some materials used for the works

### 1.2.1. Collection of data

#### - Socio-economic surveys

The so-called “participatory mapping” method (Clouet, 2000), which consists of using the knowledge of local actors to map their land, was applied. With the help of the populations of Nafadji, a sketch on paper of the various land use zones that they recognize on their land is made. These areas, as well as the limits of the land following the natural limits (watercourses, hills), were then surveyed using a Garmin 90 GPS, circumscribed on the spot with the collaboration of the villagers.

Beforehand, a documentary search was made to summarize the work and studies carried out in the Baoulé reserve (Block of Badinko), the Social and Economic Development Programs of the circle of Kita and other documents (annual reports of the Water Cantonment and Kita Forests) served to deepen knowledge of the environment and especially of the wildlife resources of the study area. Individual interviews and group meetings based on an interview guide developed for this purpose are organized with institutional actors, associative structures and resource persons. The approach taken is much more semi-structured leaving a large part to the discussion and exchanges with the interlocutors. The purpose of these meetings was to discuss in order to identify the different wildlife species in the current environment, endangered species, potential habitats, natural water points on the site, identification of migration corridors, human pressure on wildlife resources, wildlife conservation methods at the local level, etc. The villages and hamlets bordering the Nafadji forest were covered by the interviews.



### - Wildlife inventory work

The nature of the environment and the means available were the determining criteria in the choice of the inventory method. After reading the Topo funds to 200 thousandths (Sandaré ND\_29 XIV and Bafoulabé ND\_29 VIII) and prospective field visits, the transect sampling method (King, 1930) was used (figure 2). It consists of counting on foot to cover a restricted area in order to provide clues to the presence of animals (Norton-Griffiths, 1978];

Bouché, 2001). In the field, the chosen route is traveled by counting the fauna by visual or auditory contact on either side of the line of progression. The length of the transect, defined beforehand on a map with the coordinates of the entry and exit points varies from 8 to 11 km, and in an East-West direction and separated from each other by 2 to 5 km to minimize the risk of double counting. The census in the transects was done early in the morning and in good weather when the animals showed up more vigorously.

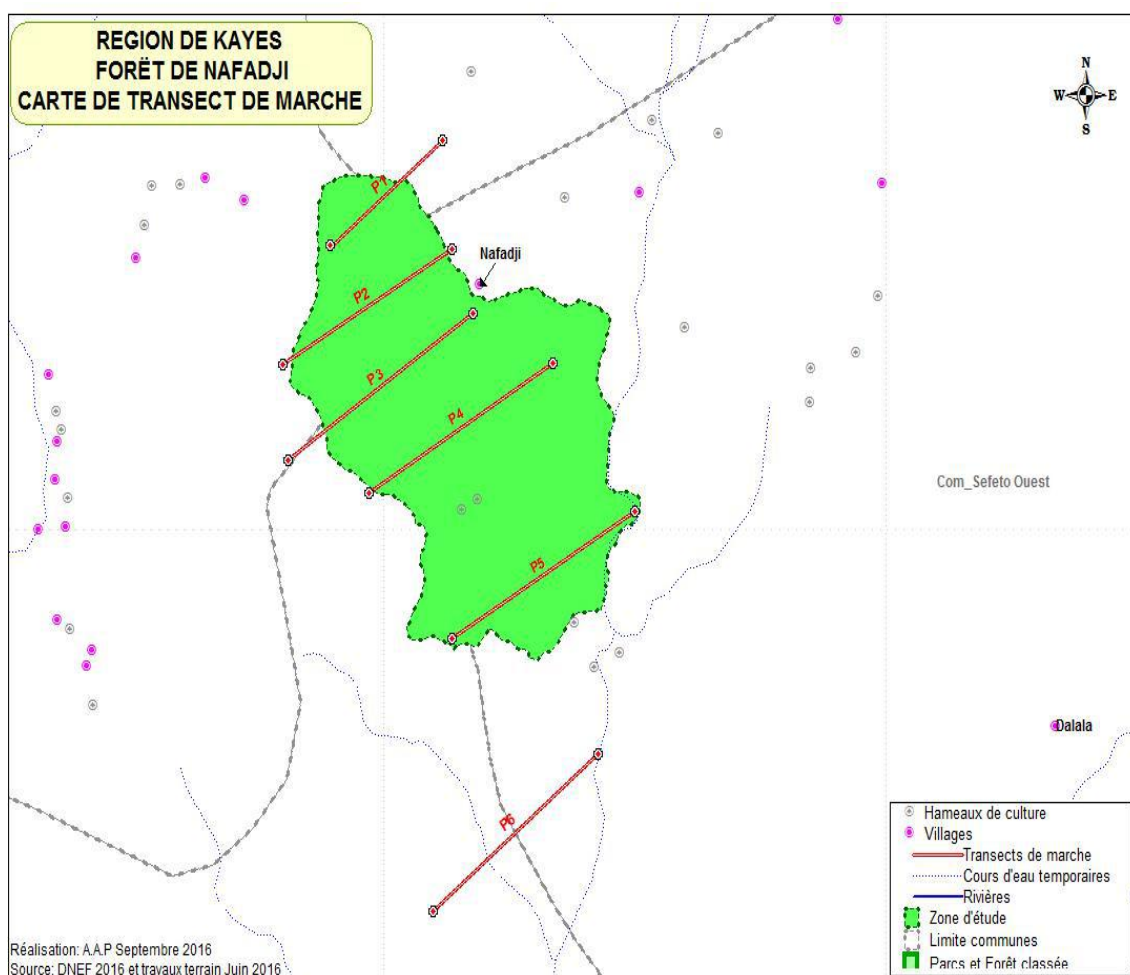


Fig.2. Wildlife transect map of Nafadji Forest.

### - Determination of global wildlife diversity

Wildlife diversity was established by counting all the species encountered their traces (droppings, prints, carcasses, etc.). The document "Guide to Mammals-Reptiles and Birds by Clark and Niagaté (2004) was used to identify the mammals and determine, with the support of the hunting guide, the sex of the species either by the difference in size, or by peeling, etc.

### - Determination of the relationship between wildlife species and their habitat

It was made using principal component analysis (PCA), which is an indirect gradient analysis method that applies to quantitative or semi-quantitative variables (Fallissard, 1998 in Diallo, 2014) in order to look for the existing correlations between them.

In our case, it is the correlation between wildlife species and their preferred habitat. For this purpose, we used a matrix of 6 transects (224 observations) x 14 faunal species. The "flexible beta" or soft aggregation method was combined with Sorensen's relative similarity distance (McCune & Grace, 2002) to assess distance differences between count transects and group identification of

wildlife species. The choice of the "flexible beta" method is justified by the fact that it allows a better understanding of the ecological processes involved in the distribution of fauna species.

#### - Determination of wildlife migration corridors

The determination of the wildlife migration corridors was made by reconciling the technical-scientific knowledge with the local traditional knowledge of the hunters and a forest guide. Thanks to the accompaniment of the latter and a hunter from Nafadji, some passages frequented by various species and groups of species of wildlife reported by hunters were followed. It is therefore about the passages of the places of stay and the places frequented to stop and to move. The layout of the routes taken, qualified as passage corridors by Bonnin (2008), was carried out both by direct visual observation of fauna species and indirect visual observation of these through their presence indices (borrowed, faeces among others) along the routes followed or frequented.

#### - Data processing and analysis

The counting, processing and analysis of the data collected were carried out using Word and Excel programs used respectively for data entry and processing.

The PcOrd software was used to establish the link between the species and their habitat and that of MapInfo 10.5 was used to produce the maps and also to generate the boundary coordinates of the study area.

### III. RESULTS

#### 3.1. Wildlife diversity

It was counted in the forest of Nafadji (Table 1), a total number of 366 individuals divided between 5 orders, 10 families, 14 species. This number of species is low. For all the species observed, *Papio anubis* (25.37%) and *Erythrocebus patas* (17.91%) are the most abundant. These two species are followed in terms of abundance by the species: *Phacochoerus aethiopicus*, *Canis adustus*, *Sylvicapra grimmia* and *Gazella dorcas*. In terms of frequency, *Erythrocebus patas* and *Canis adustus* are the most frequent at 15% and 12% respectively. The weakly observed species are among others *Redunca redunca*, *Tragelaphus scriptus* and *Viverra civetta*.

Table 1. Inventory of Individuals species identified.

Order	Family	Species	effective	%
Primates	Cercopithecidae	<i>Papio anubis</i>	66	17.91
Artiodactyle	Bovidae (Cephalophinae)	<i>Sylvicapra grimmia</i>	22	5.97
Carnivores	Canidae	<i>Canis adustus</i>	27	7.46
Carnivores	Viverridae	<i>Viverra civetta</i>	5	1.49
Artiodactyles	Bovidae (Gazelophinae)	<i>Gazella dorcas</i>	22	5.97
Artiodactyles	Bovidae (Tragelaphinae)	<i>Tragelaphus scriptus</i>	5	1.49
Carnivores	Hyenidae	<i>Hyena hyena</i>	16	4.48
Artiodactyles	Bovidae (Hippotrague)	<i>Hippotragus equinus</i>	11	2.99
Tubilidentes	Oryctéropidae	<i>Orycteropus afer</i>	16	4.48
Artiodactyles	Suidae	<i>Phacochoerus aethiopicus</i>	44	11.94
Rongeurs	Hystricidae	<i>Hystrix cristata</i>	16	4.48
Carnivores	Mustelidae	<i>Mellivora capensis</i>	16	4.48
Primates	Cercopithecidae	<i>Erythrocebus patas</i>	93	25.37
Artiodactyles	Bovidae (Reduncinae)	<i>Redunca redunca</i>	5	1.49
<b>Total (5)</b>	<b>(10)</b>	<b>(14)</b>	<b>366</b>	<b>100</b>

#### 2.2. Relationship between wildlife species and their environment or habitat

Principal component analysis (PCA), an indirect gradient analysis on the matrix of 6 transects (224 observations) x 14 faunal species gives a discrimination of 4 distinct

groups. The eigenvalues and corresponding variance percentages are summarized in Table 4. According to this table, the first 4 axes explain 94.17% of the total variance and highlight the dispersion of information on the factorial

axes. Axes 1 and 2 represent more than 61.77% of this variance.

Table 4: Values of the variance of the faunal composition explained by the 3 axes or principal components of the matrix of 6 transects (224 observations) x 14 species.

ACP Axes	Inertia value	% variance explained	% cumulative explained variance	own value
1	4,885	34,890	34,890	3,252
2	3,764	26,888	61,778	2,252
3	2,770	19,782	81,560	1,752
4	17,66	12,616	94,176	1,418

The ecological interpretation of the axes from the observations made on the 6 transects (figure 3) are on the whole correlated with the positive axis 1 and the positive axis 2 with a marked tendency of the groups. This figure

shows the breakdown of four (4) groups within individualized wildlife species.

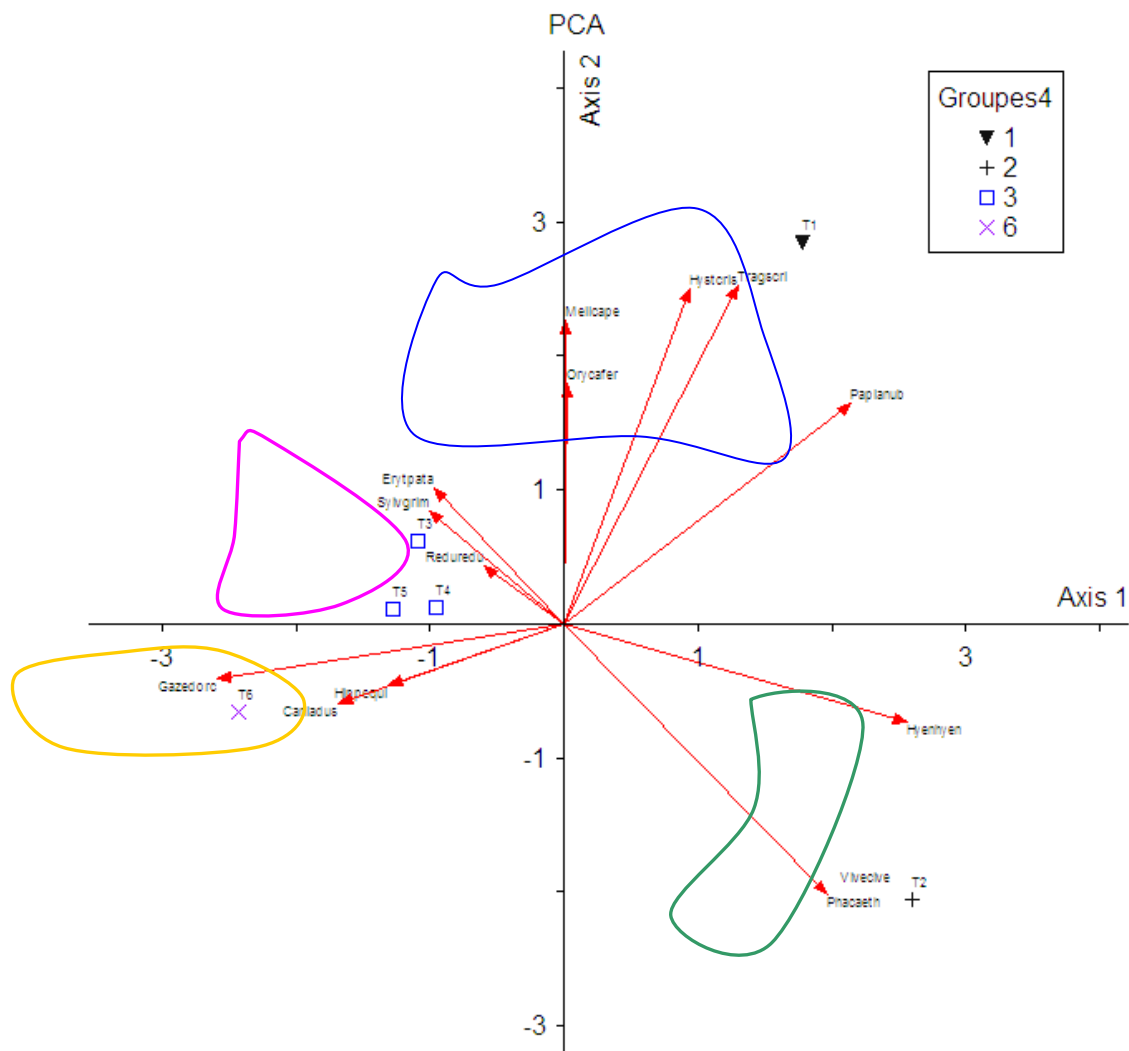


Fig.3: Factorial plane of the principal component analysis (PCA) of the groups of 224 observations along the 6 transects x 14 species. The sites are represented by small circles and the species by vectors, the score of the species corresponds to that of the small circle indicated by the end of the vector.

The first G1 group made up of the *Mellivora capensis*, *Hystrix cristata*, *Papio anubis*, *Orycteropus afer*, *Tragelaphus scriptus* subservient to transect 1 is practically correlated with the positive axis 1 corresponding to the middle of hills or plateaus with very steep valleys and escarpments (510 m altitude according to the GPS readings on the top of the Sagourou hill). The G2 group is represented by the species *Erythrocebus patas*, *Redunca redunca*, *Sylvicapra grimmia*, *Phacochoerus aethiopicus* recorded on transects T3, T5 correlated to the positive axis 2 corresponding to the valley, to the temporary flood zones. The G3 group with species such as *Canis adustus*, *Gazella dorcas*, *Hippotragus equinus* which are distributed between transects T4 and T6 corresponding to the vast plains with savannah formations correlated to the negative axis 1 and the G4 group with species such as *Hyena hyena*, *Viverra civetta*, *Papio*

*anubis* also corresponding to the vast plains with savanna formations correlated to the negative 2 axis.

Four habitats (Table 5) including G1 (in the plateaus), G2 (the valleys), G3 (intermediate zone) and G4 (the grassy plains) are discriminated.

Each group represents the distribution of a set of faunal species with similar affinities for habitat, as well as indices of abundance, specificity and interdependencies between species for each type of habitat. For example, the species *Canis adustus* is characteristic of G1 and *Redunca redunca* for G2. On the other hand, certain species such as *Erythrocebus patas*, although having a specific preference for one environment, can adapt to others, as evidenced by their presence in three of the four ecological environments. The results of this analysis show that each species is characteristic of a habitat to which it is dependent and which determines its survival (food, shelter, etc.).

Table 5: Distribution of species by group with similar habitat affinities.

Espèces	G1	G2	G3	G4
<i>Canis adustus</i>	-	+	-	-
<i>Erythrocebus patas</i>	+	+	+	-
<i>Gazella dorcas</i>	-	+	-	-
<i>Hippotragus equinus</i>	-	+	-	-
<i>Hyena hyena</i>	+	-	+	+
<i>Hystrix cristata</i>	+	+	-	-
<i>Mellivora capensis</i>	+	+	-	-
<i>Orycteropus afer</i>	+	+	-	-
<i>Papio anubis</i>	+	-	+	+
<i>Phacochoerus aethiopicus</i>	-	-	+	-
<i>Redunca redunca</i>	-	+	-	-
<i>Sylvicapra grimmia</i>	+	+	-	-
<i>Tragelaphus scriptus</i>	+	-	-	+
<i>Viverra civetta</i>	-	+	+	+
<b>Total species</b>	<b>8</b>	<b>10</b>	<b>5</b>	<b>4</b>

(+) = presence; (-) = absence.

#### 2.4. Identification of seasonal migration corridors of wildlife large and medium-sized.

The study area from Nafadji, including that from the said forest to the river, constitutes a migration corridor for large fauna. If no status exists for this area, it receives various wildlife species from the Badinko block reserve, in the Baoulé and Fina blocks and sometimes in the Kongosambou classified forest between January and

February (which is the period decline of the Badinko River) and the return at the beginning of the rains (May-June) in search of more favorable environmental conditions and tranquility according to the managers of the Baoulé reserve. This corridor (Figure 4) over 60 km long is rich in terms of grazing for wildlife, water resources (tributaries of the Badinko River) and less occupied today

by agro-pastoralists. Only the village of Sonki was listed on the axis during the inventory work.

The types of plant formations are composed of galleries along the courses, sandstone plateaus with shrubby savannahs, vast floodplains and wooded to shrubby savannahs including *Combretum glutinosum*, *Combretum nigricans*, *Combretum velutinum* to which must be added *Pterocarpus erinaceus*, *Pterocarpus lucens*, *Strignos spinosa*, *Crossopteryx febrifuga*, *Hexalobus monopetalus*, *Terminalia avicennioides*, *Terminalia macroptera*, *Lannea acida*, *Lannea velutina*, *Pteleopsis suberosa*, *Detarium microcarpum*, *Grevia sp.*, etc.

The wooded savannahs of the area are the domain of tall grasses with a continuous herbaceous cover and exceeding 2 meters in height, in particular composed of *Antropogon pseudapricus*, *Andropogon gayanus*, *Cymbopogon gigantus*, *Pennisetum pedicelatum*. These plant formations are well appreciated by wildlife for their food and tranquility.

#### IV. DISCUSSION

##### - Diversity

The results of the wildlife inventory in the Nafadji forest made it possible to count 366 individuals divided between 14 species, 10 families and 5 orders. The number of species is very low compared to that of the 70 species of terrestrial mammals listed by the IUCN (1989) in Mali, which once had a capital of extraordinary faunal species (Afrique Nature Internationale, 2009) declining and most of which only survive today in small groups considered endangered (IUCN/BRAO, 2008 ; Nomoko, 2008). As in Mali, in many African countries wildlife has declined due to harsh living conditions marked by overexploitation and habitat destruction (Ntiama-Baidu, 1998 ; Craigie *et al.*, 2010 ; Ogutu *et al.*, 2011). However, with the isolation of the Nafadji forest and the favorable conditions of its current habitat, a biological rise is perceptible in terms of species and individuals according to the populations who have become aware of the need to protect wildlife.

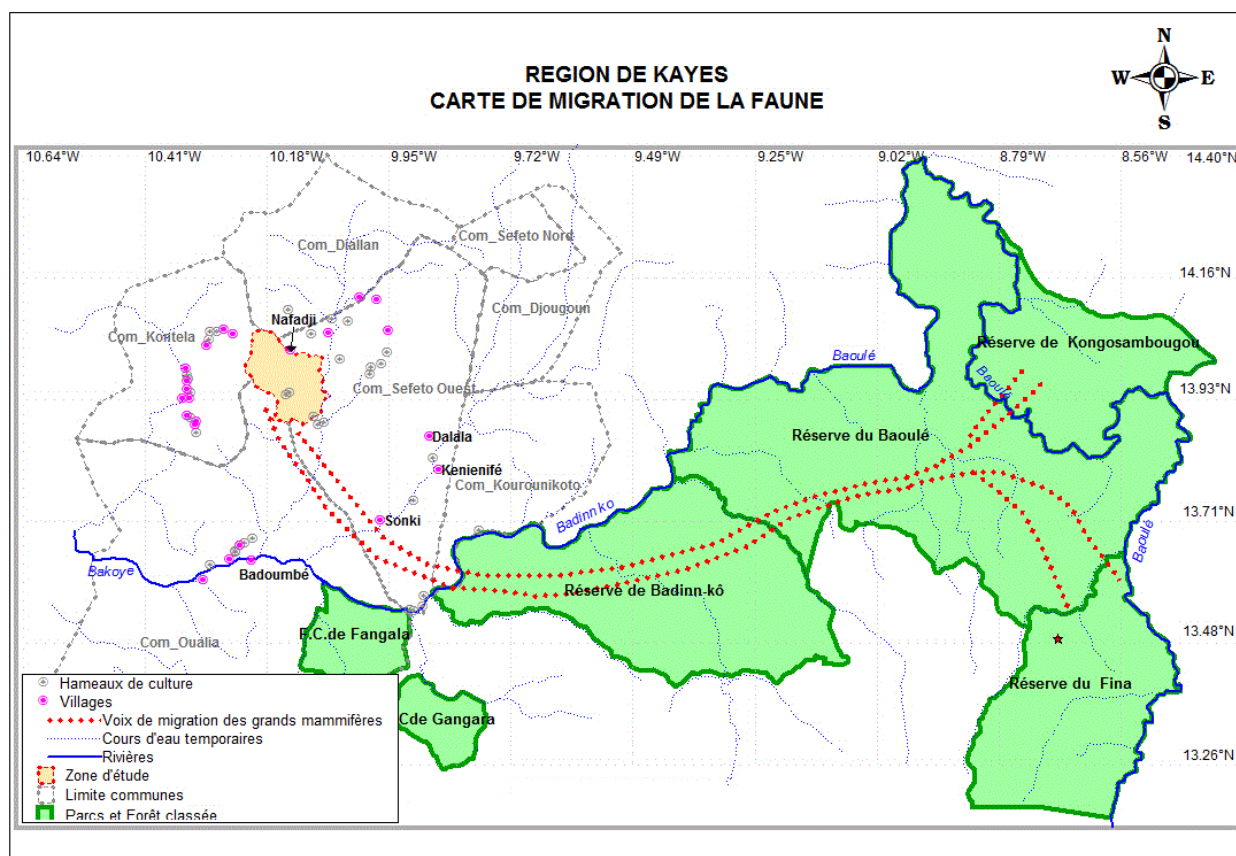


Fig.4: migration map of medium and large wildlife.

##### - Link between wildlife and its habitat

In the Nafadji forest, wildlife has a close relationship with its habitat. The analysis of the distribution of faunal

species reveals that the maximum number of species is located on the topographic units of valleys rich in water resources and under vegetation in dense thickets and gallery forests. Bélem (2008) and Sgard (2010)

emphasized the close link between species and suitable habitat conditions. The 4 groups of species G1, G2, G3 and G4 identified according to their topographical position respectively on the plateaus, the valleys, in the intermediate zone and on the grassy plains of the Nafadji forest indicate that each species is subservient to a group according to of this positioning which conditions either its survival for a preference in food (food, water), in shelter for more security or peace of mind. In the same space, species can be linked by a relationship of prey-predator interdependence, as is the case in the groups of species G1 of the prey *Tragelaphus scriptus* a bovidae and its predator *Hyena hyena* and G2 with prey species *Hippotragus equinus*, *Sylvicapras grimmia*, and their prey *Canis adustus*, *Mellivora capensis*, *Viverra civetta*. Other species and groups of species find themselves in competition on the same space to exploit the same resources (competitive relationship for the resource) between herbivores on the different grazing areas, particularly in grassy plains and valleys. The same observations were made by Czudek (2001) and Bélem (2008). The latter emphasizes that the relationship of interdependence between prey and predators makes it possible to regulate the populations of species in the same space and promote the maintenance of the biological balance.

#### **- Identification of seasonal migration corridors of wildlife large and medium-sized.**

Movements of species in the Nafadji forest along the two identified corridors which all lead into the Baoulé loop biosphere reserve. The frequentation of this reserve by species is motivated by an interest such as the natural conditions (natural habitat) favorable for the needs of a large area, tranquility (along natural obstacles that are often inaccessible) and above all a wealth of food that can allow each individual to meet basic needs on a daily basis. These movements illustrate not only the needs to be satisfied in terms of area and richness of species habitats, the presence of congeners (Bonnin, 2008) but also reflect the preferences and capacities of these species to avoid being in competition for the resource (water, fodder, etc.), to be protected from their predator and thus ensure their survival (Geerling *et al.*, 1988). Many authors (Bonnin 2008 ; IUCN, 2009 ; Buard, 2013) stipulate that the living environment of wild animals does not offer unlimited resources, especially when this number is increasing, those to avoid being in competition on the same space for the resources or the same places migrate towards the spaces with ecological conditions favorable to their development. However, it is clear that the wildlife suffers from the presence of transhumant livestock that has increased more and more in recent decades and herders who are both poachers on the corridors and in the Baoulé reserve. This

presence constitutes a handicap to the seasonal movements of wild animals (especially ungulates) and to their development, even though they are less dependent on running water, and can exploit the rangelands better than domestic herds (Hibert *et al.*, 2010). In addition, to minimize spatial interference and competition for resources with livestock, some wild animals take refuge on inaccessible natural obstacles, others on the other hand would adopt nocturnal behaviors. These same observations were made by Binot *et al.* (2006); IUCN (2009); Hibert *et al.* (2010) who point out that the presence of livestock can now hinder the recovery of wildlife through spatial interference or competition over resources.

## **V. CONCLUSION ET IMPLICATION POUR LA CONSERVATION**

The study contributes to the knowledge of the fauna and its habitat in the Nafadji forest and along the corridors. The study showed that faunal diversity is very low. Species have been classified into four groups in relation to natural habitats. Spatially, the ecosystems of the valleys (in the center) contain more faunal species than those of the plateaus to the north and the grassy savannahs to the south. From a methodological point of view, the device used for the inventory (transects) offers more visibility of large fauna in fairly open natural formations. However, the nature of the terrain (escarpment) and the rainy season for the second phase (September) constitute limits in the estimation of individuals in the middle. Also, the inventory period was considered not conducive to identify all the species. The ideal period for the inventory would be the period (October-December) or (May-June) which would correspond respectively to the departure and return of the large fauna from the Badinko reserve to the Guinean part in transit in the Nafadji forest. Corridors frequented by wildlife have not been extensively tracked. Only the resting places and certain places in the corridors frequented were observed. It would be necessary to continue the monitoring of the fauna on these corridors to better refine the routes in order to protect the fauna in its migration.

The study showed that habitat and food factors play an important role in the distribution of fauna. The management of this factor, which will have to be explored further for the rest of the study, would therefore be essential in the management of wildlife.

Finally, the Nafadji forest has a strong link with the Baoulé National Park and its adjacent reserves with a strong commitment from the local population for its protection. However, the sole involvement of this one and that of the neighboring hamlets is not enough for its good

management, it will require close collaboration with the communities, the technical services and other forest stakeholders (transhumant farmers, operators, etc.).

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# Effect of Gibberellin and Nitrogen Fertilizer on Growth and Zucchini (*Cucurbita pepo* L.) Yield In Tropical Regions

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**Abstract** — Zucchini is the Cucurbitaceae family and rich in nutrients. In Indonesia, cultivation of zucchini is still low and zucchini has the potential to be developed. Improvement of cultivation technology is required to ensure optimal growth and yield of zucchini. The research to study effect of gibberellin and nitrogen fertilizer on growth and yield of zucchini plants. Was conducted from July to October 2023 in Batu City, East Java, Indonesia. The Research was a factorial experiment using a randomized complete block design with two factors, first factor was gibberellin concentration with 3 treatment levels (0, 150 and 300 ppm) and the second factor was the dose of nitrogen fertilization with 5 treatment levels (50, 100, 150, 200 and 250 kg/ha). The observation data results were analyzed using analysis of variance (ANOVA) and continued honest significant difference test HSD at 5% error level. To determine the relationship pattern between observation variables, regression test was conducted. The results showed that significant effect of gibberellin and nitrogen fertilization on growth and yield of zucchini plants. There were increasing in plant length, number of leaves, leaf area, fresh weight, dry weight, number of fruits and fruit weight. Nitrogen fertilization caused increasing in plant length, number of leaves, leaf area, fresh weight, dry weight, number of fruits, fruit weight and chlorophyll index. The results of this study revealed that gibberellin and nitrogen fertilization positive effect in increasing the growth and yield of zucchini plants. Based on the results of this study recommended that the optimum gibberellin and nitrogen are 150 ppm and 150-250 kg/ha.



**Keywords**— Zucchini, Gibberellic acid, Nitrogen, Growth and Yield.

## I. INTRODUCTION

Zucchini plants belong to the Cucurbitaceae family. Rich in nutrients and bioactive compounds such as flavonoids, vitamin A, vitamin B2, vitamin C, vitamin E, amino acids, carbohydrates, minerals and high of fiber. (Tamer *et al.*, 2010). Zucchini cultivation in Indonesia is still low and have the potential to be developed. Zucchini fruit shows promising results and can be beneficial for health, zucchini has safe components and able to significantly inhibit damage caused by H<sub>2</sub>O<sub>2</sub> and shows anti-proliferation properties and pro-apoptotic properties against HL60 tumor cells. (Martínez-Valdivieso *et al.*, 2017). Therefore,

zucchini plants has the potential to be developed in the Indonesian region. Therefore, zucchini cultivation required the right cultivation technology including the application of gibberellins and nitrogen fertilizers.

Gibberellins is a plant growth regulator group that have a role as growth promoters. One of the important hormones in controlling development and regulating several physiological mechanisms in plants (Miceli *et al.*, 2019). Some plant responses controlled by gibberellin hormones include seed germination, stem and root elongation as well as increased leaf area and flowering (Al-Harathi *et al.*, 2021). In addition to increasing plant growth and yield

through the addition of growth hormones, plants can also grow optimally due to the availability of sufficient nutrients. Nitrogen is a macro nutrient that needed in large quantities to grow and develop. Nitrogen is required in the formation of proteins that are associated with all important processes in plants therefore nitrogen elements can lead to increased yield and crop quality (Leghari *et al.*, 2016). However, fertilization activities are often excessive and have a negative impact on the environment and increase farmers production costs. Impact of excess of inorganic nitrogen fertilizer inputs can reduce the growth and number of soil microbes. (Zhang *et al.*, 2018). The study aim to determine the effect of gibberellin concentration and nitrogen fertilizer dosage on growth and yield of zucchini.

## II. MATERIALS AND METHODS

### 2.1. Research Site

The research was conducted in July-October 2023 in Batu City, East Java Province. Batu city has an average air temperature of 22,2°C with annual rainfall of 2028 mm in 2022 (BPS, 2023). The research site lies at 7°54'35"S 112°31'34"E and 1005 m above sea level.

### 2.2. Tools and Materials

The tools used in this study were polybag measuring 40 x 40 cm, hoe, ruler, Oven Memmert type 21037 FNR, Leaf Area Meter type LI – 3100, Scales Nict Voor type PS 1200 and sprayer 2 L. The material used is Zucchini seed variety Jacky Z-6, gibberellin, goat manure, urea fertilizer 46 % N, Fertiphos fertilizer 20 % P<sub>2</sub>O<sub>5</sub> dan ZK fertilizer 50 % K<sub>2</sub>O.

### 2.3. Experimental Design and Treatments

The Research was a factorial experiment using a randomized block design with two factors and three replications. The first factor is gibberellin concentration with three level is G0 (0 ppm), G1 (150 ppm) and G2 (300 ppm). The second factor is dose of nitrogen fertilizer with five level N50 (50 kg/ha), N100 (100 kg/ha), N150 (150 kg/ha), N200 (200 kg/ha) and N250 (250 kg/ha). There are 15 treatment combination units so that there are 45 treatment units. Each treatment combination has 8 plant sample.

Gibberellin application was carried out at 14, 28, 35 and 42 day after planting by spraying the plant until the gibberellin was evenly distributed to all part of the plants. Gibberellin application at 07:00 – 09:00 AM.

Fertilization is applied at a distance of 5 cm from the base of stem. Phosphorus and potassium fertilizers according to

the recommendation of 150 kg/ha. Fertilization is done at 7 and 21 day after planting each 50 % dose of fertilizer.



Fig. 1: Fertilization and gibberellin application

Growth observation variable include plant length, number of leaves and leaf area observed periodically at 14, 28 and 42 day after planting. Fresh weight, dry weight and chlorophyll index at 30 day after planting and yield variables include number of fruits and fruit weight.

Leaf area observations were made using the ALA method (*Average Leaf Area*). Calculation of plant leaf area with the ALA method using the formula showed below Widaryanto *et al.* (2019) :

$$LA \text{ (cm}^2 \text{ plant}^{-1}\text{)} = ALA \text{ (cm}^2 \text{ leaf}^{-1}\text{)} \times \sum \text{Number of leaf (leaf plant}^{-1}\text{)}$$

### 2.4. Data Collection and Analysis

The results of the observation data were analyze using analysis of variance (ANOVA) and carried out with the F test at the 5% error level, then if there is an effect, continued honest significant difference test (HSD) at the 5% error level and linear regression test to determine relationship between the two variable

## III. RESULTS

### 3.1. Effect of Gibberellin and Nitrogen on Growth of Zucchini

Gibberellin and nitrogen treatments showed that no significant interaction on plant length but gibberellin and nitrogen had significant effect on plant length of zucchini. Plant length due to gibberellin and nitrogen fertilizer is show in (Table 1).

Gibberellin concentration showed no significant effect at 14 DAP, but at 28 and 42 DAP the concentration of gibberellin increased the length of zucchini plants compared to plants without gibberellin. The difference in gibberellin concentrations of 150 ppm and 300 ppm did not show significant differences in the length of zucchini plants. Increasing the dose of nitrogen fertilizer increased the length of zucchini plants.

Nitrogen fertilizer 150 kg/ha produced higher plant length than nitrogen fertilizer 50 kg/ha but did not show significant difference with nitrogen fertilizer 100, 200 and 250 kg/ha at 14 and 42 DAP (Tabel 1).

Table 1: The Effect of Gibberellin and Nitrogen on Plant Length

Treatment	Plant length (cm) at age (DAP)		
	14	28	42
Gibberellin			
G0	20,21	41,77 a	62,73 a
G1	20,73	46,99 b	66,13 b
G2	20,76	47,56 b	66,08 b
HSD (5%)	ns	3,070	3,091
Nitrogen			
N50	18,67 a	40,91 a	60,91 a
N100	19,65 ab	43,24 ab	63,96 ab
N150	21,33 b	48,00 c	66,85 b
N200	21,63 b	47,39 bc	66,48 b
N250	21,56 b	47,56 bc	66,79 b
HSD (5%)	2,103	4,667	4,698

\*means followed by the same letter in the same column are not significantly different according to HSD test at 5% level.

Observation at 28 DAP, 150 kg/ha fertilizer showed higher plant length than 50 and 100 kg/ha fertilizer doses, but did not show significant differences with 200 and 250 kg/ha nitrogen fertilizer. Nitrogen fertilizer 50 kg/ha had the lowest plant length compared to other fertilizer doses.

Gibberellin and nitrogen treatment showed that no significant interaction on the number of leaves but gibberellin and nitrogen had significant effect on the number of leaves of zucchini. The number of leaves as effect of gibberellin and nitrogen fertilizer is presented at (Table 2).

Table 2: The Effect of Gibberellin and Nitrogen on Number of leaf

Treatment	Number of Leaf ( <i>leaf</i> ) at age (DAP)		
	14	28	42
Gibberellin			
G0	3,24	6,27 a	8,91 a
G1	3,33	6,93 b	9,57 b
G2	3,33	7,00 b	10,1 b

HSD (5%)	ns	0,33	0,60
Nitrogen			
N50	3,37	5,51 a	8,92 a
N100	3,37	6,51 b	9,37 ab
N150	3,29	6,92 bc	9,77 ab
N200	3,03	7,14 cd	9,74 ab
N250	3,44	7,55 d	9,96 b
HSD (5%)	ns	0,51	0,92

\* means followed by the same letter in the same column are not significantly different according to HSD test at 5% level.

Gibberellin and nitrogen had no significant in 14 DAP. Gibberellin concentration increased the number of plant leaves compared without gibberellin. The difference between gibberellin concentrations of 150 and 300 ppm showed no significant difference. Plants without gibberellin application produced a lower number of leaves than the gibberellin application treatment. Increasing the dose of nitrogen fertilizer increases the number of leaves of zucchini plants and 250 kg/ha nitrogen fertilizer produces the highest number of leaves. Observation at 28 DAP showed that nitrogen fertilizer 250 and 200 kg/ha no significant difference but nitrogen application 250 kg/ha was significantly different from nitrogen 50, 100 and 150 kg/ha. Observation at 42 DAP, nitrogen 250 kg/ha produced a higher number of leaves than nitrogen 50 kg/ha, but with nitrogen 250 kg/ha was not significantly different from nitrogen 100, 150 and 200 kg/ha.

Gibberellin and nitrogen showed were no significant interaction on the leaf area but gibberellin and nitrogen had significant effect on leaf area of zucchini. The leaf area as effect gibberellin and nitrogen fertilizer is presented at (Table 3).

Table 3: The Effect of Gibberellin and Nitrogen on leaf area

Treatment	Leaf area (cm <sup>2</sup> /plant) at age (DAP)		
	14	28	42
Gibberellin			
G0	313,77	1630,51 a	2649,79 a
G1	322,37	2281,02 b	3602,34 b
G2	322,37	2751,68 c	4182,97 c
HSD (5%)	ns	181,44	240,99
Nitrogen			

N50	325,95	1916,85 a	3247,86 a
N100	325,95	2138,25 ab	3414,36 ab
N150	318,79	2255,81 bc	3559,80 ab
N200	293,71	2325,32 bc	3547,49 ab
N250	333,11	2469,12 c	3622,33 b
HSD (5%)	ns	275,82	366,33

\*Means followed by the same letter in the same column are not significantly different according to HSD test at 5% level.

Gibberellin and nitrogen showed no significant effect at 14 DAP. Increasing the concentration of gibberellin causes an increase in leaf area of zucchini plants. gibberellin concentration at 300 ppm produced a higher leaf area than the concentration at 150 ppm. Plants without gibberellin produced lower leaf area compared with gibberellin. Increasing nitrogen fertilizer dose increases leaf area of zucchini plants. Nitrogen fertilizer dose of 250 kg/ha produces higher leaf area. Observation at 28 DAP the dose of 250 kg/ha is significantly different from nitrogen 50 and 100 kg/ha but not significantly different from nitrogen 150 and 200 kg/ha. Observation 42 DAP, nitrogen fertilizer 250 kg/ha was only significantly different from nitrogen 50 kg/ha and not significantly different from nitrogen 100, 150 and 200 kg/ha.

Gibberellin and nitrogen treatments showed that no significant interaction on plant fresh weight but gibberellin and nitrogen had significant effect on fresh weight of zucchini. Fresh weight due to gibberellin and nitrogen fertilizer are presented (Fig. 2 & Fig. 3).

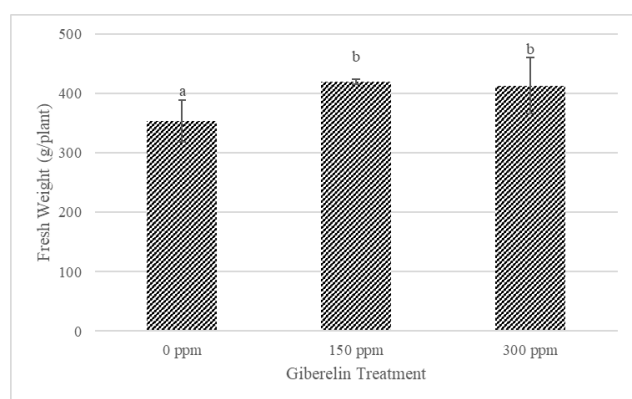


Fig. 2: The Effect of gibberellin on fresh weight

Gibberellin concentration increased the fresh weight of zucchini plants (fig 2). The difference between 150 ppm and 300 ppm gibberellin concentration showed no

significant. Zucchini plants without gibberellin had a lower fresh weight than plants with gibberellin application.

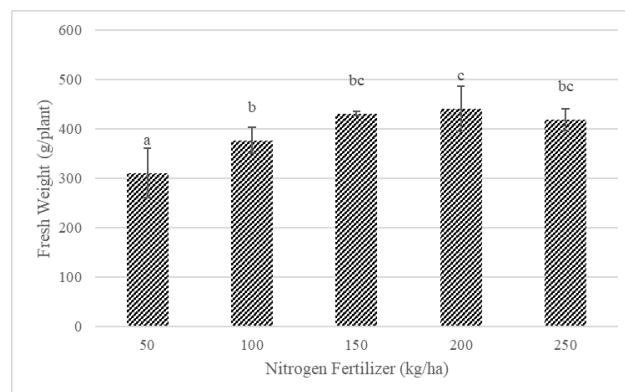


Fig. 3: The Effect of nitrogen in fresh weight

Increasing the dose of nitrogen fertilizer increased the fresh weight of zucchini plants (fig 3). Nitrogen dose of 200 kg/ha produced higher fresh weight than 50 and 100 kg/ha nitrogen fertilizer but not significantly different from 150 and 250 kg/ha fertilizer. Nitrogen fertilizer 50 kg/ha produced lowest plant fresh weight than other nitrogen fertilizer doses.

Gibberellin and nitrogen fertilizer interaction on dry weight of zucchini showed at (Table 4).

Table 4: The Effect of gibberellin and Nitrogen on Dry Weight

Treatment	Dry weight (g/plant)				
	Dose of nitrogen fertilizer				
Gibberellin	N50	N100	N150	N200	N250
G0	22,40	30,56	38,10	35,90	36,13
	a	b	c	bc	bc
	A	A	A	A	A
G1	33,06	42,10	43,93	45,33	42,30
	a	b	b	b	b
	B	B	AB	B	A
G2	43,26	43,73	47,93	45,06	49,40
	a	a	a	a	a
	C	B	B	B	B
HSD (5%)	6,671				

\* means followed by the same letter in one column or same letter in one row are not significantly different according to the HSD test at 5% level.

Gibberellin concentration increased the dry weight of zucchini plants at each nitrogen fertilization level and

nitrogen fertilizer increased the dry weight of zucchini plants in plants without gibberellin and 150 ppm gibberellin concentration. At 300 ppm gibberellin concentration application, nitrogen fertilizer showed no significant effect.

The response of zucchini plants without gibberellin, increasing nitrogen fertilizer dose increases dry weight of zucchini. Nitrogen fertilizer 150 kg/ha produced higher dry weight than nitrogen fertilizer 50 and 100 kg/ha, but not significant from nitrogen 200 and 250 kg/ha. And 50 kg/ha nitrogen fertilizer produced the lowest dry weight compared to other nitrogen fertilizer doses. At 150 ppm gibberellin concentration, 100 kg/ha nitrogen fertilizer produced higher dry weight than 50 kg/ha nitrogen fertilizer and was not significantly different from 150, 200 and 250 kg/ha nitrogen fertilizer, and at concentration of gibberellin 300 ppm nitrogen fertilizer not showed a significant effect.

The response of gibberellin concentration at each fertilization level showed that gibberellin treatments increases the dry weight of zucchini plants. At 50 kg/ha nitrogen fertilizer, the concentration 150 and 300 ppm showed a significant difference but at 100, 150 and 200 kg/ha nitrogen, the concentration difference did not show a significant difference and at 250 kg/ha nitrogen, the increase in plant dry weight occurred at 300 ppm gibberellin concentration.

### 3.2. Effect of Gibberellin and Nitrogen on Yield of Zucchini

Gibberellin and nitrogen treatment showed no significant interaction on fruit number and fruit weight but gibberellin and nitrogen had significant effect on fruit number and fruit weight of zucchini. Number and weight of plant fruits due to gibberellin and nitrogen fertilizer are presented (Table 5).

Table 5. The Effect of Gibberellin and Nitrogen on Number of Fruit and Fruit Weight

Treatment	Number of fruit	Fruit weight (g)
<b>Gibberellin</b>		
G0	2,22 a	327,30 a
G1	2,44 b	346,50 b
G2	2,51 b	351,60 b
HSD (5%)	0,17	18,59
<b>Nitrogen</b>		
N50	2,07 a	306,45 a
N100	2,29 ab	319,55 ab

N150	2,48 b	342,05 bc
N200	2,55 b	354,05 c
N250	2,55 b	386,90 d
HSD (5%)	0,27	28,42

\*means followed by the same letter in the same column are not significantly different according to HSD test at 5% level.

Gibberellin concentration increased fruit weight and fruit number of zucchini plants. However, differences in gibberellin concentrations of 150 and 300 ppm did not show significantly different in fruit number and fruit weight. Increasing the dose of nitrogen fertilizer also increased the number of fruits and fruit weight of zucchini. The number of fruits at 150 kg/ha nitrogen fertilizer significantly different compared with 50 kg/ha, but not different from 100, 200 and 250 kg/ha fertilizer. On fruit weight, 250 kg/ha nitrogen fertilizer produced the highest fruit weight. In addition, 150 kg/ha nitrogen fertilizer showed no significantly different with 100 and 200 kg/ha nitrogen fertilizer. And 50 kg/ha nitrogen fertilizer resulted in lower fruit number and weight compared to higher nitrogen fertilizer doses.

### 3.3. Effect of Gibberelin and Nitrogen on Chlorophyll Index

Gibberelin and nitrogen treatments showed no significant interaction on leaf chlorophyll index and gibberellin concentration showed no significant effect but nitrogen had significant effect on leaf chlorophyll index. Leaf chlorophyll index due to nitrogen fertilizer is presented (fig. 4).

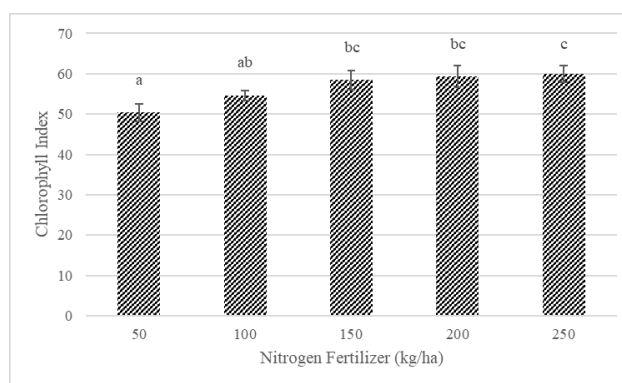


Fig. 4: The Effect of nitrogen on chlorophyll index

Increasing the dose of nitrogen fertilizer increased the chlorophyll index (fig 4). At 250 kg/ha nitrogen fertilizer produced the higher chlorophyll index but not significantly different with 150 and 200 kg/ha nitrogen. Nitrogen

fertilizer 50 kg/ha had a lower chlorophyll index compared to higher fertilizer doses.

#### IV. DISCUSSION

Gibberellin is one of the hormones in plants that regulates plant life from germination, growth to flowering. The research showed that gibberellin application increased the growth and yield of zucchini plants. Increase in growth due to gibberellin concentration can be seen from the increase in plant length, number of leaves, leaf area, fresh weight and dry weight of cultivated plants. Gibberellin hormone has a role to trigger stem elongation, roots, leaf expansion and dormancy in cultivated plants (Hedden & Sponsel, 2015). The increase in growth is due to gibberellin hormone inducing the transcription of genes involved in cell elongation and cell division that occur during the growth process (Miceli et al., 2019). In addition, gibberellin hormone is also able to increase components in the photosynthesis process such as stomatal conductance, water use efficiency and transpiration rate (Emamverdian et al., 2020) This certainly increases the ability of plants to carry out the photosynthesis process. The increase in plant photosynthesis 6% and 10% results in a 30% increase in plant biomass (Lawson et al., 2012). This is in line with the results of the research, which showed an increase in fresh and dry weight of plants due to the application of gibberellin.

Positive effects were also seen with increasing doses of nitrogen fertilization. The application of nitrogen fertilizer is effective in supporting plant growth because it causes an increase in plant length, number of leaves, leaf area, fresh weight and dry weight and leaf chlorophyll index. Nitrogen fertilization will cause an increase in nitrogen nutrients in the plant, making it easier for plants to absorb nitrogen nutrients. Nitrogen is an essential element in the formation of chlorophyll and protein and is related to leaf color, plant vigor, N content in plants, yield and crop quality (Syed et al., 2016). in line with the results of the study showed that plants given 150 kg/ha nitrogen fertilizer had a higher chlorophyll index of 15.95% compared to zucchini plants with 50 kg/ha fertilizer. According to research Liu et al. (2017), 240 kg/ha nitrogen fertilizer on rice plants caused an increase in chlorophyll content by 68.99% compared to plants without nitrogen fertilization. Chlorophyll is an important part of the Calvin cycle which is responsible for receiving light in the photosynthesis process and the photosynthesis process is the most significant factor in grain yield and plant biomass (Liu et al., 2019).

Gibberellin application and nitrogen fertilization produce a higher number and area of leaves, the number of leaves and leaf area will affect the yield of zucchini plants. The effect is related to the ability of the leaves to carry out the photosynthesis process, the process of photosynthesis will produce photosynthate and will be transported from the leaves to all parts of the plant organs and stored as food reserves in the fruit (Nasrulloh et al., 2016). Fruit weight is related to the amount of photosynthate translocated to the fruit. The greater the photosynthate that is translocated to the fruit caused fruit weight will increase (Waskito et al., 2017). In addition, nitrogen fertilizer also causes an increase in leaf chlorophyll index. Total chlorophyll in plants shows a positive correlation with photosynthetic rate, so increasing photosynthetic rate has a significant impact on growth, development and yield (Gai et al., 2017). It can be seen from the results of the fruit weight of zucchini, there is an increase in fruit weight along with the provision of gibberellins and an increase in the dose of nitrogen fertilizer.

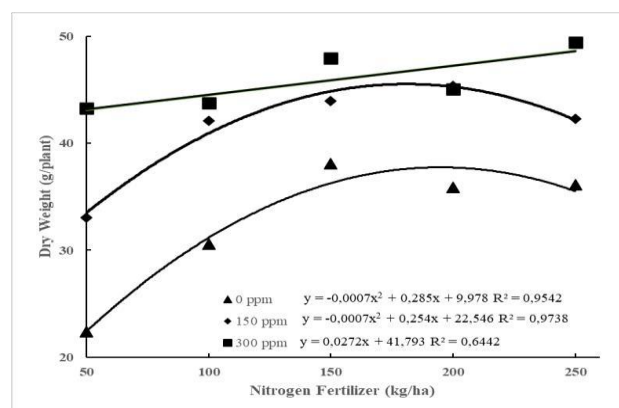


Fig. 5: The Effect of nitrogen fertilization on dry weight of zucchini plants in each gibberellin treatment

Fig 5. is a regression analysis to determine the optimal fertilizer dose at gibberellin concentration by looking at plant dry weight. The increase in dry weight occurred along with the increase in nitrogen fertilization dose in the condition without gibberellin and gibberellin 150 ppm. The resulting equation for the effect of nitrogen fertilization on plant dry weight in conditions without gibberellin is  $y = -0,0007x^2 + 0,285x + 9,978$  from the equation the optimal nitrogen fertilization value is 203,5 kg/ha to produce maximum dry weight. While in plants with 150 ppm gibberellin the equation is  $y = -0,0007x^2 + 0,254x + 22,546$  so that the optimal nitrogen fertilizer dose is 181,4 kg/ha nitrogen. To produce optimal dry weight, plants without gibberellin application need higher nitrogen fertilizer than plants with 150 ppm gibberellin application.

The application of gibberellin can reduce the need for nitrogen fertilizer and produce a higher dry weight than the dry weight of plants without gibberellin application. Therefore, the application of gibberellin with a concentration of 150 ppm with the optimal nitrogen fertilization dose can increase the growth and yield of zucchini plants.

## V. CONCLUSION

Gibberellin application increased the growth and yield of zucchini plants, the effect of gibberellin was seen at 7 days after application. The recommended concentration of gibberellin is 150 ppm. The optimal nitrogen fertilizer is 150-250 kg/ha. The application of gibberellin is effective in reducing the use of nitrogen fertilizers. Further research needs to determine the type of organic fertilizer that can have a positive effect on plant growth and yield with the aim of reducing the use of inorganic nitrogen fertilizers.

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# Land use dynamics and socio-economic impacts of wetlands in the Oti plain in the face of climate change

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**Abstract**— *Wetlands are one of Togo's most important natural resources. However, human pressures on these already vulnerable environments are contributing to a reduction in their surface area and, consequently, to a loss of biodiversity. The aim of this study is to assess the vulnerability of wetlands to climate change through a diachronic analysis of changes in climatic parameters over the period 1970-2021. To do this, we first mapped land use units from 1987 to 2019 in order to identify the various pressures on wetland ecosystems in the Oti plain in northern Togo (Ramsar site of the Oti-Kéran-Mandouri protected areas). Secondly, we identified wetland degradation factors and the socio-economic impacts of this degradation on local populations. The results show that the surface area of the natural land-use units of the Oti plain has declined over the last thirty years. Over the same period, the climate in the area has been highly variable, alternating between dry and wet periods, with a general trend towards lower rainfall and higher temperatures. The effects of this climate change, coupled with human-induced degradation factors, are having a negative impact on the socio-economic situation of local populations. The Participatory Analysis of Poverty and Livelihood Dynamic (PAPOLD) also enabled farmers to be classified into three prosperity classes (Poor, Moderately Well Off and Well Off). The results provide a decision-making tool for sustainable, participatory management of the wetlands of the Oti plain.*



**Keywords**— *Climate change, Prosperity classes, Dynamics, Wetlands, Land use, Oti plain.*

## I. INTRODUCTION

Because of the many functions and ecosystem services they provide to mankind, wetlands have become indispensable to our existence. In addition to the fact that humans have learned to take advantage of them for livestock farming, various crops, navigation, recreation and landscaping, wetlands regulate the water and climate regime, protect the soil and drinking water, purify water and are incredible reservoirs of biodiversity (DORIANE, 2014). In Togo, and particularly in the northern part of the country, wetlands offer additional food security, which is all the more important given that rainwatered crops can fail due to drought. In fact, 62.3% of the rural population lives off natural resources in general and the resources of catchment areas and their wetlands in particular.

Despite the many goods and services they provide, wetland areas are still being degraded. The causes of this degradation are not only linked to climatic hazards, but above all to human activities. The socio-economic and ecological issues surrounding wetlands, and the conflicts they generate, mean that there is a growing need for more comprehensive management, taking into account the environments, resources and activities that develop there (DETRIEUX and al., 2000). In 1987, following the publication of the Brundtland Report by the United Nations Commission on Environment and Development, the RAMSAR Convention formalised the concept of the wise use of wetlands. The wise use of wetlands is the maintenance of their ecological character achieved through the implementation of ecosystem approaches in the context of sustainable development.



As of January 2020, four (04) sites in Togo have been included on the list of wetlands of international importance, representing a wide diversity of aquatic and terrestrial ecosystems covering almost 12,104 km<sup>2</sup>, or approximately 21% of the country's surface area. These RAMSAR sites have various protection statuses, some of which are classified as national parks and wildlife reserves (Kéran National Park and Togodo Wildlife Reserve), while others are classified as watersheds, protected areas and coastal areas (Oti-Mandouri watershed and coastal wetlands).

The Oti plain wetlands in question are the Oti-Kéran-Mandouri protected areas (OKM for short). The current dynamics of these wetlands is guided by the interaction of climatic hazards and anthropogenic factors marked by intense agricultural and pastoral activity. These wetland ecosystems are also used for internal population movements to conquer good land for agricultural purposes, which is highly coveted by various stakeholders such as farmers, fishermen, livestock breeders and loggers. Wetlands and other land-use units in these ecosystems are being degraded, making them highly vulnerable. This degradation is not without consequences for the socio-economic status of local populations. As a result, when these wetlands are degraded, the services they provide to humanity are reduced, with negative socio-economic consequences for rural communities in particular, who have no other source of income.

Several wetland management strategies have been initiated over the last thirty years. Among these strategies, the people-centred approach developed by WOOD et al (2013) seems to be the most appropriate. This approach considers populations as active managers-users (natural resources), and not as conservation managers.

Many scientific studies on wetlands have been carried out in Togo. These studies have focused on plant species and degradation factors, and have paid little attention to the land-use dynamics of wetlands over the past thirty years. The present study, which falls within this framework, was carried out to add to the existing literature. Our approach

consisted firstly of a diachronic analysis, which enabled us to understand the evolution of land-use units, then we analysed the evolution of climatic parameters over the period 1975-2021. The study ended with a review of wetland degradation factors and an assessment of the socio-economic impact using the PAPOLD method.

The results obtained constitute a decision-making tool for the development and implementation of protection, conservation and development measures for natural ecosystems such as wetlands.

## II. MATERIALS AND METHODS

### 2.1 Presentation of the study environment

The Oti plain, our study area, is a wetland located in the northern region of Togo, extending between latitudes N 9°30' and N 11°00' and longitudes E 0°15' and E 0°55'. It is a sub-unit of the greater Volta basin (Fig 1). It is located in ecological zone I (ERN, 1979), which corresponds to the northern plains covered mainly by dry Leguminosae and Combretaceae savannahs, harbouring significant biological diversity (KOU MANTIGA and al., 2018; BADABATE and al., 2012) and a hydrographic network that drains the entire plain. The Oti is the major river, and its tributaries, the Kéran, Koumongou, Kara, etc., are the main watercourses. The Oti plain is a vast peneplain that experiences major seasonal flooding between September and October. It is a biodiversity sanctuary that provides ecosystem

goods and services to the local population, most of whom live in rural areas. It covers the protected areas of the Oti-Kéran/Oti-Mandouri (OKM) complex, covering an area of 179,000 ha straddling four prefectures, three of which are located in the Savannah region: Oti-North, Oti-South and Kpendjal prefectures, and the other in the Kara region: Kéran prefecture. The protected areas of the OKM complex have been classified as a RAMSAR site since 2007 and recognised as a Biosphere Reserve by the UNESCO MAB Commission in 2011 (KOU MANTIGA and al, 2018).

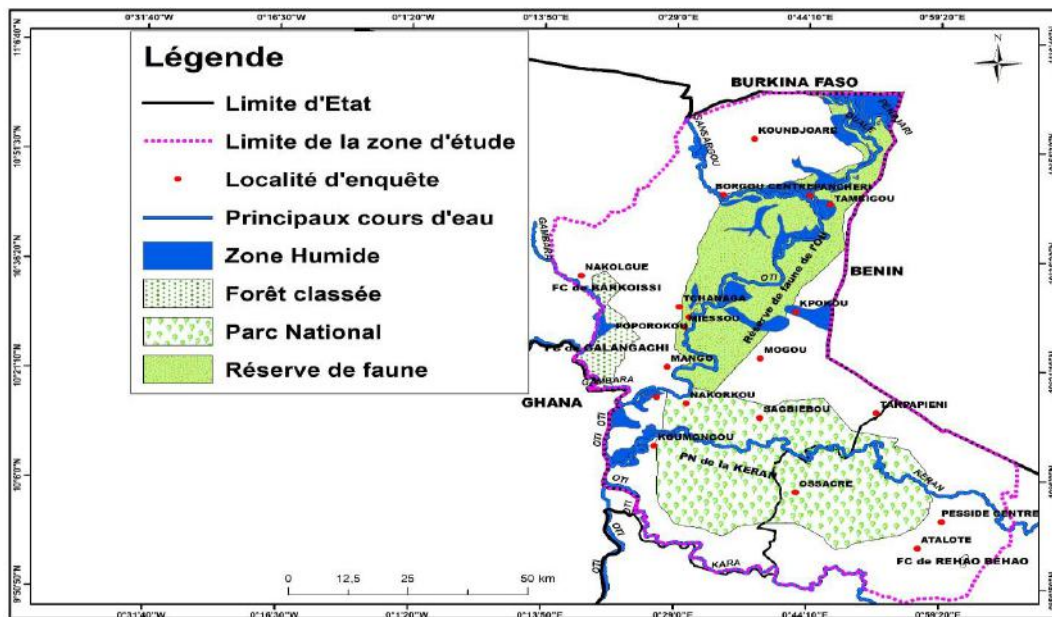


Fig.1 : Map of the study area

## 1.2 Methodological approach and data collection tools

### 1.2.1 Spatial analysis of land use in the wetlands of the Oti plain between 1987 and 2019

The cartographic approach is based on a diachronic study of land-use maps of the Oti plain between 1987 and 2019. In order to assess the dynamics of landscape change and land use in the wetlands of the Oti plain, a spatial analysis of land use was carried out. This spatial analysis was carried out using Landsat 8 images with high spectral resolution and Google Eart.

#### ➤ Image processing tools

Image processing, GIS and statistical analysis tools were used. These tools are :

- ERDAS Imagine 2011 and Leica Geosystems Geospatial Imaging software for digital image processing, radiometric and geometric corrections, band assembly, mosaicking and supervised image classification;
- ArcGIS and ENVI software for map production;
- Excel spreadsheet for graphical representation of statistics extracted from cartographic results, conversion and import.

#### ➤ Processing satellite images

The study of the dynamics of land use in the wetlands of the Oti plain over the period 1987-2019 consisted firstly of a supervised classification of each satellite image. The classifications were then followed by a pixel-by-pixel change detection analysis to identify and correct unlikely trajectories. Once these images had been classified and corrected, the quality of the

post-processing was finally assessed in order to validate or not the corrections made.

#### ➤ Diachronic analysis of land use between 1987 and 2019

Once the land cover maps for 1987 (T1) and 2019 (T2) had been drawn up, a comparative analysis of the two states T1 and T2 was carried out by calculating the Rate of Expansion ( $\Delta$ ) given by the formula proposed by the Food and Agriculture Organization (FAO) in 1996

(Ecological Monitoring Centre et al., 2012):  $\Delta = S1-S2$  where S1 and S2 represent respectively the percentage proportion of the area of a land cover class in the most recent year and the oldest year.

If:  $\Delta = 0$  then there is stability (S);

$\Delta > 0$  then there is progressive change (P);

$\Delta < 0$  then there is regression (R).

This method was used to assess the evolution of the different land cover units from 1987 to 2019 and to determine the different orders of regression (1, 2, 3, ...n), progression (1, 2, 3, ...n) and stability, since dynamics reflect the effects of population activities on the vegetation cover. Using the formula proposed by the FAO (1995), which is widely used (HOANG and al., 2002; NOYOLA-MEDRANO, 2006; PUYRAVAUD, 2003; SPARFEL, 2012; OLOUKOI, 2013; Brun and al., 2018), the balance (T) is calculated by the formula:  $T = (1/(t2-t1)) \times \ln (S2 / S1)$ .

where S1 and S2 correspond respectively to the area of a land cover category in year t1 and year t2; T is the number of years of change (balance); ln is the natural logarithm.

**1.2.1 Climatic vulnerability of the Oti plain wetlands: rainfall and temperature**

Climatological data such as temperature and rainfall from 1970 to 2021, either 50 years, have been taken into account in the analysis of this climatic vulnerability; this is to comply with WHO (1980) requirements according to which a minimum of 30 years is needed for a valid study of climate change (BADJANA and al., 2011). The diachronic analysis of changes in these two climatic parameters in the region over the time periods 1970-1995 and 1996-2021 enabled us to assess the trends in these parameters over 50 years and their impacts on the wetlands of the Oti plain. This analysis was based on rainfall and temperature data from the synoptic stations at Mango, Dapaong and Niamtougou.

**1.2.2 Anthropogenic factors in the degradation of Oti wetlands**

In order to study the relationship between the wetlands and the anthropogenic factors (agriculture, logging, livestock

farming, fishing and hunting) responsible for their degradation, surveys and field trips were carried out among the target groups selected for this purpose.

Socio-economic data was collected on the basis of survey sheets using direct and semi-direct interview techniques, interview guides and focus groups.

- Sampling technique

The random selection method was adopted as the sampling technique. Given the specific nature of the wetlands in the study area, the localities surveyed were selected taking into account 03 factors: their spatial distribution in the study area, the presence of human activities that degrade the wetlands and also the strategic position that wetlands occupy in socio-economic activities. Three localities were surveyed. These were Tchanaga, Sadori and Péssidè.

In these localities, the stakeholders taken into account in the survey are: farmers, stockbreeders, hunters, fishermen and loggers. The people interviewed were aged between 35 and 50, and their activities had a direct influence on the wetland ecosystems. A total of 135 people were surveyed in all three localities. Table 1 below shows the spatial distribution of the people surveyed by sector of activity.

Table 1: Spatial distribution of respondents by sector of activity.

Locations surveyed	Sectors of activities surveyed	Workforce by business sector	Total workforce by location
Tchanaga	Farmers	15	40
	Breeders	8	
	Fishermen	7	
	Hunters	5	
	Foresters	5	
Sadori	Farmers	20	50
	Breeders	10	
	Fishermen	10	
	Hunters	5	
	Foresters	5	
Péssidè	Farmers	20	45
	Breeders	10	
	Fishermen	5	
	Hunters	5	
	Foresters	5	
TOTAL			135

### - Processing of socio-economic data

The survey data were processed using Kobocollect and Excel 2013 software. Based on this processing, the factors causing wetland ecosystem degradation in the various localities were identified.

#### 2.2.4 Assessment of the socio-economic impacts of wetland degradation on local populations

The socio-economic impacts of wetland degradation on local populations were assessed using the PAPOLD method (Participatory Analysis of Poverty and Livelihood Dynamics). This method is based on a technique called "Stages of Progress", which was developed by Prof. Anirudh Krishna of Duke University in the United States to obtain the local community's conceptions of poverty, prosperity and the phases through which households could emerge from their extreme poverty and become prosperous (KALINGANIRE and al, 2005). For this assessment, three categories of producers were taken into account because of their direct influence on the wetlands. These were farmers, livestock breeders and fishermen in the three localities surveyed.

The first step was to get the producers to identify the criteria for prosperity in the local terms of Poor or Deprived, Moderately Well Off, Well Off. To do this, we asked key questions such as: what are the most important goods for a young farmer setting up in business, and what are the priority needs to be met as his means increase? What are the minimum needs of the poorest farmer in the village?

People are capable of listing these goods and needs, which constitute the criteria for prosperity. We had to reach a consensus on the order of the criteria and number them. In this way, all the prosperity criteria for the locality were ranked and we proceeded to draw the poverty line and the prosperity line.

We considered two different periods chosen by the people themselves in order to study the dynamics of poverty. The choice of each period was dictated by an event that had marked the locality, such as starvation, flooding, drought, etc.

The socio-economic data obtained using the "PAPoLD" method was entered in tabular form using Excel 2013 software. The various prosperity criteria selected for all three localities were codified and then harmonised using the

formula below:  $Y_i = (n_i - 1) / (N_i - 1)$  where  $n_i$ : the value of the criterion in locality;  $N_i$ : the total number of criteria cited in locality.

To take account of the fact that the number of criteria in the three localities is not the same, the prosperity classes for all the localities were normalised using the formula :  $M_i = \sum Y_i / 4$  with  $Y_i = (n_i - 1) / (N_i - 1)$ .

#### Movements in household poverty and the magnitude of the movement

The movement in poverty was measured by comparing the level of prosperity of the producer in 2002 and that in 2022. To do this, 4 classes were defined using the PAPOLD method:

- A: poor in 2002 and still poor in 2022
- B: poor in 2002 and not poor in 2022
- C: non-poor in 2002 and poor in 2022
- D: not poor in 2002 and still not poor in 2022

A class was assigned to each producer according to their situation.

Movements in poverty were assessed by calculating the percentage of prosperity classes (Deprived, Moderately Well Off, and Well Off) and poverty movement classes (A, B, C, and D), for all four localities from 2002 to 2022. Similarly, the prosperity classes according to poverty movements were determined by percentage calculations.

Based on the two prosperity levels in 2002 and 2022 and the prosperity movement class, we can assess the magnitude of the prosperity movement. This can be positive, negative or zero. It is used to classify producers. The magnitude of poverty movements was determined by calculating the difference between the 2022 prosperity class and that of the historical year used in the locality.

### III. RESULTS

#### 3.1 Spatial analysis of land use in the wetlands of the Oti plain between 1987 and 2019

Spatial analysis of the land-use change map (Fig. 2) of the wetlands between 1987 and 2019 shows that the hierarchy of land-use types has remained the same, but the relative importance of the units has varied over time.

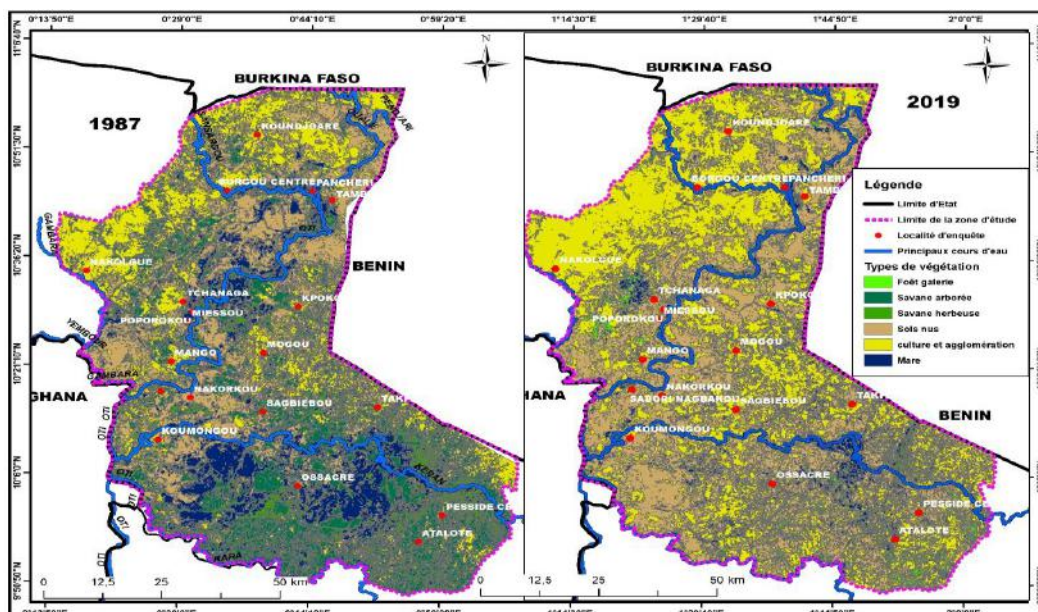


Fig. 2: Land use in the wetlands of the Oti plain between 1987 and 2019.

Examination of Figure 2 shows that from 1987 to 2019, land-use units have changed in time and space. For example, ponds and swamps, gallery forest, wooded savannah and grassy savannah have declined in favour of bare soil and cultivated and built-up areas. This explains the various pressures that these formations are subject to from man.

➤ Evolution of wetland land use units between 1987 and 2019

In 1987, the land-use units of the Oti plain were dominated by wooded savannah, which occupied 25% of the total surface area; bare soil, crops and settlements covered 23%. Ponds and swamps accounted for 13%, while grassy savannahs made up 9% of the total wetland area. The remaining 1% was gallery forest (Table 2).

Table 2: Change in hectare area of land use units from 1987 to 2019.

Occupancy units	1987		2019		Expansion rate
	Surface area in Ha	Proportion in %	Surface area in Ha	Proportion (%)	$\Delta = S_{2019} - S_{1987}$ in %
Bare floors	171792,15	23,34	296739,14	40,32	16,98
ponds and swamps	132310,98	18,00	55990,60	7,61	-10,39
Cultures and urban areas	171098,75	23,24	352780,63	47,93	24,69
Gallery forest	7776,50	1,00	6233,56	0,85	-0,15
Wooded savannah	183686,71	25,00	14876,00	2,02	-22,98
Grassy savannah	69333,74	9,42	9378,91	1,27	-8,15
Total	735998,84	100,00	735998,84	100,00	-----

Analysis of Table 2 shows that in 2019, wooded savannah has completely regressed, falling from 25% of the total area in 1987 to 2% in 2019, i.e. a rate of expansion of -22.98%.

At the same time, grassy savannah, ponds and swamps have also declined, from almost 10% of the total area to 1.27% for grassy savannah and from 18% of the total cover to

almost 8% for ponds and swamps. Forest galleries, which occupied 1% of the total surface area in 1987, have also declined, representing just 0.85% of the total surface area in 2019, with an expansion rate of -0.15%. However, there has been a significant increase in bare land, crops and built-up areas. The area of bare land rose from 23.34% to 40% between 1987 and 2019. The area under crops and built-up areas rose from around 23% to almost 48% between 1987 and 2019.

This analysis shows that the trend in land use units is positive overall for man-made formations, with positive expansion rates, while it is regressive for natural vegetation formations, with negative expansion rates (Fig 3).

The extent of the changes observed is a consequence of the strong human pressures leading to growing needs for arable land and the uncontrolled exploitation of the natural resources of the wetlands of the Oti plain.

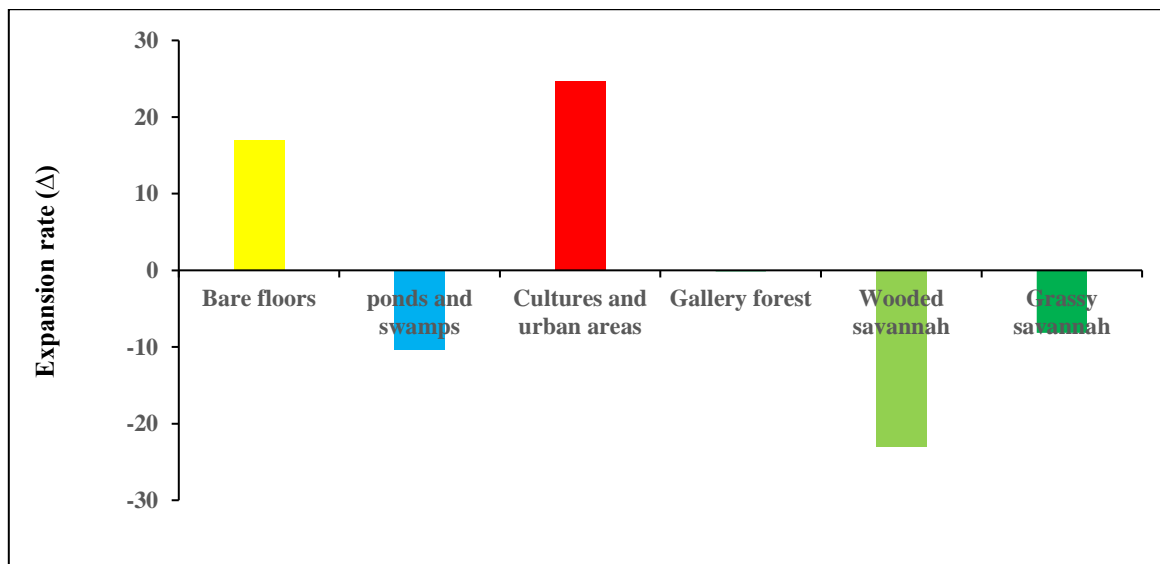


Fig. 3: Evolution of the expansion rate of land cover classes.

➤ **Assessment of the dynamics of land use in wetlands between 1987 and 2019**

The spatio-temporal dynamics of land use units in the wetlands of the Oti plain is expressed in terms of:

- ❖ **Stability:** it takes into account the portions which, apparently, have not experienced pronounced changes or at least have kept the same appearance between 1987 and 2019. The dynamics of occupation reveals that no unit has experienced stability from 1987 to 2019. All have either regressed or progressed.
- ❖ **Regression:** it concerns units which have experienced a deterioration or reduction in their surface area. There are three orders of regression (R1, R2 and R3). In our case, only the R1 regression was observed and relates to the portions having undergone very pronounced degradation. This is the case for units such as: ponds and swamps, gallery forest, tree savannah and grassy

savannah. These units are gradually transformed either into agricultural fields (crops), or into towns or into bare soil. This demonstrates the impact of the various anthropogenic activities carried out on the components

of the humid ecosystems of the Oti plain.

- ❖ **Progression:** it applies to units which have evolved to become denser in 2019 compared to 1987. There are also three orders of progression (P1, P2 and P3). In the present case, an order of progression (P3) was observed. It takes into account the units of wooded and grassy savannahs, ponds and swamps and part of the gallery forests in 1987, which are gradually being transformed into agricultural fields, towns and bare soils in 2019.

Figure 4 below presents the summary in area of the different land use units in the Oti plain.

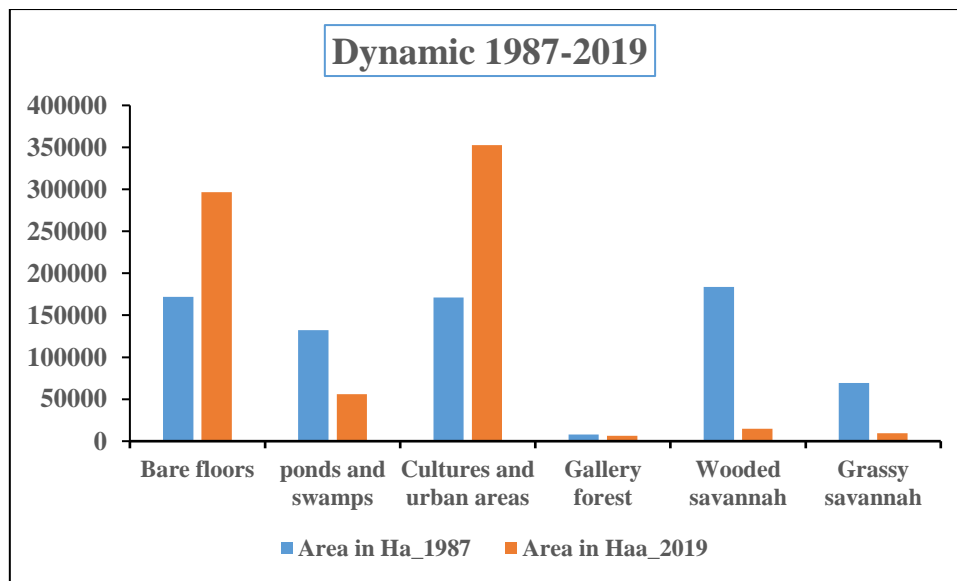


Fig. 4: Variation in the evolution of the surface areas of land use units (1987-2019).

Source: Data processing results, 2023.

### 3.2 Climatic vulnerabilities of the wetlands of the Oti plain: precipitation and temperature

#### ➤ Climate change in Togo

As a West African country, Togo's climate is warming globally, with an increase in average temperatures ranging from 0.8°C to 1.2°C between 1961 and 2018 (CNCC, 2022). In addition, the National Climate Change Adaptation Plan (PNACC) indicates that between 1962 and 2012, the climate warmed by 1°C compared with previous periods. The general temperature has risen throughout the country. Other aspects of the climate have also changed, such as rainfall. Annual rainfall has fallen by between 3 mm and 81 mm (PNACC, 2017, p.18). The risk of the climate drying out is growing all the time, as rainy periods are no longer long enough to compensate for the evaporation of water from the soil, especially in northern Togo, the region covering our study area,

which is close to the Sahelian zone. Also, the climate change scenarios for Togo drawn up by the PNACC predict that there could be a rise in average temperature ranging from 0.9 to 4.5°C. In the coming years, if the climate evolves as it is currently happening, Togo will have to face various climate risks such as floods, droughts, extreme heat, irregularities in the seasons, rainfall shifts, more violent winds, major soil erosion (the Savanes Region is already experiencing all these elements but they will be amplified), and finally, landslides and a rise in sea levels (PNACC, 2017, p.19).

#### ➤ Spatio-temporal evolution of climatic parameters in the region covering the wetlands of the Oti plain by time intervals 1975-1995 and 1996-2021

##### - Spatio-temporal evolution of temperatures.

The Spatio-temporal evolution of temperatures in the study area over a quarter of a century can be seen by studying the isotherm maps shown in Figure 5.

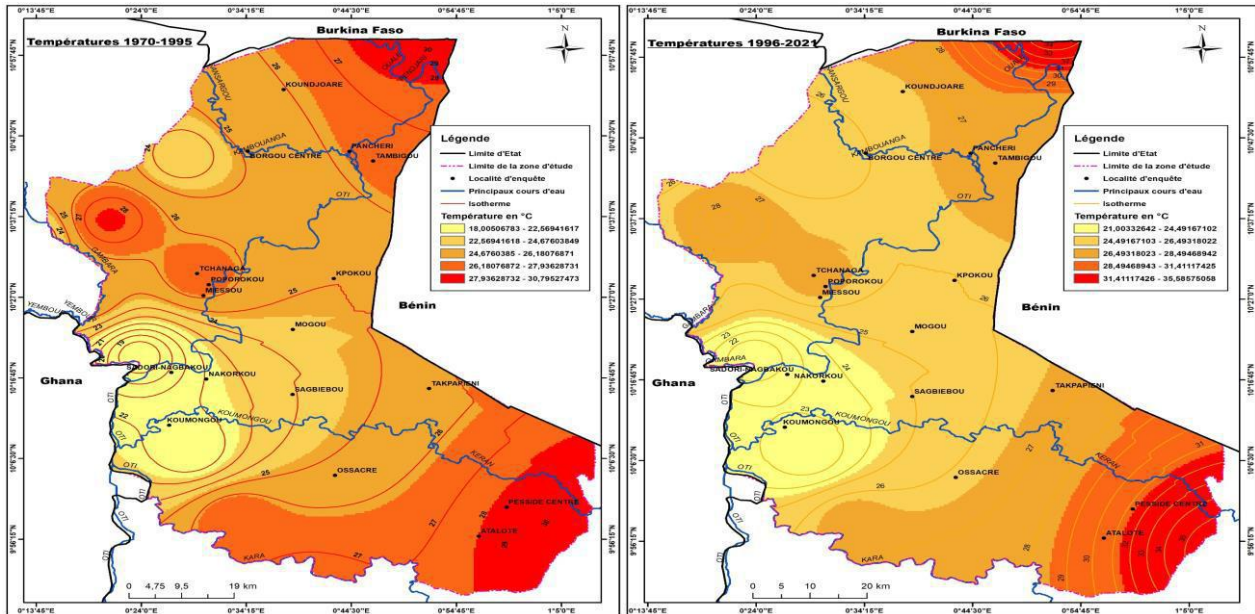


Fig.5 : Isotherms of the average annual temperature over twenty-five years in the region of the study area

Analysis of Figure 5 shows that, overall, average temperatures have risen over the last half-century (1970-2021). The period 1970-1995 is the warmest, with temperatures around 22°C, limited by the 23°C isotherm. Beyond this isotherm, temperatures rise towards the south-east and north-west, reaching 30°C. The maximum temperature is 30.5°C in the Pendjari area towards Mandouri.

Over the period 1996-2021, temperatures are around 24°C, limited by the 25°C isotherm, and the Pendjari and Kanté

areas reach 35°C, indicating a warmer climate than in the previous twenty-five years. In fact, temperatures have risen by around 1°C.

- **Spatio-temporal evolution of precipitation**

The spatio-temporal evolution of rainfall in the study area over a quarter of a century is shown by studying the isohyet maps presented in Figure 6.

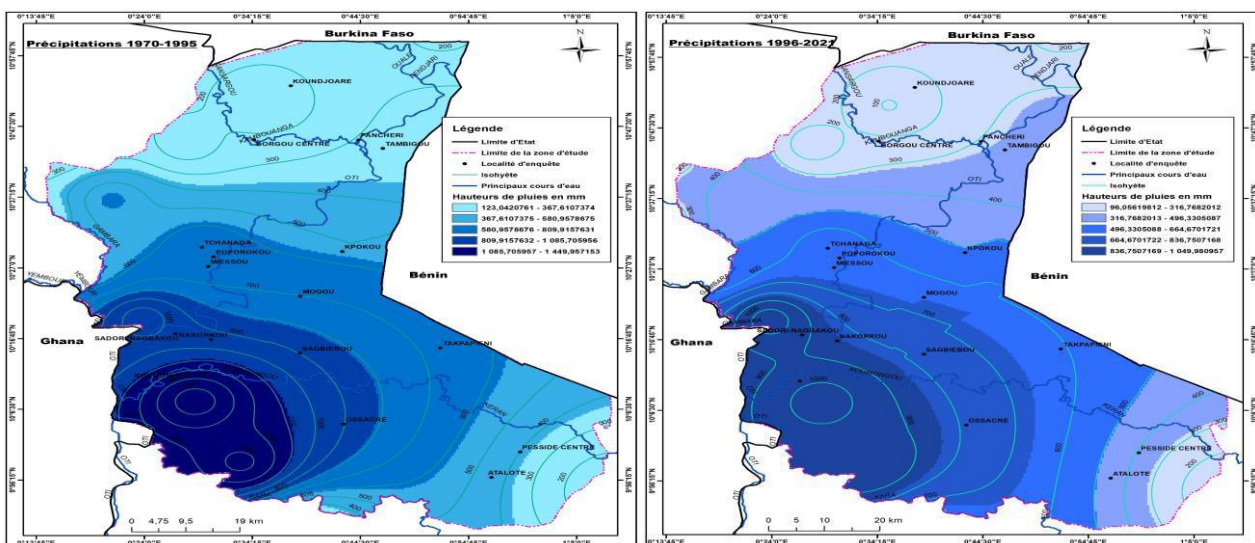


Fig.6: Isohyets average annual precipitation over twenty-five years in the region of the study area



Analysis of Figure 6 shows that rainfall has fallen over the last five decades (1970-2019). The period 1970-1995 was marked by significant inter-annual fluctuations, with rainfall of around 1,400 mm limited by the 1,100 mm isohyet. Above this isohyet, rainfall decreases towards the south-east and north-west to 200 mm. Maximum rainfall is 1,450 mm at Koumongou.

Over the period 1996-2021, rainfall is around 1,000 mm, limited by the 800 mm isohyet, and Koumongou reaches 1,050 mm. This reflects a drop in rainfall of around 400 mm in less than 30 years, accompanied by a migration of the 1400 isohyet towards the south of the country.

**3.3 Anthropogenic factors in the degradation of Oti wetlands**

Anthropogenic factors in the degradation of Oti wetland ecosystems are activities that have a negative impact on the environmental components of wetlands. The direct determinants identified during our field trip are: agriculture, lumbering, livestock farming, hunting and fishing.

The importance of these degradation factors was assessed according to the perception of local populations (Figure 7). Analysis of this figure shows that agriculture, forestry and livestock farming are the most important factors in wetland degradation. Agriculture, through activities such as land clearing and the use of herbicides, is leading to the gradual disappearance of natural wetland formations and, by extension, wetlands. As a result, the Oti plain is witnessing an alarming gradual degradation of its natural potential, with rich wetland forest formations disappearing in favour

of bare soil and farmland. In the search for land suitable for agriculture, forest galleries are being devastated or even destroyed.

Forestry activities such as charcoal production (carbonisation) and wood energy convert natural plant formations into bare soil and agricultural land. Livestock farming is cited by the local population as a major factor in the degradation of wetland components, due to the damage caused by transhumant animals and overgrazing. In fact, the lack of grazing in Sahelian countries over a good part of the year (from December to May), the search for outlets for livestock products and the low rate at which national meat consumption is covered by local production have considerably encouraged the transhumance of Sahelian cattle to Togo. This transhumance is expanding rapidly, leading to the degradation of natural resources through the destruction of flora by tree pruning and cutting, overgrazing and the degradation of pasture and soil quality, the destruction of hydraulic structures and water pollution.

With low importance values, fishing and hunting were also seen as factors in the degradation of wetlands. Poor fishing practices, such as illegal fishing, failure to select the right species and size of fish, and the use of inappropriate techniques, lead to the over-exploitation of fishery resources and the destruction of aquatic ecosystems. As for hunting, it is perceived by local populations as a factor in the degradation of vegetation because hunters, by seeking to make game areas accessible, provoke bush fires which are powerful determinants of the degradation of wetland vegetation.

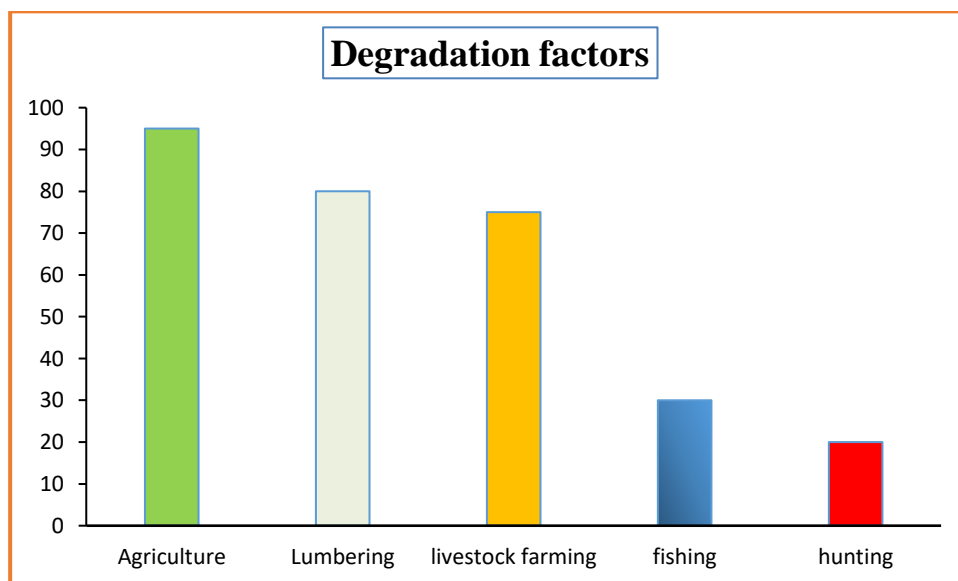


Fig.7 : Wetland degradation factors

**3.4 Assessment of the socio-economic impact of wetland degradation on local populations**

➤ **Prosperity criteria in the three localities bordering the wetlands of the Oti plain**

The prosperity criteria listed by the producers are sometimes similar, although they vary from one locality to another. In Sadori, 21 prosperity criteria were listed. The first criterion is the straw-built house. The poverty line is located at the limit of criterion 5 (small solar panel). The line of prosperity begins at criterion 12 (sheet metal house) The PAPoLD revealed 22 prosperity criteria in Tchanaga, with the poverty line limited to criterion 6 (bicycle) and the prosperity line limited to criterion 13 (sheet metal house). As in Péssidè, the first criterion corresponds to a daba/hoë, while the last corresponds to a television set and a Canal+ aerial.

The different criteria listed in each locality, along with the poverty and prosperity lines, are summarised in Tables 3

and ends at criterion 21 (goods vehicle). This means that in Sadori, building a house made of sheet metal is a starting point for any wealthy person. The average farmer would therefore fall between the two lines of poverty and prosperity, i.e. criteria 5 and 12.

In Péssidè, 22 criteria were identified, with the poverty and prosperity lines located respectively at the limit of criterion 8 (Small solar plate) and criterion 15 (Sheet metal house). The first criterion corresponds to the daba/hoë and the last to a general food shop.

and 4, Daba/hoë, poultry, plots of land and bicycle occupy more or less similar positions in the different localities, while other criteria occupy different ranks from one locality to another or are only mentioned in one of the 3 localities. Although the position and importance of each criterion varies from village to village, the criteria as a whole reflect the socio-economic realities of the three localities.

*Table 3: Sadori and Péssidè prosperity criteria*

N°	Classes	Sadori Prosperity Criteria	N°	Classes	Péssidè Prosperity Criteria
1	Destitute	Daba/hoë	1	Destitute	Daba/hoë
2		Field plot	2		Poultry
3		Straw banco house	3		Field plot
4		Bike	4		Straw banco house
5		Small solar panel	5		Primary education
		<b>Poverty line (1-5)</b>	6		Radio set
6	Moderately well off	Endure house	7		Bike
7		Electricity at home	8		Small solar panel
8		Motorbike			<b>Poverty line (1-8)</b>
9		Poultry			
10		Goats/Sheep	9	Moderately well off	House in tiled banco
11		Solar panel	10		Motorbike
		<b>Line of prosperity (12-21)</b>	11		Level of secondary education
12	Nantis	Sheet metal house	12		Goats/Sheep/Pigs
13		Electricity at home	13		Solar panel
14		Solar panel	14		Increase in field area
15		Motorcycle/tricycle			<b>Prosperity line (15-22)</b>
16		Shop	15	N	Sheet metal house
17		Goats/Sheep	16		Electricity at home
18		Steers	17		Solar panel

19	large agricultural area	18	Motorbike
20	Télé+ Canal+ antenna	19	Large agricultural area
21	Goods vehicle	20	Goats/Sheep
		21	Steers
		22	General food shop

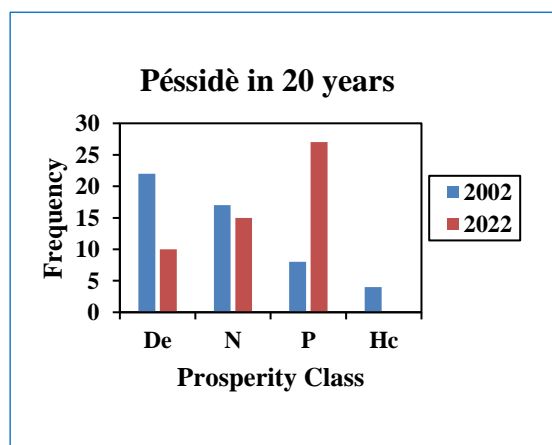
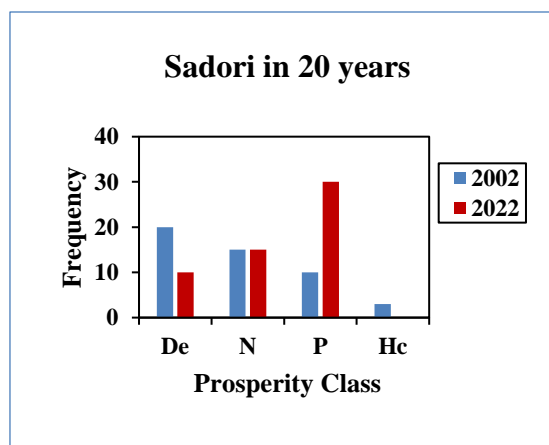
Table 4: Tchanaga prosperity criterion

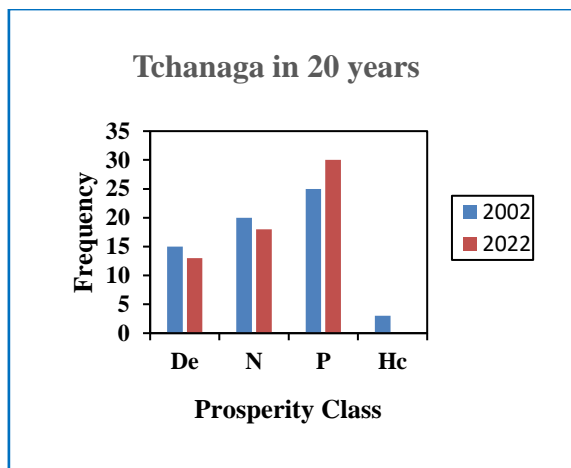
N°	Classes	Tchanaga Prosperity Criteria
1	Destitute	Daba/ho
2		Poultry
3		Field plot
4		Radio set
5		House in banco
6		Bike
		<b>Poverty line (1-6)</b>
7	Moderately well-off	banco house in sheet metal
8		motorbike
9		secondary education
10		Goats/Sheep/Pigs
11		solar panel at home
12		Increase in field area
		<b>Prosperity line (13-22)</b>
13	Nantis	Sheet metal house
14		Electricity at home
15		Solar panel
16		Motorbike
17		Shop
18		Goats/Sheep
19	Steers	

➤ Evolution of prosperity classes by year, by locality

Figure 8 shows the evolution of prosperity classes from the year of the major event to 2002, in the three localities studied. It can be seen that the local populations of Tchanaga, Sadori and Péssidè are distributed among the three prosperity classes (Deprived, Moderately Well Off, Well Off). As the gap between the year 2002 and 2022 was large in the three localities, individuals outside the class were identified. Because of their young age, these individuals were hardly working at all in 2002.

The main finding is that there has been an increase in the number of affluent people and a decrease in the number of middle-income and poor people. The major events have obviously had negative repercussions on the economies of the local populations at both individual and local levels. After these difficult years, the repercussions can be seen in the movement of farmers in the Oti wetlands from one class to another or simply from one level of the class to another.





Legend: P: Well off N: Moderately well off De: Deprived HC: Out of class

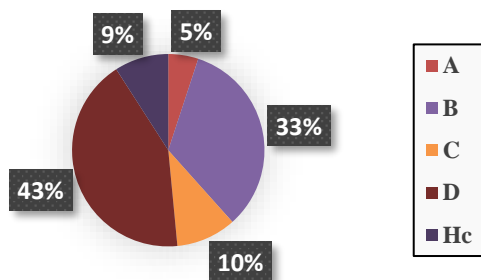
Fig.8: Evolution of prosperity classes in the 3 localities according to the year

➤ **Breakdown of poverty movements by year, by locality**

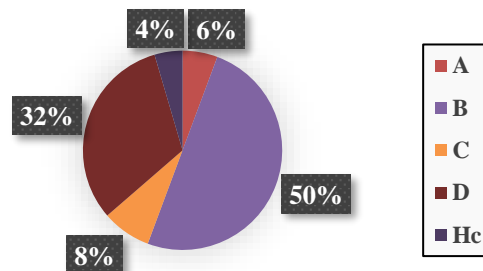
The study of the various movements has made it possible to distinguish four categories of individuals between 2002 and 2022: those who were poor and remained poor (A); the poor who became moderately well-off or well-off (B); the well-off who became poor (C); and finally the non-poor who retained their non-poor status (D). Figure 9 shows the distribution of poverty movements in the three localities. It shows that, in all these localities, the highest proportions

were found among individuals in category B, which means that the majority of moderately well-off farmers either remained as they were or moved into the well-off category. The affluent individuals either remained affluent or became affluent. Assessing the magnitudes of these movements enables us to determine whether they are positive or negative.

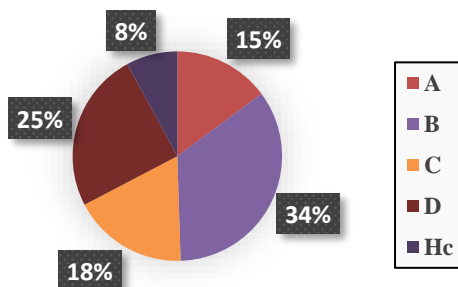
Distribution of prosperity movement in Pésside



Distribution of the Prosperity Movement in Sadori



Distribution of the prosperity movement in Tchanaga



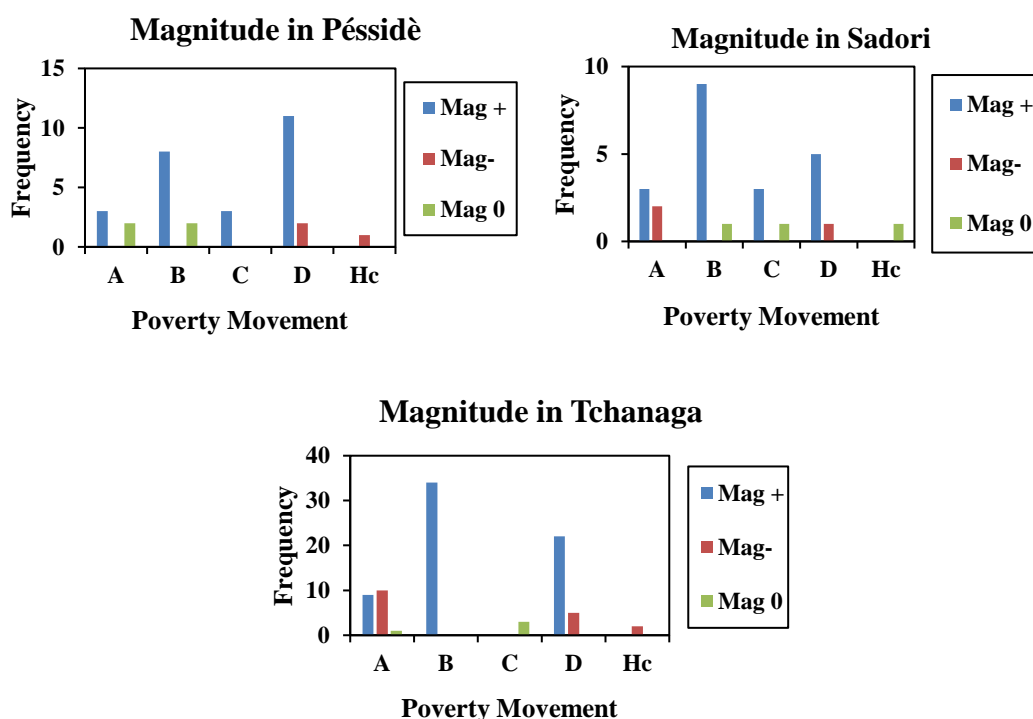
Legend: A: still poor in 2022; B: poor in 2002 and not poor in 2022 ; C: not poor in 2002 and poor in 2022; D: not poor in 2002 and still not poor in 2022; HC: out of class.

Fig.9: Distribution of poverty movements by locality

➤ **The magnitude of the poverty movement in the three localities**

Figure 10 shows the evolution of the magnitude of movements in the three locations studied. In category A, and only in Péssidè, there were no negative magnitudes, but rather positive and zero magnitudes. This means that without having left the prosperity class that characterised them at the outset, individuals have moved to a higher level

or remained stable on the prosperity scale. The exception is Tchanaga, where the movements of individuals in category B are characterised by positive and zero magnitudes. It can also be seen that for individuals who were once rich but are now poor in Péssidè, the magnitudes are positive, while in Sadori and Tchanaga they are positive and zero. The diversity of magnitudes is found in category B insofar as poverty status has changed in Nantis.



Legend: A: always poor in 2022; B: poor in 2002 and not poor in 2022 ; C: not poor in 2002 and poor in 2022; D: not poor in 2002 and still not poor in 2022; HC: out of class: Mag + = Positive magnitude; Mag - = Negative magnitude Mag 0 = Zero magnitude

Fig.10: Magnitude of poverty movement in the 3 localities

**IV. DISCUSSION**

➤ **Spatio-temporal dynamics of land-use units in wetlands**

The results of our study showed that the dynamics of land-use units in the wetlands of the Oti plain over the period 1987-2019 saw a decline in the natural units characteristic of wetlands, in particular water bodies (ponds and swamps), forest gallery, wooded and grassy savannahs, in favour of bare soil, crops and built-up areas.

In fact, the area of wooded savannah and grassy savannah has shrunk exponentially over the last thirty years, with rates of regression of -22.98% and -8.15% respectively. Crops and settlements have increased by 24.69% of the area they occupied in 1987. As a result, a large portion of the wetland that was covered by natural formations in 1987 is now covered either by man-made units (crops and settlements) or by bare soil in 2019. The forest gallery, which represented 1% of wetland vegetation cover in 1987

and protected the banks of watercourses, has regressed with an expansion rate of -0.15% and has been transformed into arable land. The ponds and marshes that make up the lungs of the wetlands have dried up due to the droughts experienced by the country in the 1980s or have gradually been transformed into cultivated areas, which justifies the reduction in their surface area from 18% in 1987 to 7.61% in 2019.

Overall, the surface area of man-made formations (crops and settlements) in the wetlands of the Oti plain increased over the period 1987-2019. The increase in the surface area of these units is caused by urbanisation coupled with galloping demographics and the ever-growing need for biological resources and arable land, most often to the detriment of savannah areas. This confirms the results of the work of IWEDIGA and al, (2012), DIMOBE and al, (2012), DIOP and al, (2018), BRUN and al, (2018) concerning the loss of dense plant formations in favour of agricultural areas. Indeed, the work of IWEDIGA and al, (2012) showed that off-season agricultural practices on the banks of watercourses in the Oti plain had a negative impact on riparian vegetation, particularly gallery forests. Similarly, DIMOBE and al (2012) showed that human activities (hunting, fishing, medicinal plant harvesting, wood cutting and collection, charcoal burning, straw harvesting in the savannah, etc.) had a negative impact on riparian vegetation, particularly gallery forests, bush fires and transhumance) carried out in the Oti-Mandouri reserve have resulted in the rarefaction of certain plant species, a reduction in forest cover, the loss of animal habitats and biodiversity, soil erosion, changes in water regimes and

climate disruption (Photo 1). In addition, the results of BRUN and al (2018) on the dynamics of land use in the wetlands of the Commune of Allada in southern Benin have highlighted the negative impact of man on wetlands. They showed that the major trends reveal a decline in natural formations in favour of man-made formations. The total disappearance of the shrub savannah shows man's strong hold on his natural environment, which provides immeasurable wealth to satisfy his vital needs.

Agreeing with his predecessors, ADJAKPA (2020) confirmed that the main factors in the degradation of the natural resources of wetlands are human activities that devastate the environment, such as shifting cultivation, the exploitation of forest products and transhumant livestock rearing, which are practised to an alarming degree. Heavy migration towards the wetlands is leading to the systematic over-exploitation of all natural resources. To reverse this trend, strategies based on ecological restoration, which consists of assisting the self-regeneration of degraded or destroyed ecosystems, should be implemented. This involves attempting to re-establish pre-existing plant formations, rather than forcing nature by trying to impose other biological models. Among the best strategies for safeguarding and saving ecosystems are the restoration and rehabilitation of terrestrial and aquatic ecosystems, ecological improvements in the use of biological resources, and the establishment or improvement of human behaviour that incorporates knowledge of the importance of natural capital, as well as its conservation and management.



*Clearing and cutting wood*



*Transhumant breeding*



*Using wetlands to grow rice*

*Photo 1: A few shots showing how wetland resources are exploited.*

*Source: LAMBONI, 2023.*

➤ **Climatic vulnerability of the wetlands of the Oti plain**

The IPCC's assessment of climate change in September 2013 confirms that since 1901, temperatures measured at the Earth's surface have risen overall on average. According to the same report, global warming in Africa is likely to be greater than at global level (IPCC, 2013).

The warming detected in the north of Togo in the study area by analysing changes in average temperatures over the period 1970-2021 is part of this global warming trend. This analysis shows that average temperatures have increased over the comparative periods 1970-1995 and 1996-2021. There has been an increase in average minimum and maximum temperatures. The last quarter of the century has been the warmest, with temperatures rising by around one degree Celsius. These results are comparable to those carried out by ADAMELI and DUBREUIL, (2015) on temperature trends in Togo between 1961 and 2010. The results show a temperature increase of between 0.3 and 1.6°C, which has been more pronounced in the lowlands than in the mountain ranges since the late 1970s.

In the case of precipitation, the results of our work revealed a decline in precipitation over the last five decades (1970-2019). The period 1970-1995 was marked by significant

inter-annual fluctuations, with rainfall of around 1,400 mm, compared with the period 1996-2021, when rainfall was around 1,000 mm. This represents a significant drop compared with the 1970s. This situation has resulted in a southward shift in isohyets.

All the results point to a downward trend in rainfall and an upward trend in temperature, with significant inter-annual variability accompanied by breaks in rainfall.

These changes are having a disastrous impact on wetland environments and populations. Global warming is causing land to gradually dry out and deteriorate, increasing farmers' dependence on wetlands in search of fertile wetlands (ADJONOU, 2009; BADJANA, 2010; DJENONTIN, 2010). Falling rainfall is leading to a reduction in the area of wetlands. One of the consequences of the general deterioration in the climate is the proliferation of floating vegetation (water lettuce, water hyacinth, typha, etc.), due in particular to the reduction in the flow velocity of watercourses, the change in their temperature and the deterioration in water quality. They are suffocating several bodies of water in the region, including wetlands whose biological diversity is recognised as being of worldwide importance (NIASSE et al, 2004) (photo2).



*Photo 2: Pond invaded by water hyacinth near Gando  
Source: LAMBONI, 2022.*

➤ **Assessment of the socio-economic impact of wetland degradation on local populations**

It is widely recognised that the survival of Africa's rural populations depends on agricultural produce, timber and non-timber products. Local populations, mainly rural and agro-pastoralists, are highly dependent on natural resources that are highly sensitive to climate, which explains their vulnerability, given their low capacity to adapt (IPCC, 2004). The various adaptation strategies developed by these populations to cope with the adverse effects of climate change are increasing the pressure on wetland ecosystems in the Oti basin. Wetlands, particularly the floodplains of the Oti, represent a rich ecosystem that provides people with a means of subsistence as they strive to achieve food security. They also provide drinking water and are a last refuge for biodiversity. Against a backdrop of climate change and demographic pressure, these areas are increasingly coveted and under threat. Our research reveals a correlation between the level of prosperity and the state of natural resources. In all three localities, the number of wealthy people has increased, indicating a decline in poverty compared to the reference year. This is largely due to the exploitation and marketing of agricultural, fishing, hunting and livestock products. Many farmers have made their fortunes marketing oilseed crops such as soya and sesame over the last ten years. Since 2021, soya exports have generated nearly CFAF 50 billion in Togo (INSEED, 2022). This agricultural revolution, encouraged by government efforts, has had a positive impact on the socio-economic situation of farmers. However, it is increasing pressure on natural resources.

It is also recognised that poverty in rural areas is an obstacle to the sustainable management of natural resources (BELEM et al, 2006). This is why the World Bank's environmental action plan focuses on the poorest sections of society. It believes that reducing poverty will make it possible to improve the quality of the environment and reverse the spiral of degradation (WORLD BANK, 1997). Thus, the fight to maintain and preserve wetlands will involve reducing people's dependence on natural resources. Investment in reforestation and the creation of agroforestry parks will also create a favourable climate for maintaining diversity in the biosphere reserves.

## V. CONCLUSION

This study has enabled us to show that the areas of natural land use units on the Oti plain have been declining over the last thirty years. The main trends show a decline in natural formations in favour of man-made formations. Over the same period, the climate of the Oti plain has become highly variable, alternating between dry and wet periods, with a general trend towards lower rainfall and higher temperatures. The effects of this climate change, coupled with human-induced degradation factors, are having a negative impact on the socio-economic situation of the local population. The consequences of these human activities are leading to the degradation of natural resources. Work is therefore urgently needed to set up local structures with internal rules for the management, preservation and protection of the highly exploited natural resources of wetlands in order to preserve their sustainability.



### ACKNOWLEDGEMENTS

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# Evaluating the Effects of Different Sowing Dates and Tillage Practices on Faba bean Yield Based on DSSAT Model

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**Abstract**— The faba bean is a crucial pulse crop known for its high protein content and its ability to fix nitrogen from the atmosphere. The growth and yield of this crop are influenced by various agronomic practices, such as sowing date and tillage method. To investigate these factors, a two-year field experiment was conducted in the dry and hot environment of Egypt's western desert. The experiment aimed to evaluate the yield and its components of faba bean under four different sowing dates and three tillage practices. To accurately simulate the outcomes, the Decision Support System for Agro-technology Transfer (DSSAT) model was employed. This model allowed for the prediction of seed yield, total above-ground biomass, and harvest index based on the varying sowing dates and tillage practices. It was found that the highest seed yield, reaching  $4011 \text{ kg ha}^{-1}$  in the first season and  $4115 \text{ kg ha}^{-1}$  in the second season, was achieved when the plants were sown on October 15<sup>th</sup>. Additionally, the seed yield peaked at  $3690 \text{ kg ha}^{-1}$  in the first season and increased to  $4074 \text{ kg ha}^{-1}$  in the second season when the no-tillage practice was implemented. The other yield components followed a similar trend to the seed yield, responding to the different sowing dates and tillage practices. The DSSAT model proved to be a reliable tool for simulating the seed yield and total above-ground biomass, exhibiting a Nash and Sutcliffe Efficiency (NSE) above 0.80 when compared to the calculated values. However, for the harvest index, the NSE was 0.669 in the first year and 0.772 in the second year. Despite this slight discrepancy, the DSSAT model remains a valuable decision support tool for predicting faba bean yield under various agronomic practices.



**Keywords**— Sowing date, tillage practices, faba bean, New Valley, DSSAT, dry-hot environment

## I. INTRODUCTION

Egypt has a long-standing tradition of both producing and consuming the faba bean (*Vicia faba* L.), which has played a vital role in the Egyptian diet. In fact, it holds the distinction of being the nation's first leguminous food crop (Ouda and Zohry, 2017). However, the production of faba beans in Egypt has encountered numerous challenges over the years. Extensive studies have revealed a concerning decline in the cultivation areas of faba beans, plummeting from 271.5 thousand feddanes (1 feddan =  $4200 \text{ m}^2$ ) in 2000 to 175.4 thousand feddanes in 2019. Consequently, the total production has also suffered, dropping from 252.4 thousand tons in 2000 to 242.07

thousand tons in 2019. This decline has resulted in a significant decrease in self-sufficiency rates, which fell from 31.4% to 67.4% during the same period (Abdelaal and Soliman, 2022). Despite its historical significance, faba bean production in Egypt has faced considerable difficulties due to a shrinking cultivable area and fierce competition from other winter crops.

Heat stress has the potential to significantly reduce crop productivity and undermine global food security, particularly in the context of climate change (Lamaoui et al., 2018; Jagadish et al., 2021). Drought and heat are two major abiotic stresses that lead to significant decreases in the growth and yield of various important crops (Fahad et al.,

2017). High temperature is a critical stressor that restricts the growth and productivity of plants (Zhao et al., 2020). Extensive research has been conducted on the vulnerability of faba bean to heat and drought stress. It has been observed that heat stress during the flowering stage can result in a decline in the seed yield and its components of faba bean (Bishop et al., 2016). Moreover, the current rapid climate changes in arid regions have had severe adverse effects on faba bean production, particularly due to drought stress (Abdelhaleim et al., 2022). To mitigate the impact of heat stress on different crops, the adjustment of the sowing date has been identified as a potential strategy. This approach holds promise in alleviating the detrimental effects of heat stress on crop growth and productivity.

The impact of sowing dates on faba bean production in Egypt has been the subject of extensive research due to its significant influence on growth, production, and maturity. Several studies have shed light on this matter, providing valuable insights for farmers. In a study conducted by Tarek et al. (2020), it was concluded that late sowing of faba beans resulted in a shorter maturation period. Additionally, certain genotypes exhibited higher seed yields when sown on November 1<sup>st</sup>, which was the second sowing date used in the experiment. Another study by Badran and Ahmed (2010) explored the effects of different sowing dates on faba beans, revealing that it impacted the number of days to maturity, growth characteristics, and seed yield. The study also evaluated various sowing methods.

It is crucial for farmers to take into account local conditions, climate, and available resources when determining the optimal sowing date for faba beans in Egypt. For the cultivars Sakha 1 and Giza 461, it was found that seed yield and its attributes increased when sown early on October 31<sup>st</sup> (Badr et al., 2013; Hegab et al., 2014). Conversely, cultivating faba beans after the first of November led to a decrease in seed and biological yields. The lowest values for these yields were observed when faba beans were cultivated on the first of December in the Nile delta.

Tillage is the process of preparing the soil for planting or seeding by utilizing techniques such as plowing or turning the soil (Claassen et al., 2018). While it has long been a common practice in crop farming, tillage can have detrimental effects on soil health, leading to issues such as nutrient run-off and carbon sequestration (Bhattacharyya et al., 2022). The consequences of regular tillage include the loss of topsoil, reduced yields due to the depletion of organic matter and nutrients, and a decline in soil structure and overall quality (Zikeli et al., 2013). However, experienced organic farmers have found ways to mitigate

the negative impacts of tillage by operating equipment with care and maintaining optimal soil conditions (Mader and Barner, 2011). One alternative approach gaining attraction is conservation agriculture, a farming method that emphasizes the preservation of a continuous layer of soil cover, minimal disturbance of the soil, and the utilization of diverse plant species. The primary objectives of conservation agriculture are to enhance biodiversity, improve water and nutrient efficiency, and sustain crop productivity. To achieve these goals, three guiding principles are followed: (1) minimizing soil disturbance, (2) maintaining permanent soil cover through crop residues and/or cover crops, and (3) implementing crop rotations with a variety of plant species, including legumes (FAO, 2010). In Egypt, the implementation of these three principles has resulted in increased productivity of cultivated crops (Harb et al., 2015).

No-till farming is a highly beneficial practice that enhances soil quality by effectively retaining soil moisture, organic matter, and nutrients. It also plays a crucial role in reducing erosion, increasing biodiversity, and promoting the development of robust root systems in crops (Muñoz-Romero, 2011). However, it is important to acknowledge the challenges associated with no-till farming, such as the need for specialized equipment and the potential for increased weed growth due to undisturbed soil. Despite these challenges, no-till farming has consistently demonstrated its feasibility and sustainability as an approach to crop production, particularly when implemented with proper management techniques and in specific environments (Su et al., 2021). In fact, no-tillage systems have shown remarkable potential in enhancing soil fertility and quality while minimizing environmental impact in faba bean production. These systems effectively reduce soil N<sub>2</sub>O emissions and significantly increase crop productivity (Tedone et al., 2023). Reduced tillage and no-tillage practices are being extensively studied as viable alternatives to conventional faba bean farming. These practices have shown promising results in enhancing soil quality and boosting crop yield (López-Bellido et al., 2003; Amami et al., 2021).

Crop growth models are valuable tools that allow us to integrate our understanding of physiological processes with the ability to formulate and evaluate crop management strategies. One such model, the CROPGRO-Faba bean model, was developed by Boote et al. (2002). This model builds upon the foundation of the CROPGRO-Soybean model (Boote et al., 1998) and has been further refined through extensive growth analysis of two faba bean cultivars i.e., Alameda and Brocal cultivated in Cordoba, Spain. These cultivars were subjected to two seasons of irrigation, both under N-fixing and N-fertilized conditions

(Sau and Minguez, 2000). The model successfully predicted the total accumulation of dry matter in crops, the mass of pods, and the distribution of resources to different parts of the plant. The model accurately predicted high yields of faba bean, with yields exceeding 6000 kg ha<sup>-1</sup>, which aligned with the actual data. Sensitivity analyses on the timing of sowing indicated that the best yields were achieved with early winter sowing in Cordoba and late winter sowing in northern Europe. Using an existing mechanistic model like CROPGRO had its benefits as it allowed for the simulation of similar processes across different species (Manschadi et al., 1998 and Bogale 2021).

The primary agronomic factors that significantly impact the growth and yield of faba bean are the sowing date and tillage practices. In order to gain a better understanding of how these factors affect faba bean, this study aims to investigate the response of faba bean's yield and its various components to different sowing dates and

tillage practices. The study specifically focuses on faba bean cultivation under hot and dry conditions. To achieve this, a crop growth model called DSSAT was utilized to simulate the yield in two consecutive growing seasons.

## II. MATERIALS AND METHODS

Two field experiments were conducted at a private farm located in El-Moneera village, Kharga Oasis, New Valley governorate, situated in the western desert of Egypt (25.62°N, 30.64°E). The objective of these experiments was to assess the impact of sowing dates and different tillage practices, as well as their interaction, on the yield and its components of faba beans (specifically the Giza 716 variety). To provide a visual representation of the experimental period (1<sup>st</sup> of October to 30<sup>th</sup> of April) during the growing seasons of 2021/2022 and 2022/2023, Figure 1 displays the recorded average temperatures.

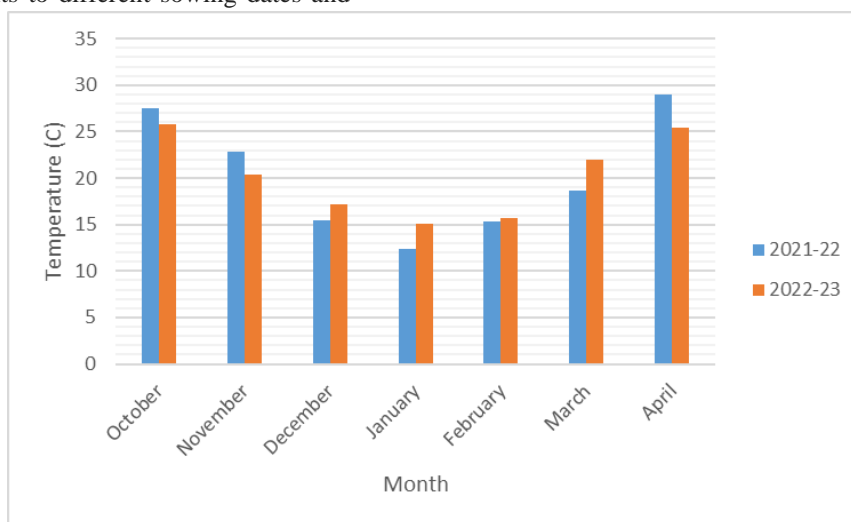


Fig.1: Mean monthly temperature at the study location in 2021-2022 and 2022-2023 seasons

The treatments consisted of four sowing dates: 1<sup>st</sup> of October, mid-October, 1<sup>st</sup> of November, and mid-November. Additionally, three tillage treatments were employed: conventional tillage (involving three passes with the removal of maize residues), mulch tillage (which incorporated maize residues into the conventional tillage

process), and no-tillage (direct drilling without any prior preparation).

The chemical properties of the soil used in the experiment were analyzed prior to cultivation, and the findings have been presented in Table 1.

Table.1: Some physical and chemical properties of the soil in the experimental site.

Depth (cm)	pH	EC (dS m <sup>-1</sup> )	OM (%)	Soil texture	Available macro-nutrients			Available micro-nutrients			
					(mg kg <sup>-1</sup> )			(mg kg <sup>-1</sup> )			
					N	P	K	Fe	Mn	Zn	Cu
0-30	8.2	12.1	0.24	Clay loam	38.5	3.5	454.6	10.2	13.8	1.5	0.44
30-60	8.7	9.0	0.24	Clay loam	30.8	1.0	569.0	13.9	3.8	1.1	0.56

The experiment was designed in a split plot arrangement with three replications. Sowing dates were distributed in the main plots, and tillage treatments were allocated in the subplots. Sub-Plot area was 15 m length × 3 m width, occupying an area of 45 m<sup>2</sup>. Plant distances were 0.30 m apart; the distances between rows were 0.60 m. All other agriculture practices of faba bean cultivation were done in accordance with standard recommendations for commercial growers by the Ministry of Agriculture (MALR, 2013).

At harvest, samples of ten plants of each experimental plot were taken to determine plant height (cm), number of seeds/plant, 100-seed weight (g). Seed yield and biological yield (kg ha<sup>-1</sup>) were determined from each plot.

The analysis of variance (ANOVA) was conducted to determine the effects of treatments on the obtained data as described by Gomez and Gomez (1984). Least significant difference test (LSD) was used to test the differences between means at  $P < 0.05$ .

The CROPGRO model, which is part of the DSSAT; Decision support system for Agro-technology Transfer, (Jones *et al.* 2003), system, has been adapted to simulate the growth and yield of faba beans. The model is a suite of crop models developed to simulate the growth, development, and yields of several crops, as well as changes in soil water, carbon, and nitrogen. It was calibrated using the data collected from the field experiments of faba bean crop for the years 2021-22 and 2022-23, respectively. Calibration was done using seed yield, total aboveground biomass (referred to as biological yield in other places in this text) and harvest index. Local soil and weather parameters, initial conditions of experiment and management practices were used for running the model

To evaluate the model performance and to compare the simulated grain yield, biomass and harvest

Table.2: Effect of sowing date on the faba bean's yield and its component during the 2021-22 and 2022-23 growing seasons.

Sowing date	Plant height (cm)		No. seeds/plant		100-seed weight (g)		Seed yield (kg ha <sup>-1</sup> )		Bio. yield (kg ha <sup>-1</sup> )		Harvest index (%)	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
1 <sup>st</sup> Oct.	92.3	88.6	41.2	48.7	79.3	81.6	2970	3678	9171	10233	32.34	35.92
15 <sup>th</sup> Oct.	95.6	97.8	44.3	52.2	84.8	92.1	4012	4115	11644	11534	34.44	35.43
1 <sup>st</sup> Nov.	86.3	88.0	42.4	51.2	82.9	85.0	3514	3648	10694	10822	32.85	33.64
15 <sup>th</sup> Nov.	83.0	83.5	36.2	46.0	71.1	73.7	3166	3079	10558	10017	29.98	30.76
LSD at 0.05	2.77	1.61	1.73	1.49	1.38	1.51	129.6	72.07	283.0	117.2	0.59	0.48

When considering the number of seeds per plant, the highest significant value was achieved through

index versus the observed data, three statistical measurements were used: the coefficient of determination ( $R^2$ ), Nash- Sutcliff efficiency (NSE) (Nash and Sutcliffe, 1970), and the root mean square error (RMSE) (Eq. 1,2 and 3).

$$R^2 = \frac{[\sum_{i=1}^n (O_i - \bar{O})(P_i - \bar{P})]^2}{[\sum_{i=1}^n (O_i - \bar{O})^2][\sum_{i=1}^n (P_i - \bar{P})^2]} \tag{1}$$

where,  $P_i$  are the predicted values,  $O_i$  are the observed values,  $n$  is the total number of observations,  $\bar{O}$  is the mean of the observed data and  $\bar{P}$  is the mean of the predicted data.  $R^2$  ranges from 0 to 1, with higher values indicating less error variance

$$NSE = \frac{\sum_{i=1}^n (O_i - \bar{O})^2 - \sum_{i=1}^n (P_i - O_i)^2}{\sum_{i=1}^n (O_i - \bar{O})^2} \tag{2}$$

NSE, ranges between  $-\infty$  and 1, The value of  $NSE = 1$  corresponds to a perfect match between predicted and observed data

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (O_i - P_i)^2}{n}} \tag{3}$$

where, The RMSE is defined as the square root of the mean squared error. In modeling this is used to measure the geometric difference between observed and modeled data.

### III. RESULTS AND DISCUSSION

#### 3.1 The effect of sowing date

Table 2 illustrates the impact of sowing date on faba bean yield and its various components throughout the two seasons under study. In terms of plant height, the most noteworthy results were observed during the first and second seasons, specifically when cultivation took place on October 15<sup>th</sup>, resulting in heights of 95.6 cm and 97.8 cm, respectively.

cultivation on October 15<sup>th</sup> in the first season, with a value of 44.3. Similarly, sowing on October 15<sup>th</sup> and November

1<sup>st</sup> yielded values of 52.2 and 51.2, respectively, during the second season. The 100-seed weight also exhibited notable variations, with the highest significant values recorded as 84.8 g in the first season and 92.1 g in the second season, both achieved through sowing on October 15<sup>th</sup>.

In terms of seed yield, the first season recorded the highest significant value of 4012 kg/ha, while the second season reached 4115 kg/ha, both obtained through sowing on October 15<sup>th</sup>. Conversely, the lowest yield was observed during the first season with sowing on October 1<sup>st</sup>, resulting in a value of 2970 kg/ha, and during the second season with sowing on November 15<sup>th</sup>, resulting in a value of 3079 kg/ha. The biological yield also displayed significant variations, with the highest values recorded as 11644 kg/ha in the first season and 11534 kg/ha in the second season, both achieved through sowing on October 15<sup>th</sup>.

Lastly, the harvest index exhibited notable differences, with the highest significant values of 34.44% achieved through sowing on October 15<sup>th</sup> during the first season, and 38.92% achieved through sowing on October 1<sup>st</sup> during the second season.

These results indicate that sowing faba bean on October 15<sup>th</sup> consistently resulted in the highest seed yield across both seasons. This suggests that this particular sowing date is optimal for achieving maximum productivity in terms of seed yield. On the other hand, sowing faba beans on October 1<sup>st</sup> or November 15<sup>th</sup> led to the lowest yields. This implies that these specific sowing dates may not be suitable for obtaining high seed yields in faba bean cultivation. The difference in seed yield between the two seasons is also worth noting. While the first season had a higher overall yield compared to the second season, both seasons still showed a similar trend with respect to sowing dates. This suggests that sowing date plays a significant role in determining seed yield regardless of seasonal variations.

The choice of sowing date plays a crucial role in determining the ultimate seed yield of faba bean. The importance of selecting an appropriate sowing date cannot be overstated, as it directly influences various physiological processes and growth stages, thereby affecting overall productivity. Optimum sowing dates for faba bean cultivation are influenced by several factors such as photoperiod sensitivity, temperature requirements, and management practices (McDonald et al., 1994; Adisarwanto and Knight, 1997; Yasmin et al., 2020; Manning et al., 2020). Planting at the right time (October 15<sup>th</sup>) ensures that the crop goes through key developmental stages under favourable environmental conditions, allowing for optimal vegetative and reproductive growth. A well-timed sowing date helps synchronize plant maturity with conducive environmental conditions during flowering, pollination, and pod filling stages, thus maximizing seed yield potential. Failure to choose the correct sowing date can result in reduced flowering period duration, increased susceptibility to pests and diseases, uneven pod set or abortion, impaired grain filling process, and ultimately diminished seed yields. Successful management strategies incorporating precise sowing dates have shown significant improvements in achieving maximum seed yield potential in faba bean crops across various regions (Sharaan et al., 2004; Hasanvand et al., 2015; Megawer et al., 2017; Zeleke and Nendel, 2019).

### 3.2 The effect of tillage practices

Table 3 illustrates the impact of different tillage practices on faba bean yield and its various components throughout the two seasons under study. The practices examined were conventional tillage, conventional tillage with mulching, and no-tillage. In terms of plant height, the most noteworthy results were observed with the no-tillage practice. During the first season, the plant height reached a significant value of 102.2 cm, while in the second season, it reached 96.8 cm.

Table.3: Effect of tillage practices on the faba bean’s yield and its component during the 2021-22 and 2022-23 growing seasons.

Tillage	Plant height (cm)		No. seeds/plant		100-seed weight (g)		Seed yield (kg ha <sup>-1</sup> )		Bio.yield (kg ha <sup>-1</sup> )		Harvest index (%)	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
Conventional	78.7	82.1	35.0	44.6	74.23	77.3	3226	3155	10156	9913	31.73	31.93
Con.+mulch	87.1	89.5	40.5	49.8	80.85	82.8	3330	3661	10408	10642	31.93	34.36
No tillage	102.2	96.8	47.7	54.2	83.62	89.2	3690	4074	10985	11397	33.55	35.53
LSD at 0.05	2.40	1.39	1.50	1.29	1.20	1.31	112.3	62.4	245.1	101.5	0.51	0.42

When considering the number of seeds per plant, the no-tillage practice also yielded the highest significant

values. In the first season, the number of seeds per plant reached 47.7, and in the second season, it increased to 54.2.

Furthermore, the 100-seed weight was found to be significantly higher with the no-tillage practice. In the first season, the weight reached 83.62 g, and in the second season, it increased to 89.2 g.

The seed yield, a crucial measure of productivity, also demonstrated the superiority of the no-tillage practice. In the first season, the seed yield reached a significant value of 3690 kg/ha, and in the second season, it increased to 4074 kg/ha. It is worth noting that the conventional tillage treatment resulted in the lowest yields in both seasons, with values of 3226 and 3155 kg/ha, respectively. Additionally, the biological yield, which encompasses all plant material produced, showed significant increases with the no-tillage practice. In the first season, the biological yield reached 10985 kg/ha, and in the second season, it increased to 11397 kg/ha.

Finally, the harvest index, a measure of the proportion of harvested yield to total biological yield, also favoured the no-tillage practice. In the first season, the harvest index reached a significant value of 33.55%, and 35.53% in the second season.

This data clearly indicates that adopting the no-tillage practice has a positive impact on the seed yield of faba bean. The significant increase in seed yield from the first season to the second season further reinforces this superiority. The fact that the conventional tillage treatment resulted in the lowest yields in both seasons is noteworthy. This suggests that traditional methods of tilling the soil may not be as effective in promoting optimal growth and productivity for faba bean. Recent research studies have provided compelling evidence that the no-tillage treatment

of faba bean cultivation consistently yields higher seed productivity compared to conventional tillage (Stringi et al., 2004; Ali et al., 2018; Badagliacca et al., 2018; Volpi et al., 2018; Kimbirauskiene et al., 2023). This technique allows for improved water infiltration, reduced erosion risks, and increased microbial activity in the soil (Reicosky and Saxton, 2007; Blanco-Canqui and Ruis, 2018). By maintaining an undisturbed top layer of soil, no-tillage treatment promotes optimal root development and actively facilitates nutrient uptake by the plants (Triplett Jr and Dick, 2008; Mehra et al., 2020). In contrast, conventional tillage commonly disrupts soil structure and exposes it to erosion risks while also causing loss of organic matter content crucial for future crop production (Madarász et al., 2016; Kuhwald et al., 2017; Rahmati et al., 2020). The higher seed yield obtained through no-tillage treatment signals its potential as an environmentally-friendly and sustainable approach with considerable economic benefits for farmers growing faba bean.

### 3.3 The effect of sowing date × tillage practices

Table 4 presents the findings on the impact of different sowing dates of faba bean and tillage practices on the yield and its components in two consecutive seasons. The results indicate that the interaction between no-tillage and sowing on October 15<sup>th</sup> had a significant effect on plant height. In the first season, the highest recorded value was 111.8 cm, while in the second season, it reached 113.4 cm. These findings highlight the importance of considering both the sowing date and tillage practices in order to achieve optimal plant height.

Table.4: Effect of the interaction between sowing dates and tillage practices on the faba bean's yield and its component during the 2021-22 and 2022-23 growing seasons.

Treatment	Plant height (cm)		No. seeds/plant		100-seed weight (g)		Seed yield (kg ha <sup>-1</sup> )		Bio. yield (kg ha <sup>-1</sup> )		Harvest index (%)	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
1 <sup>st</sup> Oct.+Con.	79.3	84.4	34.3	42.9	75.1	77.5	2582	3145	8025	8961	32.17	35.10
1 <sup>st</sup> Oct.+Con.and mulch	93.8	88.8	41.9	49.3	79.5	80.9	2941	3784	9333	10270	31.52	36.85
1 <sup>st</sup> Oct.+No tillage	103.7	92.5	47.4	54.0	83.4	86.4	3385	4104	10153	11467	33.33	35.79
15 <sup>th</sup> Oct.+Con.	84.3	84.4	37.4	46.8	77.9	85.3	3872	3319	11297	10279	34.27	32.29
15 <sup>th</sup> Oct.+Con.and mulch	90.8	95.8	42.0	52.1	84.5	92.9	3902	4028	11510	11459	33.90	35.15
15 <sup>th</sup> Oct.+No tillage	111.8	113.4	53.5	57.5	92.1	98.0	4262	4999	12123	12864	35.15	38.86
1 <sup>st</sup> Nov.+Con.	75.6	81.8	36.1	46.7	76.8	77.1	3445	3193	10860	10259	31.69	31.12
1 <sup>st</sup> Nov.+Con.and mulch	85.3	89.6	43.6	52.1	84.4	83.3	3346	3737	10441	10789	32.04	34.64

1 <sup>st</sup> Nov.+No tillage	98.0	92.6	47.4	54.8	87.6	94.5	3750	4014	10780	11419	34.79	35.16
15 <sup>th</sup> Nov.+Con.	75.4	77.9	32.0	41.9	67.1	69.4	3003	2965	10443	10156	28.76	29.19
15 <sup>th</sup> Nov.+Con.and mulch	78.4	83.9	34.5	45.7	75.0	74.0	3131	3095	10348	10054	30.26	30.79
15 <sup>th</sup> Nov.+No tillage	95.3	88.8	42.3	50.4	71.4	77.7	3363	3178	10882	9840	30.90	32.30
LSD at 0.05	4.80	2.79	2.99	2.57	2.39	2.62	224.5	124.8	490.2	203.1	1.02	0.84

Furthermore, the study also examined the number of seeds per plant. Similar to plant height, the interaction between no-

tillage and sowing on October 15<sup>th</sup> resulted in the highest significant value in both seasons. In the first season, the number of seeds per plant reached 53.5, while in the second season, it increased to 57.5. These results emphasize the positive impact of the selected sowing date and tillage practices on the number of seeds produced per plant.

In addition, the study investigated the 100-seed weight. The interaction between no-tillage and sowing on October 15<sup>th</sup> yielded the highest significant value in both seasons. In the first season, the 100-seed weight was recorded at 92.1 g, while in the second season, it increased to 98.0 g. These findings suggest that the selected sowing date and tillage practices contribute to achieving a higher weight per seed, which can have implications for overall yield.

Moreover, the study examined the seed yield. Once again, the interaction between no-tillage and sowing on October 15<sup>th</sup> resulted in the highest significant value in both seasons. In the first season, the seed yield reached 4262 kg/ha, while in the second season, it increased to 4999 kg/ha. It is worth noting that the lowest significant values were observed with conventional tillage and sowing on October 1<sup>st</sup> in both seasons, with values of 2582 kg/ha in the first season and 3145.7 kg/ha in the second season. These findings highlight the importance of considering the sowing date and tillage practices in order to achieve optimal seed yield.

In terms of biological yield, the first season witnessed a significant value of 12,123 kg/ha. This result was attributed to the synergistic effect of implementing no-tillage techniques and sowing on October 15<sup>th</sup>. Impressively, the same combination of practices also yielded the highest significant value in the second season, reaching an impressive 12,864 kg/ha.

Shifting the focus to the harvest index, the first season showcased a significant value of 35.15%. This outcome was once again attributed to the successful interaction between no-tillage practices and sowing on October 15<sup>th</sup>. Astonishingly, the same combination of

techniques also produced the highest significant value in the second season, reaching an impressive 38.86%.

No-tillage and sowing faba bean on October 15<sup>th</sup> has been proven to be the most effective method for achieving optimal yield and yield components. This agricultural practice involves eliminating conventional tillage methods, such as ploughing or discing, and directly sowing the beans into untilled soil. By doing so, the natural structure of the soil is preserved, minimizing erosion and conserving moisture content (Fengyun et al., 2011; Komissarov and Klik, 2020; Bekele, 2020; Mondal and Chakraborty, 2022). The specific timing of sowing on October 15<sup>th</sup> ensures that the faba beans are planted early enough to take advantage of cooler temperatures required for optimum growth. Additionally, this timing allows for an extended growing season during which the plants can accumulate enough biomass, leading to high yields (Catt and Paull, 2017; Manning et al., 2020; Alharbi, and Adhikari, 2020). Overall, adopting no-tillage techniques combined with sowing faba bean on October 15<sup>th</sup> represents a best-practice approach for maximizing yield and optimizing various growth factors in faba bean cultivation.

### 3.4 DSSAT model simulation results

The crop and soil parameters of the DSSAT model were iteratively adjusted until close agreement was achieved between the measured and simulated seed yield, total biomass and harvest index. The agreement was measured using different statistical fitting parameters (NSE, R<sup>2</sup> and RMSE). Figure 2 shows the measured and simulated values of these variables and values of statistical fitting parameters. The fitting of the calibration data and model simulation was very good with statistical parameters as indicated; grain yield (NSE = 0.879 and 0.84, R<sup>2</sup> = 0.88 and 0.855, and RMSE = 157.5 and 227.7) total above ground biomass (NSE = 0.839 and 0.829, R<sup>2</sup> = 0.886 and 0.831, and RMSE = 406.2 and 402.2) and harvest index (NSE = 0.669 and 0.772, R<sup>2</sup> = 0.752 and 0.884, and RMSE = 1.07 and 1.28) in the 2021-22 and 2022-23 seasons, respectively (Figure 2).



Data reveals that DSSAT model accurately reflected the sowing dates and tillage practices on the faba bean grain yield, total biomass yield and harvest index. Same trends were reported by Zeleke et al. (2019) when the Aqua Crop model was used to simulate the impact of sowing dates, sowing rates and supplemental irrigation on faba bean growth and yield. Several studies also reported the successful application of the crop growth models to simulate the different management practices on plant

growth and yield of faba beans using APSIM (Keating et al., 2003), STICS (Falconnier et al., 2019) and CROPGRO (Hassanein et al., 2007 and Boote et al., 2002)

Same results were obtained by Osman et al. (2014) when using Cropsyst model to simulate the growth and yield of faba bean, they reported that the  $R^2$  values were above 0.9 when the predicted versus measured grain yield and total biomass were compared.

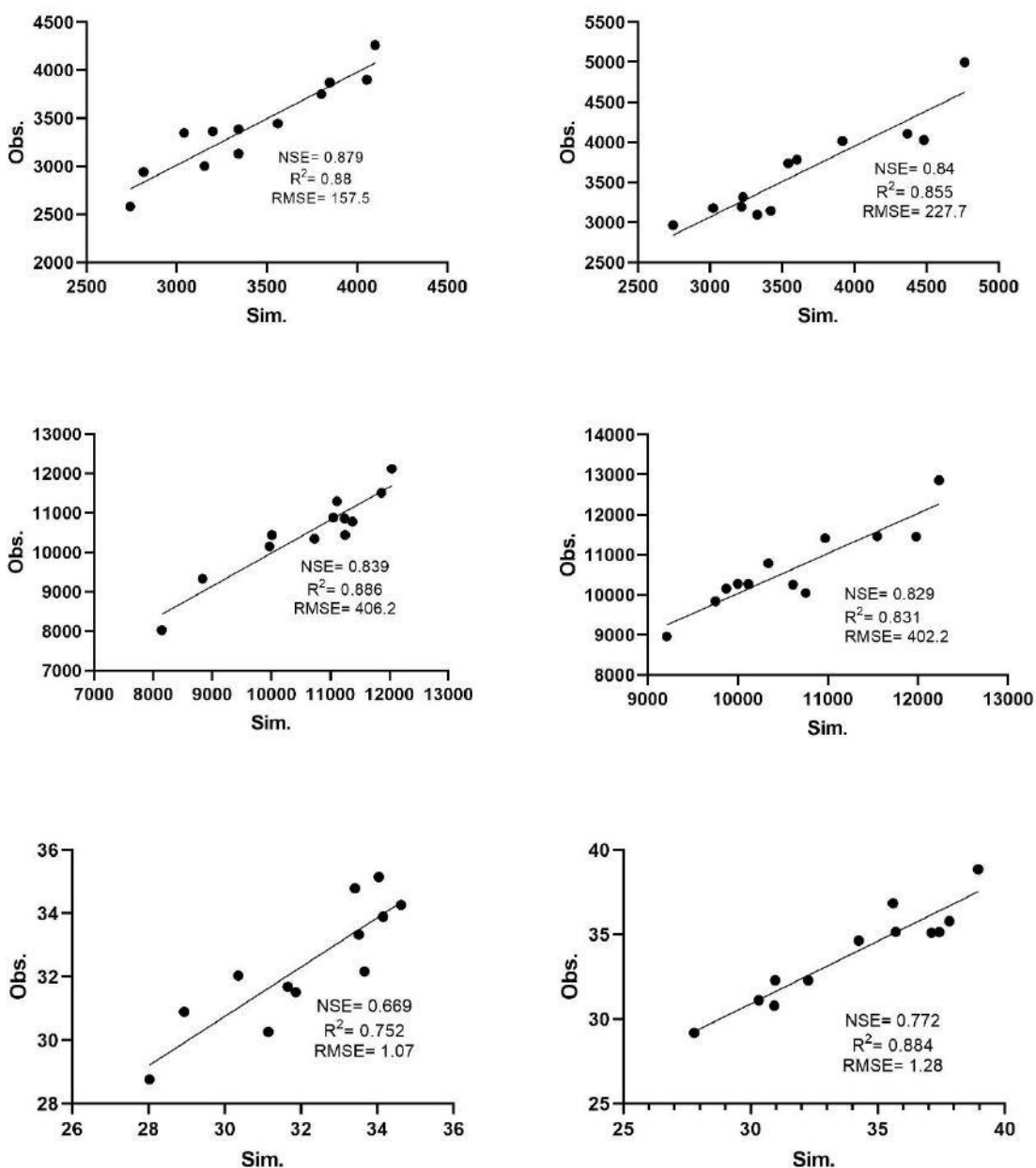


Fig.2: Simulated vs. observed seed yield (upper), total biomass (middle) and harvest index (lower) in the first (left) and second (right) seasons.

#### IV. CONCLUSION

The study revealed that the timing of sowing and the tillage practices exert a significant influence on the

yield of faba bean seeds and their components. Notably, the DSSAT model demonstrated remarkable accuracy in simulating the seed yield, total above-ground biomass, and

harvest index. The model achieved an NSE value exceeding 0.80 for both the seed yield and the total biomass, effectively capturing the impact of various sowing dates and tillage practices across two seasons. In the specific context of Kharga, New Valley, the study identified mid-October as the optimal sowing date for faba beans. Deviating from this timeframe, whether by sowing earlier or later, resulted in reduced seed yield. Furthermore, the adoption of a no-tillage practice proved advantageous in enhancing both faba bean yield and soil health. These findings underscore the potential value of the DSSAT model as a valuable decision-making tool for determining faba bean sowing dates and implementing effective tillage management strategies.

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# Remodeling in Microbial Fuel Cell (MFC) design and parameters for the sustained production of electricity

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**Abstract**— In the recent years Microbial fuel cells (MFCs) have gained much attention as an alternate source of sustainable power production. In MFCs the bacteria at anode are used as catalyst for extraction of electrons from biodegradable substrate. In the present study bacteria having electricity production potential were isolated from pond's sludge and were identified using different microscopic, staining techniques and with the help of different biochemical tests. Double chambered MFCs were constructed to check the ability of those bacteria for current generation. Initially double chambered MFC was constructed using 250 ml of sludge as a source and maximum current produced was 119 mV. This experiment was repeated using 500 ml of mixed culture and the maximum current production of 169 mV was recorded. Glucose, peptone and yeast extract were used as a substrate for the growth and current production by bacteria. Different parameters such as chemical mediators, different electrode types and sizes and salt-bridge concentrations were utilized for production and amplification of current generated in MFC. Methylene blue indicator dye was found suitable for enhancing current in MFCs for short time. Two modes of feeding were used to increase the lifetime of cell i.e., and from the results of current study, it was concluded that fed batch mode was more effective as compared to non-fed batch culture.



**Keywords**— Double chambered microbial fuel cells, Electricity production, fed batch mode, Microbial fuel cell (MFC's), Salt-bridge.

## I. INTRODUCTION

Microbial fuel cell (MFC) is a device which uses bacteria as the catalysts to oxidize organic and inorganic matter for the generation of electric current. When these bacteria produce electron from these substrates, then they are transferred to the anode i.e., negative terminal and then it streams to the cathode i.e., positive terminal which is connected through a conductive material and that is composed of a salt bridge

[1]. So MFC essentially converts the energy which is provided in bio-convertible substrate into the electric current reliably [2].

From the 1960s till very lately, it was believed that the exogenous mediators are required to be added into the microbial fuel cell to produce sensible amounts of power. However, Kim and his co-workers verified that power could be produced by a naturally existing association of bacteria

in the absenteeism of exogenous mediators. Other workers then disclosed that by simply placing produced to power subsurface devices Now it appears that the current can be produced from any material that is biodegradable, extending from pure compounds for instance acetate, bovine serum albumin, ethanol, glucose and cysteine to complex mixtures of organic matter that involves domestic (of human), animal, food-processing and meat-packing wastewaters. The bacteria which are electrochemically active in MFCs are considered to be the iron-reducing bacteria such as *Shewanella* and *Geobacter* species, but the examination of such communities shows a diversity of bacteria much greater than these model iron reducers persevering in the biofilm community. The maximum power densities are limited due to the high amount of internal resistance. And the comparisons made with different systems by using pure cultures or mixed cultures cannot establish which microbial community is accomplished by the highest power density [3].

### 1.1.1 APPLICATIONS

MFC has many uses but we will discuss some of its dominant uses for instance as environmental sensors, production of hydrogen gas in MFCs, for the production of electricity, biosensors, as a pollutant degrader, as removers of fermentation inhibitors and for the empowerment of implanted medical devices etc.

### 1.1.2 AS ENVIRONMENTAL SENSORS

In order to understand and indicate the response of ecosystem the information about normal atmosphere can be helpful. The sensors that are dispersed in the natural atmosphere want power. MFC can be used to give power to these devices, mostly in areas such as deep-water and river environments where it is difficult to habitually approach the structure to change the batteries. Sedimental fuel cells are being recognized to screen the environmental systems for instance streams, brooks and the ocean. The organic matter in the residues function as power for these devices.

### 1.1.3. PRODUCTION OF HYDROGEN GAS BY MFCs

Hydrogen can be considered as a significant constituent of an energy structure that decreases CO<sub>2</sub> emissions if hydrogen is produced from non-fossil fuel sources and then it is used in microbial fuel cells. High substrates of sugar when fermented then it can produce hydrogen gas through this biological process at more concentration of 60% [4]. By eradicating oxygen at the cathode and the addition of a small voltage through the biochemically aided microbial reactor (BEAMR) process, the MFCs can yield hydrogen gas when altered through this pattern. Bacteria produces an anode working potential of approximately -0.3 Volt. The electrons as well as protons that are fashioned at the anode can

combine at the cathode to produce hydrogen gas with only an additional total cell potential of 0.11 Volt. Though in practice 0.25 V or more than this should be placed into the circuit to make hydrogen due to the reason of overpotential at the cathode. Through the process of BEAMR the production of biohydrogen is not constrained or limited to glucose only. Any substrate which is biodegradable and generates electricity in an MFC will work in the system of BEAMR. This pattern works with domestic wastewater, but the issue is that the hydrogen recoveries in current reactor designs are still too low to make hydrogen production with BEAMR likely to be as viable as electricity production with MFCs. High-strength wastewaters appeared to be the most instant potential for hydrogen recovery in the BEAMR process [5].

### 1.1.4. PRODUCTION OF ELECTRICITY

Microbial fuel cell is given importance Because of the ability of MFC to produce sustained current for longer duration it has given much importance. At present MFC is considered as the active method of current generation. The series of algae or aquatic plants acts as a feedstuff are used where the hydrogen production in manure plants and the greenhouse gases are used in electric current. The processed sugars and other compounds that caused the explosion of steam for corn wastage could be used as feedstuff of MFC while these microbial cells don't follow the Carnot cycle because the chemical energy from the fuel particles to oxide reliably converted into electricity instead of conversion into heat. In the hypothetical opinion, the proficiency of microbial fuel cell is over 70% though still producing electricity from MFCs in a very small quantity. The solution of this issue in a one way is to perform practically by storing it in the rechargeable power device. The appropriate method for MFCs offering energy is the automated communications process that consumes less energy. For example, for power production the wireless sensors for signal broadcast in distant areas. These MFCs can also be used as natural power distribution systems in areas which are not much developed all over the world.

### 1.1.5. BIOSENSOR

MFCs can be used hypothetically as a sensor for monitoring the in-situ process, directing and for inspection of contaminants [6]. The organic matter content in the wastewater is usually assessed in relations of biological oxygen demand (BOD). The examination of BOD according to ancient procedures take 5 or 7 days incubation period at 20°C +1 or -1 in the dark. For the guesstimate of water and to make a guess of the wastewater quality the best procedures are BOD<sub>5</sub> and BOD<sub>7</sub>. Most of the time the samples of wastewater are calculated through this conventional procedure. The expensive equipment's are not

required here. The limitations involve that it consumes time and needs expertise and much experience to get the reproducible results. So, the ancient procedures of BOD are not good for on-line monitoring and control of biological wastewater treatment processes due to the important need of rapid feedback. Consequently, it is important to change such old methods and introduce a method that could give fast measurements to demonstrate the lively changes in the process of treatment. To construct biosensors the concept of MFC has been applied for fast BOD estimation, in which a biological sensing element that is bacteria in anode compartment and a transducer that is electrodes and proton exchange membrane, they are combined. In 1977 Karube et al. developed an MFC biological oxygen demand biosensor consuming the hydrogen which is produced by the help of *Clostridium butyricum* restrained on the electrode. Many kinds of MFC-based BOD biosensors have been developed by following this procedure, for instance, mediator-less MFCs and microbial fuel cells with electron transfer mediated. Such sensors which are based on microbial fuel cell have the benefit of lasting stability and can be used continuously for operational monitoring of wastewater [7].

#### 1.1.6. MFCS AS A POLLUTANT DEGRADER

Those compounds which are degraded by bacteria are converted into current. The capability of the microbial communities of microbial fuel cell to reduce a large number of pollutants environment could be more appreciable than electricity generation itself in some situations, exclusively when this technique could be used for the cleanup of environment in-situ. For the anaerobic deprivation of petroleum components and landfill leachate pollutants in ground water some species such as *Geobacter* has been shown as important. In soil, the utilization of electrode which acts as an electron acceptor is very attracting because the degradation causing microbes will co-localize with the pollutant at the anode that is of graphite. When in place those electrodes can deliver a continuous lasting electron sink for the deprivation of the destructive environmental pollutants. In such situation, the electrons that are produced by the microbes in the form of electricity is inappropriate when related to the enlarged bioremediation rate.

#### 1.1.7. FERMENTATION INHIBITOR REMOVERS

Similarly, experimentations have revealed that microbial fuel cells may likely be able to eradicate inhibitors of fermentation which gather in process water after the pretreatment of cellulosic biomass. The exclusion of the inhibitors permits for enlarged fermentation product profit while providing small quantities of energy.

#### 1.1.8. IMPLANTED MEDICAL DEVICES EMPOWERMENT

A rare application for this MFC technology is to rule the fixed medical devices using glucose and oxygen from blood. An implanted MFC could provide power indefinitely and refute the necessity for surgery to substitute batteries. Based on noble metal catalysts the abiotic fuel cells and activated carbon have been established to produce energy from blood glucose in vitro and based on enzymatic catalysts in the fuel cells have also been shown to work under functional conditions but still require much improvement to become feasible [8].

#### 1.2 CURRENT PRODUCING MICROORGANISMS

Exo-electrogenic bacteria are those organisms that have the ability to transfer electrons to the extracellular electron acceptors that are insoluble and also have the potential to be used in devices like microbial fuel cells. Presently, these exo-electrogens have been recognized in the Firmicutes and Acido-bacteria and also in Alpha-, Beta-, Gamma- and Deltaproteobacteria. Even though most current MFCs perform optimally when they contain a mixed microbial community, some pure cultures that exhibit strong electrogenic activity in the environment of MFC have been characterized. The properties of electrogenic and some characteristics of extracellular electron transfer have been well-defined for pure cultures of organisms such as *Rhodospseudomonas palustris* DX-1, *Ochrobactrum anthropi* YZ-1, *Geobacter sulfurreducens*, *Escherichia coli*, *Shewanella putrefaciens* and *Rhodoferrax ferrireducens*. The existing list of confirmed exoelectrogens includes representatives of four of the five classes of

Proteobacteria in which the Epsilon proteobacteria and the representatives of Firmicutes and Acidobacteria are not characterized. Though, it is likely that innovative electrogenic bacteria remain to be revealed [9].

##### 1.2.1 MECHANISM

The statistic that some bacteria can transport electrons beyond their cell wall and for that reason they electrically intermingle with the environment is known for over a century. This property can be utilized to grow advanced electrically enhanced bioprocesses. In such bulk electric systems, organisms interact with electrodes through exchanging of electrons which are sometimes supplied or removed sometimes through an external electrical circuit. In MFCs the microbes donate electrons to electrodes and produce electrical current. In bioremediation of groundwater and sediments (aquatic) where microbes that are metal reducing catalyzes the conversion of organic contaminants to carbon dioxide. Within such systems the oxidation of anode by bacteria is joined to the construction of chemicals such as hydrogen or methane on the cathode

and they are denoted as microbial electrolysis cells. Electrons are transferred from a low potential electron donor to an acceptor having more positive redox potential by some reactions such as redox reactions in a microbial electron transport chain. Usually, these reactions are catalyzed by compounds that are membrane bounded and uses the energy difference between donor and acceptor to launch an ion gradient across the membrane which is used for the synthesis of ATP and does conversion of the difference in electrical potential into chemical energy [10]. Bioelectrochemical systems (BES) which are typical MFCs have arisen as hopeful technologies for bioremediation as well as energy generation. Those microorganisms that are attached to the electrodes as biofilms has a main role in electric current generation and in biosynthesis in Bioelectrochemical systems. Because of the capacity of extracellular electron transfer to electrodes weather directly or indirectly the electricity generating bacteria can be well defined as exoelectrogens. The transfer of electrons from exoelectrogens to electrode can combine with the energy conservation and can support their growth so it can be considered as respiration of electrode/anode. Motivated by the increased interests in bioelectrochemical, bacterial electrode respiration and electromicrobiology has received much attention in current years. The c-Type cytochromes that are well-thought-out as one of the most significant electron transfer strategy in electricity generation by exoelectrogens are the heme-containing proteins in most archaeas as well as bacterias. Biofilms contains many microbial cells which are compactly stacked and disseminated spatially in the extracellular polymeric substances. A network of complex electrons that involves numerous components of electron transfer can be assumed in an electric current generating biofilm. Some other factors such as the diffusion co-efficiency, the prearrangement of the electron transfer components in EPS and pH gradient can have an important impact on the electron transfer in biofilms. A proper electron shuttle in bioelectrochemical system can be dissolvable, stable, reusable, environment friendly, and can have a proper redox potential. Many bacterias most importantly G<sup>-</sup> bacteria has the ability of secreting electron shuttles in bioelectrochemical systems. Also, it has been recommended that the bacterial shuttle secretion can be stimulated by electricity generation in microbial fuel cells [11]. Producing power in microbial fuel cells depends on the redox chemistry. Microbial fuel cells contain anode and cathode compartments in which each of which grasps an electrode that is separated by a cation-permeable membrane. In the anode chamber, microbial substrates such as acetate (an electron donor) are oxidized in the absence of oxygen by respiratory bacteria, producing protons and electrons. The electrons are passed through an

electron transport chain (ETC) and protons are translocated across the cell membrane to generate adenosine triphosphate (ATP). Electrons and protons exiting the ETC typically pass onto a terminal electron acceptor such as oxygen, nitrate, or Fe (III). However, in the absence of such acceptors in an MFC, some microorganisms pass the electrons onto the anode surface. Difference in redox potentials (i.e., the ability of a compound to donate or accept electrons, denoted  $E_0$  and measured in volts) between the electron donor and the electron acceptor is the determinant of the potential energy available to the microorganism for anabolic processes. In an MFC the electrochemical redox potential difference of the anode and cathode determines for bacteria to produce electricity in MFCs, the cells need to transfer electrons generated along their membranes to their surfaces. Very little information is known about bacterial interactions with electrodes. While anodes and cathodes can function in bacterial respiration, research has been focused on understanding microbial anodic electron transfer. Anode-respiring bacteria catalyze electron transfer in organic substrates onto the anode as a surrogate for natural extracellular electron acceptors (e.g., ferric oxides or humic substances) by a variety of mechanisms [12].

## II. DESIGN OF MFCs

There are basic components of MFCs which are important in constructions. Electrodes, wirings, glass cell and salt bridge have an important role. Salt bridge is replaced with Proton exchange membrane in PEM fuel cell. Though it enhances the cost but handling and the power generation both get enhanced, thus increasing the portability and efficiency of the system. Apart from that fuel cells can be classified in two types on the basis of number of compartments or chambers.

### 2.1.1 DOUBLE CHAMBERED MICROBIAL FUEL CELLS

Both the cathode and anode are housed in different compartments or chambers connected via a proton exchange membrane (PEM) or sometimes salt bridge. PEM or salt bridge mainly functions as medium for transfer of proton to make the circuit complete. This not only completes the reaction process but also prevents anode to come in direct contact with oxygen or any other oxidizers. They are run in batches and can be used for producing higher power output and can be utilized to give power in much inaccessible conditions. It can be suitable designed to scale up to treat large volume of wastewater and other source of carbon. These particular types are called up flow mode of microbial fuel cell. They practically fall between the classification of single chambered and double chambered microbial fuel cells. They are mediator-less and



sometimes membrane-less and can be used for large scale production of electricity from the wastes.

### 2.1.2 SINGLE CHAMBERED MICROBIAL FUEL CELLS

They are simple anode compartment where there is no definitive cathode compartment and may not contain proton exchange membrane. Porous cathodes form one side of the wall of the cathode chamber utilizing oxygen from atmosphere and letting protons diffuse through them. They are quite simple to scale up than the double chambered fuel cells and thus have found extensive utilization and research interests lately. The anodes are normal carbon electrodes but the cathodes are either porous carbon electrodes or PEM bonded with flexible carbon cloth electrodes. Cathodes are often covered with graphite in which electrolytes are poured in steady fashion which behaves as catholytes and prevent the membrane and cathode from drying. Thus, water management or better fluid management is an important issue in such single chambered fuel cells.

### 2.1.3 STACKED MICROBIAL FUEL CELLS

These are another type of construction in which fuel cells are stacked to form battery of fuel cell. This type of construction doesn't affect each cell's individual Coulombic efficiency but in together it increases the output of overall battery to be comparable to normal power sources. These can be either stacked in series or stacked in parallel. Both have their own importance and are high in power efficiency and can be practically utilized as power source [13].

## III. MATERIALS AND METHODS

This research took place at Biotechnology Research Laboratory, Centre of Biotechnology and Microbiology (COBAM), University of Peshawar, from April 2017 to June 2017. The samples were collected from pond as a source at University of Peshawar. In this experiment, double chamber and stacked cell microbial fuel cells were designed using fed batch and batch mode of feeding microbes for the purpose of maintenance and amplification of voltage generation for longer duration. Different mediators were checked to optimize the voltage generation and results were recorded to find out the most functional one.

All the equipment's, chemicals and materials that were used in this study were provided by the Centre of Biotechnology and Microbiology and the Department of Physics, University of Peshawar.

### 3.1. DOUBLE CHAMBERED MICROBIAL FUEL CELL CONSTRUCTION

Two double chamber microbial fuel cells were built in this experiment using different terms and conditions and were

examined to find best out of them. For feeding microbes the modes selected was Fed Batch mode.

#### 3.1.1. SAMPLE SOURCE

In this microbial fuel cell, the sample used was the sludge of pond; all the microbes present in the sludge were used as the anaerobes and were placed in the anaerobic half of the MFC. The sludge was collected from the bottom of Botany department pond at University of Peshawar. The reason of selecting from that area was that the anaerobic concentration is higher at the bottom because oxygen is in lesser amount in such areas. The collection of sample was done with the help of shovel in sterile bucket. The soil sample was dugged up to 10 inches and then the sample was collected by using clean gloves and was stored in a sterile zipper bag of polyethylene.

#### 3.1.2. AN ANAEROBIC COMPARTMENT CONSTRUCTION

A container made up of plastic having lids was taken and with the help of a driller, hole was made on one side of the container for salt bridge. All the ingredients such as sludge, table sugar and peptone in amounts 500 g, 30 g and 2 g respectively were added into the container. Electrode of Carbon of dimension 2 inches width x 3 inches length were attached with copper wire and was placed into the container in such a way that it was dipped in the sludge. The container was covered with the lid to make it airtight.

#### 3.1.3. AEROBIC COMPARTMENT CONSTRUCTION

A hole drilled in a plastic container with the help of driller on one side was taken. Ingredients such as sodium chloride salt and distilled water in amounts 100 g and 500 ml respectively were added to container. The electrode of carbon of dimension 2 inches width x 3 inches length were attached with the copper wire are dipped into the electrolyte solution in the container. A hole in the lid of the container was also drilled to allow the air to enter into it. Despite that the container was covered with the lid to prevent the entrance of excess air into the container.

#### 3.1.4. SALT BRIDGE SOLUTION CONSTRUCTION

Then salt bridge solution was made by taking 40 grams of table salt and 8.5 grams of Agar were weighed using digital weight balance. 300 ml of water was taken in the beaker and heated till boiling by using a burner. The salt and Agar were mixed into the boiling water. This mixture was carefully poured into the plastic pipe of about 1.5 Meter length with one end closed by using a tape. The pipe was shifted for some time in the refrigerator so that the mixture gets solidify. Once it gets solidified, the salt bridge is ready to use.

### 3.1.5. MICROBIAL FUEL CELL DESIGN

To design a fuel cell all parts were joined. The aerobic compartment was connected with the anaerobic compartment through salt bridge with the help of holes drilled in the compartments. The anaerobic compartment was sealed properly by applying plaster of Paris.

The anaerobic compartment acted as anode whereas the aerobic compartment as the cathode. The wires of the anode and cathode were connected to the positive and negative terminals of the multimeter. In anaerobic compartment sludge of pond was used. After designing the fuel cell no voltage was measured initially but with the passage of time the voltage started increasing gradually. The maximum voltage was measured at day 3 and was maintained for up to 50 hours. After 50 hours, the voltage decreased slowly but still the cell was generating voltage for up to 4 weeks and then the voltage became zero.

Our main focus was to amplify and sustain the current for longer time so the methodology was improved to maintain and amplify the voltage generated for longer time. The changes made were by altering the feed of microbes, by altering the concentration of feed of microbes, by changing the mode of feeding microbes such as batch or fed batch, by the Use of different mediators, by Changing dimensions and materials of the electrodes or by Increasing the quantity of sludge to amplify the voltage generated.

### 3.2. MICROBIAL FUEL CELL USING FED BATCH MODE OF FEEDING

In this case the basics were same as that of the previously described but only the concentrations of sludge were changed and instead of batch, fed batch mode for feeding the microbes was adapted. The amounts used in this case were two times more than used previously.

#### 3.2.1. ANAEROBIC CHAMBER

1-liter mixed culture of pond's sludge, 12 grams of Glucose and 3 grams of Yeast extract was used as energy sources. Electrode of copper having dimensions 4 inches widthx3 inches length was used. Here the electrode concentration was increased.

#### 3.2.2. AEROBIC CHAMBER:

1 Liter of 10X TBE Buffer was taken in the aerobic half of MFC. An electrode of copper with a copper wire was dipped into the buffer.

#### 3.2.3. DESIGNING A MICROBIAL FUEL CELL

The aerobic and anaerobic compartments were connected together by means of a salt bridge which acts as a proton exchange membrane. Aerobic act as cathode and anaerobic as anode. The wires that were attached to the electrodes were connected to the multimeter. No voltage was

measured right after the designing of a cell. After approximately 6 hours the voltage was generated by the cell and reading was measured then. In this case, the microbes were fed after every 24 hours with 12 grams of Glucose and 3 grams of Yeast's extract. The voltage generated was sustained for a longer time now and did not decline because the energy source was there in cell this time. After 72 hours, Methylene blue (a mediator) was added and it showed increase in voltage generated up to some extent. This cell generated maximum voltage for almost 4 weeks, after that the value of voltage started decreasing and after that negligible voltage was generated by the cell.

### 3.3. MICROBIAL FUEL CELL UTILIZING NON-FED BATCH MODE

When the food materials are added at once in the start of the process and then the chambers are properly sealed it's called the non-fed batch mode of feeding. Below is the process involved in making this MFC.

#### 3.3.1. MAKING OF AN ANAEROBIC CHAMBER

Anaerobic chamber was made by taking a lid containing container made up of plastic and by the use of a driller a hole was made on one side of the container for salt bridge entrance. The composition of anaerobic chamber is sludge, table sugar and peptone in amounts 500g, 30g and 2g respectively. All these ingredients were added into the container. Electrode of copper attached with copper wire was placed into the container in such a way that it was dipped in the sludge. The container was then covered with the lid so that air do not enter into it.

#### 3.3.2. MAKING OF AEROBIC CHAMBER

Aerobic chamber was made by drilling a hole by driller into a plastic container on one side of it for salt bridge entrance. Ingredients such as sodium chloride salt and distilled water in amounts 100g and 500 ml respectively were added to the container. Dipped the electrode of copper attached with the copper wire into the electrolyte solution in the container. A hole in the lid of the container was also drilled so that air can enter into it. The container was covered with the lid to prevent the container from excess entrance of air.

#### 3.3.3. SALT BRIDGE COMPOSITION

40 grams of table salt and 8.5 grams of Agar were weighed using digital weight balance.

300ml of water was taken in the beaker and heated till boiling by using a burner. The salt and Agar were mixed into the boiling water. This mixture was carefully poured into the plastic pipe of about 1.5 Meter length with one end closed by using a tape. The pipe was shifted for some time in the refrigerator to make the mixture solidify. After that it was used.

### 3.3.4. DESIGNING OF A MICROBIAL FUEL CELL

All the parts were combined to design a fuel cell. The anaerobic and aerobic compartment were joined by means of salt bridge through the holes drilled in the compartments. The anaerobic compartment was sealed properly by using the plaster of Paris. The anaerobic compartment was labeled as the anode and aerobic compartment as the cathode. The wires of the anode and cathode were connected to the positive and negative terminals of the multi meter. The source of microbe was pond's sludge. The moment, at which the cell was designed, no voltage was measured but slowly and gradually as the time passed the voltage started increasing. The maximum voltage was measured at day 8 and was maintained for up to many hours in case of sludge. After that the voltage decreased slowly.

### 3.4. MAINTAINANCE

The operating conditions for MFC was room temperature. In the cathodic chamber of microbial fuel cell of both units, oxygen was allowed to enter (that will combine with H ions and electron from the anodic chamber to form water molecule). 7g yeast and 40g glucose (feed of bacteria/for maintaining the growth of bacteria in an aerobic chamber) was added to anodic chamber of both MFC units on day 1. Current was measured of the units individually and then in stacked form. Immediately after MFC construction, no current was recorded.

After sometime current was produced as a result of microbe's accumulation on anode (biofilm formation). After 24 hours, 7g yeast was added again in anaerobic chamber of both units and then change in current was detected.

### 3.5. IDENTIFICATION OF THE ISOLATES

For identification of isolates gram staining and different biochemical tests were performed.

#### 3.5.1. GRAM STAINING

For gram staining small colony was picked from the plate and smear was made on the slide. 23 drops of crystal violet stain were added on the smear for 1-2 mins. After that the slide washed gently with the water. Then the smear was covered with gram iodine and slide was left for 1-2 mins. Then the slide was decolorized using ethanol. Safranin dye was added for 2 mins and after that the slide was washed gently with water, air dried and was observed under the microscope using oil immersion.

#### 3.5.2. MACCONKEY – AGAR

MacConkey agar was prepared by dissolving proper amounts of all ingredients in distilled water as recommended by the manufacturer. The medium was autoclaved and poured in petri plates in laminar flow hood.

To check the sterility the petri plates were placed in incubator for overnight at 37°C, medium was initially red in color. Sterile plates were then streaked and incubated.

#### 3.5.3. CATALASE TEST

In Catalase test with the help of inoculation loop we have picked the inoculum from the center of pure colony and were placed on a clean glass slide. A drop of 3% hydrogen peroxide was added to cover the colony completely. Immediate bubbling (gas liberation) was recorded as a positive result.

#### 3.5.4. ENDOSPORE STAINING

In endospore staining, inoculum of organism to be tested was taken and smear was made on a clean microscopic slide for presence of endospores and the slide was air dried. He slides was passed over flame 2 to 3 times to fix the smear. The slide was heated gently by putting the slide on a beaker that has been placed over Bunsen burner. The malachite green was added 12 drops, after 5 mins the slide was removed and allowed to cool to room temperature for 2 mints. The slide was rinsed with tap water. Then for 2 mints the smear was stained with safranin; the secondary was removed with the help of rinsing. Then the slide was dried and observed under microscope.

#### 3.5.5. TRIPLE SUGAR IRON TEST

With a sterilized straight inoculation needle was touched the top of a well-isolated colony and TSI Agar was inoculated by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant was done. The cap was left on loosely and the tube was incubated at 35°C in ambient air for 18 to 24 hours.

## IV. RESULTS

The aim of this research was to remodel a working Microbial Fuel Cell and to sustain and increase the current generated. Out of three different Microbial Fuel Cells designed, two showed satisfactory results. The voltage produced by individual cell was measured at different intervals of time. As stated earlier, inoculum from a single source (pond sludge of Botany department, University of Peshawar) was used, two out of three were built using pond's sludge. During this research, different parameters were used in order to optimize and amplify the voltage for maximum possible time. Fed batch gave best results out of two modes of feeding microbes. MFC built using pond sludge as inoculum gave suitable results.

**4.1. VOLTAGE GENERATION FROM POND SLUDGE USING DOUBLE**

**CHAMBERED MFC (1<sup>st</sup> design)**

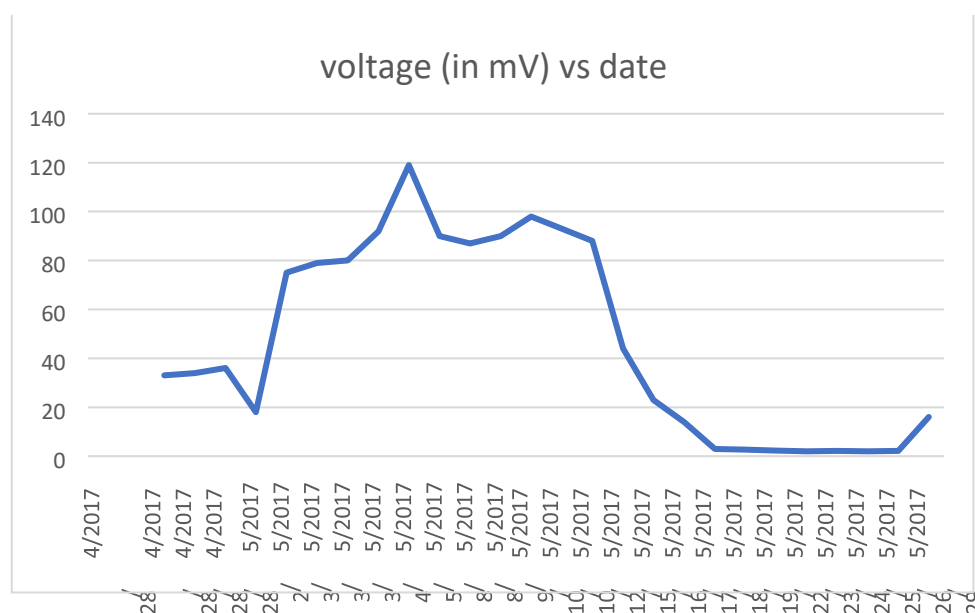
Double chamber MFC using pond sludge was performed on 28<sup>th</sup> April, 2017. Results are shown in table 1.

*Table 1. Voltage generation in a double chamber microbial fuel cell using pond sludge*

Date	Time (in hours) after the cell was designed	Voltage generated by cell built up from pond's sludge (in mV)
28/4/2017	18	30
28/4/2017	3	33
28/4/2017	2	34
28/4/2017	2	36
02/5/2017	65	18
03/5/2017	24	75
03/5/2017	5	79
03/5/2017	1	80
04/5/2017	18	92
05/5/2017	27	119
08/5/2017	60	90
08/5/2017	7	87
09/5/2017	18	90

10/5/2017	20	98
10/5/2017	7	93
12/5/2017	18	88
15/5/2017	74	44
16/5/2017	24	23
17/5/2017	23	14
18/5/2017	24	3
19/5/2017	23	2.7
22/5/2017	25	2.3
23/5/2017	24	2
24/5/2017	24	2.1
25/5/2017	27	2
26/5/2017	22	2.2
29/5/2017	24	16

This experiment performed that electricity can be generated by using pond sludge and bacteria involved in current production were already present in pond sludge. It was shown that a voltage of 30mV was generated within only 18 hours. The voltage gradually increased with time because of biological activity of bacteria. The maximum voltage (119mV) was recorded on day 8 (after 192 hours of cell construction). The voltage was generated by cell over a long period and it was observed that whenever we feed it, an increase had occurred in it (Fig 1).



*Fig 1. Voltage generation from pond sludge in double chambered MFC (1<sup>st</sup> design)*

**4.2. VOLTAGE GENERATION FROM POND SLUDGE USING DOUBLE**

**CHAMBERED MFC (2<sup>nd</sup> design)**

A 2<sup>nd</sup> Double chamber MFC design using pond water for electricity generation was constructed on 16<sup>th</sup> May, 2017. The resulting voltage generated is given in table 2.

Table 2. Voltage generated in Double chamber MFC from pond sludge (2<sup>nd</sup> design)

Date	Time (in hours) after the cell was design	Voltage measured from the cell made from pond sludge
16/5/2017	25	31
17/5/2017	22	82
18/5/2017	23	104
19/5/2017	24	119
22/5/2017	72	66
23/5/2017	24	9.6
24/5/2017	26	34

25/5/2017	23	49
26/5/2017	23	56
29/5/2017	71	137
29/5/2017	4	88
15/6/2017	17 days	169

After constructing a new design, double chambered MFC, a voltage of 31mV was given at 25<sup>th</sup> hour. After this period of approximately 22h, cell voltage exponentially increased over the next 50hrs reaching an initial maximum voltage of 119V. With the passage of time, nutrients were metabolized by bacteria, decrease in voltage occurred. Then we feed it again and again so that the microorganisms do not die and the current is sustained. After a long time if there is enough food and enough anaerobic environment is provided then an increase in current is seen like after 17 days the current measured was 169. The power generation in double chamber MFC used in our experiment was limited by internal resistance (min et al. 2005) and nutrients unavailability (as mode of feeding was some time batch and no nutrients were added during experiment (Fig 2).

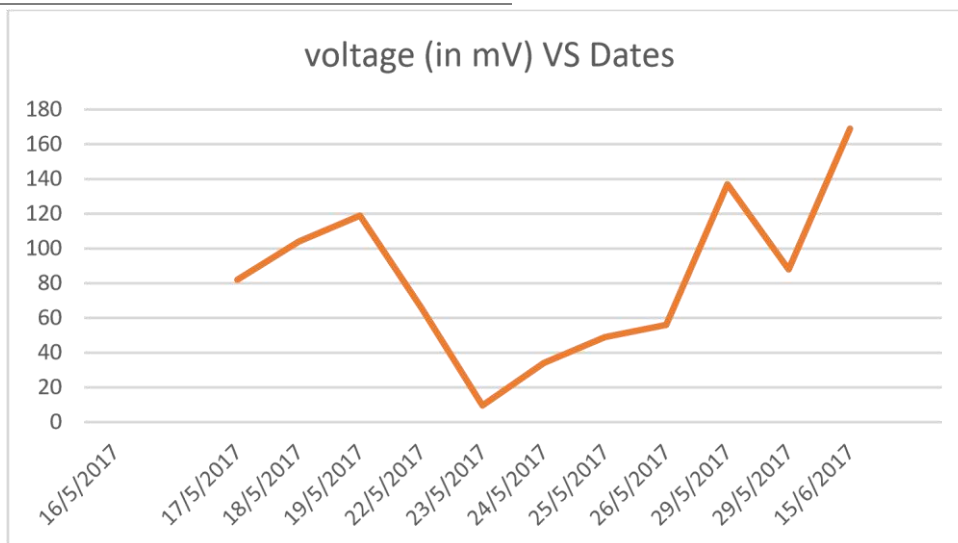


Fig 2. Voltage generation from pond sludge in double chambered MFC (2<sup>nd</sup> design)

It is clear from Fig. that initial voltage given by the MFC using pond sludge (31) was higher than that of given by 1<sup>st</sup> design (30 mV). In addition to that, voltage generated by using pond sludge was maintained for longer period of time as compared to MFC using other samples.

**4.3. COMPARISON OF VOLTAGE GENERATION IN DOUBLE CHAMBER MFC USING 1<sup>st</sup> DESIGN AND 2<sup>nd</sup> DESIGN**

Double chambered MFC using 1<sup>st</sup> design MFC and 2<sup>nd</sup> design MFC was constructed to examine the voltage generation. The anaerobic chamber was fed with glucose and yeast (as carbon and energy source) in increments after every 24 or 48 hours. The resulting voltages from 1<sup>st</sup> and 2<sup>nd</sup> design recorded are shown. The maximum recorded voltage was from pond sludge. A comparison between voltage generated from pond sludge and soil is given in Fig 3.

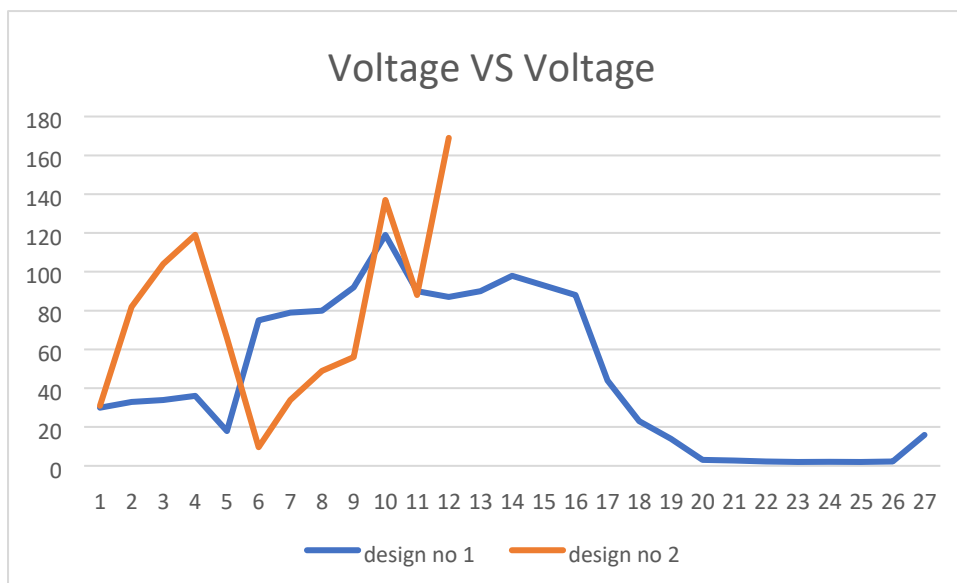


Fig 3. Comparison of voltage generated from 1<sup>st</sup> design and 2<sup>nd</sup> design

#### 4.4. OTHER FACTORS AFFECTING VOLTAGE PRODUCTION

Several other factors were also examined with respect to voltage output like dimensions and materials of electrodes were changed, energy sources were improved and mediators were used in building double chamber cells. This time the

results obtained were very good. Three different type of electrodes were used in this experiment for construction of three different MFC. Carbon electrodes showed good results as compared to aluminum electrodes used. The voltage produced using electrodes of different surface area is compared in below fig4.

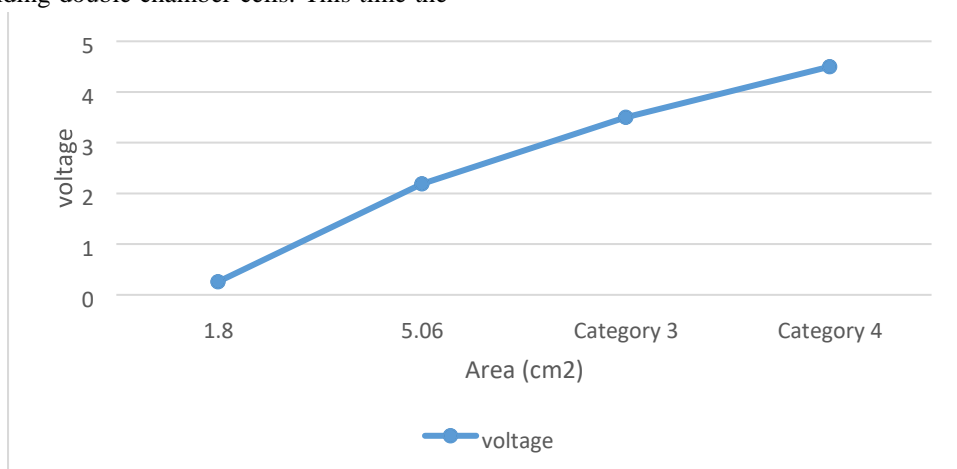


Fig 4. Comparison of voltage generated by graphite electrodes of different dimensions

The experimental findings that voltage generation is directly proportional to surface area has also been reported previously. Three mediators (methyl red, neutral red and methylene blue) were used in double chamber MFC in this experiment. Methyl red showed best results increasing the voltage from 34mV to 74mV (3 times more than the initial). Result by methylene blue was also satisfactory and the final

voltage (after adding methylene blue) was approximately two times of the initial.

Results by ethyl red were disappointing and despite of increase in voltage, tremendous decrease was shown (Fig below).

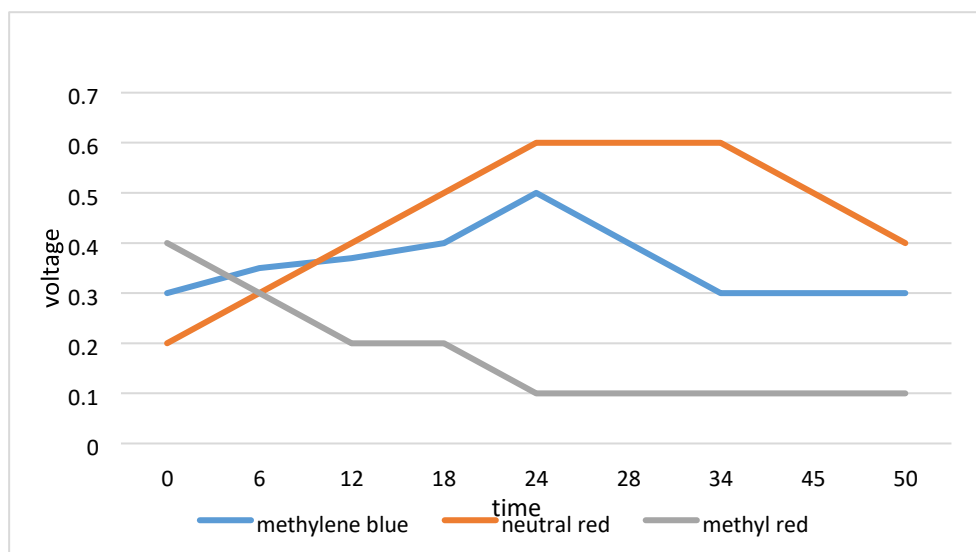


Fig 5. Effect of different mediators on voltage generation

#### 4.5. IDENTIFICATION RESULTS

##### 4.5.1. GRAM STAINING RESULTS

After subculturing, seven different colonies were obtained. For their identification gram staining was done and the seven isolates that are 1,2,3,4,5,6 & 7 found following results and shapes.

Table 3

Plates no	Gram staining	Shape	Catalase+ve	Spore forming	Oxidase test	TSI agar test
1	+ve	Rod (filamentous)	+ve	Greenish spores (reddish pink rods)	-ve	Red slant +red butt
2	+ve	Coccobacilli (non-filamentous)	-ve	Greenish spores (reddish pink rod)	-ve	Yellow slant +red butt
3	-ve	Coccobacilli (non-filamentous)	+ve	Greenish spores	-ve	Yellow slant +red butt
4	+ve	Coccobacilli- rods	-ve	Greenish spores	-ve	Yellow slant +red butt
5	+ve	Rod (filamentous)	+ve	Greenish spores	-ve	Yellow slant +red butt
6	-ve	Coccobacilli	-ve	Greenish spores	-ve	Yellow slant +yellow butt
7	+ve	Rods	+ve	Greenish spores (reddish pink rods)	-ve	Yellow slant +yellow butt

## V. DISCUSSION

In this study, an increase in voltage from 31 mV to 169 mV was observed when the sludge conc. was increased from 500mg/l (in case of batch mode) to 1000mg/l (in case of fed batch).

Similar observations have been reported by researchers while using sludges of different conc.

i.e. 1g/l, 2g/l, 3g/l, 4g/l and 5g/l etc. to find out there effect on the voltage generated by microbial fuel cells [30]. So, sludge conc. is also an important factor in amplification of voltage generated by MFC and working of MFC can be improved by considering this.

To determine the effect of PEM surface area on power output salt bridges with different sizes such as 3.5 and 6.5 were used. Similar observations have been reported by researchers used different the sizes of the anode and cathode were varied in two-chambered MFCs having PEMs with three different surface areas (APEM = 3.5, 6.2, or 30.6 cm<sup>2</sup>). This showed an increase in voltage generation due to more transference of H ions.

To find out the effect of surface area of electrode on voltage, electrodes of different dimensions were used. An increase in voltage occurred when the surface area was increased. The voltage obtained for carbon electrode (5.06 cm<sup>2</sup>) was 6 times more than that obtained by using carbon electrodes (1.8cm). The experimental findings that voltage generation is directly proportional to surface area of cathode has also been reported previously e.g., the voltage obtained by using ferricyanide cathode and Pt coated carbon cathode (22.5 cm<sup>2</sup>) was reduced from 200-300mV when the surface area was reduced from 22.5 cm<sup>2</sup> to 5.8 cm<sup>2</sup>. [31]

The effects of different mediators in different concentrations on the current generation in the cell was also observed by different researchers. In a study three different types of mediators i.e. methyl red, 2-hydroxy-1, 4-naphthoquinone and methylene blue in different concentrations (50, 100, 200, 300 and 400  $\mu$ M) were used and it was found that different mediators are effective in different concentrations. [16] In our study, best results were shown by methylene blue. The reason of methyl red poor performance is that as mediators are specie specific so may be neutral red was not effective for the strains present in our inoculums.

Some scientists focused on building low cost MFC's, they used different construction methods and built different designs of MFC's. Cheap and simple materials were used in designing the MFC as compared to the previously designed MFC's. They used different methods for reducing resistance and power loss, building single chamber MFC and making economical membranes. They designed a simple and

economical single chamber MFC and were able to generate the voltage of about 0.59 V[32]. In our study, MFC's were built up using cheap and easily available materials. Double chambered MFC's were constructed and a current maximum of 169mV was measured.

## VI. CONCLUSIONS

1. A microbial fuel cell is a device that utilizes the power of microorganisms to convert chemical energy into electrical energy.
2. Stacked cell was more efficient one because large amount of voltage was generated by the stacked cell microbial fuel cell followed by double chambered and then single chambered MFC's.
3. Mediators are specie specific and only help in amplifying voltage of specific species; it was found that one type of mediator is not effective for all types of microbes.
4. Different substrates can be used as feed for microbes; it was observed that mostly organic matters such as peptone, yeast extract, glucose, etc. help in increasing the voltage if they are used as a substrate in MFC.
5. When the substrate was added in increments, an increase in voltage produced by the cell was observed, and also the life of the cell increased as compared to those in which the substrate was added as a whole in the cell.
6. Different metals can be used as electrodes such as carbon, copper, platinum, aluminum, etc.; it was concluded that when the electrodes having large surface area were used in building a cell, the voltage generation in cell increased i.e. more was the surface area of electrode, more will be the current generated by cell.
7. Different parameters were studied to maintain and amplify the current generated by the cell, it was also observed that by increasing the slurry concentration in the cell, the current production by cell can also be enhanced.

## VII. RECOMMENDATIONS

Following recommendations should be suggested:

1. The anaerobic compartment should be properly sealed to avoid the entrance of air in the chamber.
2. Electrodes having large surface area should be used in making a cell.



3. The site rich with facultative anaerobe should be selected for sampling.
4. The substrate should be added in increments into the anaerobic compartment.
5. Minute amounts of mediators should be used.
6. Wires having minimum resistance and high conductance should be used.

### FUTURE PROSPECTS

1. To design the chambers for anaerobic compartment that could properly provide the anaerobic conditions to the microbes within the cell.
2. To build up the techniques that utilized cheap and easily available materials and generate huge amount of current for us.
3. To design an MFC that is one level higher than the present one (by changing MFC design, by using new strains, by changing concentration of substrate, by reducing power dissipations and by improving the strains)

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# Influence of Foliar Application of Salicylic Acid on Growth and Yield of Chia (*Salvia hispanica*)

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**Abstract**— Chia has become enormous recognition as a super food worldwide, as reflected by a sharp rise in both consumption and cultivation. Salicylic acid (SA), a vital signaling molecule, is essential for plant tolerance responses to biotic and abiotic stress preserving healthy plant growth and enhancing productivity in stress condition. A field investigation of carried out during Rabi season of 2021 and 2022 at experimental field of Horticulture Department, C. B. G. Agriculture PG College, BKT, Lucknow to examine the “Influence of foliar application of salicylic acid on growth and yield of chia (*Salvia hispanica* L.)”. The treatment consisted of five dose of foliar application of salicylic acid (0, 75, 150, 300 and 600 ppm). The experiment was laid out in a design of Randomized Block Design (RBD) and replicated three times. The foliar application of SA resulted in greater plant height, number of branches, fresh weight and dry weight of plant, yield and yield component of chia during the study. Generally the greatest values were registered from 300 ppm foliar application of salicylic acid.



**Keywords**— Chia, *Salvia hispanica*, Salicylic Acid, Growth, Yield

## I. INTRODUCTION

Chia (*Salvia hispanica* L.), native to southern Mexico and Northern Guatemala (Silva *et al.*, 2016, Artocos, 2018), is an annual herbaceous short day plant belonging to the family Lamiaceae. It grows naturally in tropical and subtropical environments from 400 to 2500 m above mean sea level. Chia seeds were traditionally one of the four basic elements in the diet and also a source of energy in Aztec civilization of Central America and Southern civilization in the pre-Columbian era (Ali *et al.* 2012; Cortes *et al.*, 2017). Chia grows up to 1 m tall with opposite arranged leaves with small flowers (3–4 mm) purple or white in a spike at the end of stem, with small corollas and fused flower parts that contribute to a high self-pollination rate. The seed colour varies from black - grey with black spotted, oval in shape and seeds are found in size ranging from 1 to 2 mm (Yeboah *et al.*, 2014).

Chia seeds are composed of multiple nutritionally important polyunsaturated fatty acids (PUSFA) comprising approximately 25 to 40% oil content, of which 60% and 20% is w-3 alpha linolenic and w-6 alpha linolenic acids,

respectively (Ali *et al.*, 2012). Due to the vast health and medicinal properties of Chia seeds, it has been shown to lower the triglycerides (TG) and cholesterol levels, which in turn lowers the risk of cardiovascular related diseases and lowers blood pressure (Ali *et al.*, 2012).

Chia is currently commercially cultivated in Australia Bolivia, Columbia, Guatemala, Mexico, Peru, Ecuador and Argentina (Jamboonsri *et al.*, 2012). Mexico is the largest producing centre of chia and currently exports seeds to Japan, USA and Europe (Baginsky *et al.*, 2016). The promotion of the consumption and cultivation of chia could be of value due to its unique nutrient composition and its inherited tolerance to drought and other stress factors. It has been deemed to be drought tolerant, although there is lack of research on the physiological and molecular responses of this crop to drought stress. Salicylic acid is perhaps the only compound on the surface of the earth to mediate so diverse function as ranging from curing various human ailments to protect the plant various biotic and abiotic stresses and affecting various physiological and biological processes of plants (Popova *et*

*al.*, 2012). There is a need to establish a suitable crop that can be deemed to be drought tolerant and is able to withstand such environments outside of the scope of cereals, but also pseudo cereals.

## II. MATERIALS AND METHODS

An experiment entitled “Influence of foliar application of salicylic acid on growth and yield of chia (*Salvia hispanica*)” was conducted during the Rabi season of 2021 and 2022 at Horticulture Research Farm, Department of Horticulture, C.B.G. Agriculture PG College, Bakshi Ka Talab, Lucknow, situated at 26°84 N latitude and 80°94 E longitude with an average altitude of 123 meter above mean sea level. The experiment was laid out in randomized block design consisting five levels of Salicylic Acid (control, 75ppm, 150 ppm, 300 ppm and 600ppm) with three replications. The observation on growth parameters *viz.* plant height (cm), number of branches per plant, herb fresh weight (g) and herb dry weight (g) were recorded at 30, 60 and 90 days after sowing. The data on seed weight (g/plant), 100-seed weight (g) and seed yield (kg ha<sup>-1</sup>) were noted at harvest.

## III. RESULTS AND DISCUSSION

### 1. Vegetative growth characters

#### 1.1 Plant height (cm)

The plant height was increased significantly up to SA 300 ppm at all stages, however, reduced non-significantly with SA 600 ppm. Crop received SA 300 ppm recorded significantly maximum plant height (21.11 cm) at 30 DAS (50.11 cm) at 60 DAS and (81.11cm) at 90 DAS as compared to rest of the treatment. While, minimum plant height (55.03 cm) was noted in plants sprayed with tape water (T<sub>1</sub>). Salicylic acid plays key roles in regulation of various physiological and developmental processes of plants. It is a phenolic compound that enables plants to survive under challenging soil and environmental situations (Iqbal *et al.*, 2012). Different concentration of Salicylic acid increases regulation of nutrition and hormone in plants (Shafiee *et al.*, 2010). The growth stimulated by salicylic acid can be associated by mineral nutrition, hormonal profile and photosynthesis. The positive effect on growth of salicylic acid to its positive effect on hormonal balance disturbed by metallic stress have been linked by several authors (Shakirova *et al.*, 2003), they treated wheat with SA and recorded increased ABA content, which regulate to stomata movements (Wilkinson and Davies, 2010) and genes and antioxidant enzymes activity (Bari and Jones, 2009). Shakirova *et al.* (2013) found that salicylic acid influence the endogenous

ABA, hormonal intermediary to trigger defense reactions in plants.

The increase in plant height due to salicylic acid is recognized to regulate cell enlargement and cell division during root and shoot development (Shakirova *et al.*, 2003) and also increase the number of internodes (Jaiwal and Bhambie, 1989). According to Martin-Mex *et al.* (2005), SA cause an increase in plant growth with increasing cell division in both stem and root, hence increasing plant height (23%) under greenhouse and field condition. Similar results due to application of salicylic acid were also obtained by Kaur *et al.* (2007) in chickpea, Yildirim *et al.* (2008) in cucumber, Anwar *et al.* (2014) in tuberose, (Kamkari *et al.*, 2016) in onion and (Basit *et al.*, 2018) in marigold. Furthermore, foliar application of lower concentration of SA treatment improved the plant height of *Salvia hispanica* (Fouad *et al.*, 2018).

#### 1.2 Number of branches per plant

The data indicated that different levels of salicylic acid had significantly affected the number of branches during crop growth stages. The highest number of primary branches (9.57) per plant was obtained with SA @ 300ppm, while the lowest (7.79) recorded with control (Table 1). The increase in number of branches per plant may be due to the involvement in enhancing some physiological and biochemical processes of plants such as cell elongation, cell division, cell differentiation, enzymatic activities, protein synthesis and photosynthetic activity (Raskin, 1992). The results are in agreement with Ebtsam *et al.* (2006) in tuberose, Hassanain *et al.* (2006) in chamomile, Jat *et al.* (2007) in African marigold, El-shraiy and Hegazi (2009) in pea, Mohammadzadeh *et al.* (2013) in basil and Al-Rawi *et al.* (2014) in cotton. On the contrary, Fouad *et al.* (2018) found that foliar application of SA treatment did not influence the number of branches in Chia.

#### 1.3-Herb Fresh and dry Weight (g/plant)

All the levels of salicylic acid produced more fresh weight as compared to control (Table 1). The data explicit that maximum herb fresh weight (29.90 g) was observed in treatment of 300 ppm salicylic acid (T<sub>4</sub>), followed by T<sub>3</sub> (28.64 g) and T<sub>5</sub> (28.20 g). While, minimum Herb fresh weight (27.50 g) was listed in plants sprayed with tape water (T<sub>1</sub>). The herb fresh weight produced per plant appeared to be positively correlated with height and number of primary branches per plant, as the plants in various treatments having more height and number of branches also had more herb fresh weight. These results are in close conformity with the results of (Fouad *et al.*, 2018) in chia. Similar findings were also reported by Anuprita *et al.* (2005) in gerbera, Jat *et al.*

(2007) in African marigold, Singh *et al.* (2012) in gladiolus and Anwar *et al.* (2014) in tuberose. Najafian (2009) reported that spraying salicylic acid at three levels (150, 300, and 450 mMol) on *Thymus vulgaris* L. had a significant effect on the fresh and dry weight of Thyme plant. Spraying at a concentration of 150 mM showed an increase in the dry weight of the plant and foliar application of 300 ppm salicylic acid was found to be superior in promoting herb dry weights (g) plant<sup>-1</sup> comparing to rest of the treatments which gained (14.34g) dry weight plant<sup>-1</sup>.

Application of exogenous SA also influenced significant variation in dry weight of the plant. An application of SA significantly increased the dry weight of

plant may be due to improved photosynthetic efficiency in plants (Hayat *et al.*, 2007), stabilization of chlorophyll and assimilates translocation from source to sink, which ultimately enhanced dry weight of chia plant (Fouad *et al.*, 2018). Furthermore, salicylic acid acts as defense hormone that could reduce the abiotic stress in leaves which ultimately leads to increase amount of dry matter contents production in marigold flowers (Champa *et al.*, 2015). The positive effects of salicylic acid on fresh and dry weight are corroborated with the results as reported by Gharib (2006) in basil and marjoram, Khandaker *et al.*, (2011) in red amaranth, Abdou and Mohamed (2014) in mint, Miri *et al.* (2015) in thyme, Fouad *et al.* (2018) in Chia.

Table 1. Influence of salicylic acid on plant height (cm), number of branches per plant, herb fresh weight and herb dry weight (g plant<sup>-1</sup>)

Treatments		Plant Height (cm)			Number of branches per plant			Herb fresh weight (g/plant )			Herb dry weights (g/plant )		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T <sub>1</sub>	SA 0 ppm (Control)	14.84	34.81	2.37	4.13	7.79	7.79	20.48	21.64	27.50	7.57	7.70	11.78
T <sub>2</sub>	SA 075 ppm	15.87	45.19	2.59	4.44	7.96	7.96	20.67	22.52	27.88	7.82	7.85	11.87
T <sub>3</sub>	SA 150 ppm	17.39	47.39	2.75	4.90	8.30	8.30	20.84	23.50	28.64	7.87	8.50	12.59
T <sub>4</sub>	SA 300 ppm	21.11	50.11	3.38	5.89	9.57	9.57	22.84	25.55	29.90	9.00	10.08	14.34
T <sub>5</sub>	SA 600 ppm	19.02	49.06	2.45	4.20	7.90	7.90	21.64	24.00	28.20	8.90	8.62	12.63
SE(m)		0.59	0.44	0.59	0.10	0.19	0.25	0.40	0.43	0.35	0.16	0.33	0.29
C. D. at 5%		1.97	1.44	1.98	0.36	0.63	0.83	1.35	1.40	0.81	0.52	1.09	0.95

## 2. Yield and Yield Components

The different levels of salicylic acid significantly increased the seed weight per plant as compared to control (Table 2). The treatment of SA 300 ppm (T<sub>4</sub>) showed significantly highest value of seed weight (4.36 g) and was closely followed by T<sub>5</sub> i.e. SA 600ppm. While the control treatment produced significantly lowest seed weight (3.24 g) as compared to the rest of treatment. The treatment of SA 300 ppm (T<sub>4</sub>) was also registered significantly maximum 100-seed weight (4.75 mg) as compared to rest of the treatments.

In the present investigation, highest seed weight per plant and 100- seed weight attributed highest yield per

hectare. The highest seed yield ha<sup>-1</sup> (327.86 kg) was produced by foliar application of 300 ppm SA, while the lowest (285.6 kg/ plant) was with control (Table 2). The salicylic acid affected the physiological and biochemical processes that were led to ameliorate in vegetative growth (Dawood *et al.*, 2012), active assimilation and translocation of photosynthates from source to sink in plant (Hayat and Ahmed, 2007). Results are also in the line of Gharib (2006) in basil, Jat *et al.* (2007) in African marigold, Han *et al.* (2011) in soybean. Al-Rawi *et al.* (2014) concluded that cotton plant (*Gossypium hirsutum* L.) tested with three levels of salicylic acid (50, 100, and 150 mg/l) exhibited highest total cotton yield (3371.9

kg/ha) with 150 mg/l SA in relation to other concentrations used.

Table 2. Effect of salicylic acid on seed weight (g/plant), 100 seed weight (g) / seed yield (kg/plant) at different stages of plant growth

Treatment		Seed weight (g/plant)	100-seed weight (g)	Seed yield (kg/ha)
T <sub>1</sub>	SA 0 ppm (Control)	3.24	3.94	285.60
T <sub>2</sub>	SA 075 ppm	3.56	3.95	291.60
T <sub>3</sub>	SA 150 ppm	3.78	4.29	291.46
T <sub>4</sub>	SA 300 ppm	4.36	4.75	327.86
T <sub>5</sub>	SA 600 ppm	3.97	4.50	296.26
SE(m)		0.14	0.11	1.96
C. D. at 5%		0.47	0.38	6.48

In a study on the response of the Indian mustard (*Brassica juncea* L.) to spraying with two levels of salicylic acid (35 and 70 mg/l), there was a significant increase in all the parameters of the crop (the weight of onemustard, the total yield of the seed, and the seed yield), when spraying the plants @70 mg/l in comparison to @ 35 mg/l and spraying with distilled water only (Dugogi et al., 2012). Abbas and Ibrahim (2014) worked out that the growth regulator SA was sprayed on *Nigella sativa* L. at different levels (50, 100, and 200 mg/l). Spraying of 50 mg/l salicylic acid was the best in increasing growth, yield and oil ratio indices. Al-Mohammadi and Al-Rawi (2016) were also observed that spraying with acetylsalicylic acid with 200 ppm registered the highest fruit numbers of per plant and the total yield kg ha<sup>-1</sup> as compared to non-treated plants of *Datura sp.*

#### IV. CONCLUSION

Based on the above findings it can be concluded that the application of salicylic acid @ 300ppm was found to be significantly superior in respect of improving growth, flowering and fruit set, yield attributes and yield of Chia.

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# Solid Waste Management- Mismanagement from Houseboats of Dal Lake: Assessing Strategies for Effective Waste Reduction and Resource Recovery

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**Abstract**— The current study set out to evaluate and examine the difficulties faced by houseboat owners in solid waste management as well as possible solutions to these difficulties. The study was conducted at four sites of Dal Lake: Lakut Dal, Bod Dal, Gagribal, and Nigeen Lake. A list of registered houseboats was acquired from the Lake Conservation Management Authority (LCMA) in 2023 indicates that there are 950 houseboats in Dal Lake. Data were gathered using a structured interview schedule. A sample of 100 houseboats, 25 from each site, was selected using stratified random selection; this represents about 10.5% of the total population. The study found that the restrictions faced by houseboat owners differed depending on how easily LCMA services could be accessed at each location. The absence of a nearby color-coded waste facility was cited by 92% of houseboat owners in Site 1 as their main concern. Site 2's outcomes were comparable, the absence of consistent (weekly) solid trash collection services being identified by 84% of houseboat owners as their main challenge, 88% of site 3 houseboat owners cited the absence of solid trash collection services provided by LCMA, and 56% of site 4 houseboat owners said they were happy with the existing collection services. Nonetheless, the largest obstacle faced by 40% of houseboat owners is the absence of regular garbage collection services. The houseboat owners in Dal Lake nevertheless face several challenges on a daily basis, which are discussed in more depth.



**Keywords**— Houseboats, Dal Lake, LCMA, Limitations, solid waste

## I. INTRODUCTION

Dal Lake is 3.5 kilometers wide and 7.44 kilometers long, and it is located in the Srinagar district of Kashmir. The deepest point of the lake is at Nigeen Lake, which is 20 feet deep, whereas Gagribal's depth is 2.5 meters (8.2 ft). (Bhat et al., 2017). One of the key factors in the valley's booming tourism business is the quantity of floating homes on the lake. Houseboats, also referred to as "floating palaces" or "floating hotels," are closely linked to Dal Lake. These serve as visitor accommodations, symbolize the Kashmir Valley's cultural legacy, and accentuate Dal Lake's breathtaking natural surroundings. More contemporary houseboats of a higher class often have a dining area, kitchenette with server, lounge, and

two to five bedrooms, each with its own bathroom, complete with toilet. There is a little "balcony" on the front of every houseboat, and many of them are anchored next to a small wooden deck on the lake. Some houseboats (Deluxe and Class A) may also include a little garden with piled ground in between the boats. Houseboats may be seen at Nigeen Lake and along the Boulevard Road between Dalgate and Nehru Park. For control purposes, the tourist office assigns deluxe, A to D categories to the houseboats. Because they have more amenities than other houseboat classes and generate more garbage as a result, deluxe, A, and B grade boats are regarded as high quality. (Tanveer et al., 2017) In the busiest travel seasons, houseboats in the Nigeen basin and Dal Lake are completely occupied by visitors. Waste is generated during

occupation in both liquid and solid forms. which immediately into the lake, posing a major danger to its ecology and health. As a result, this freshwater lake has undergone significant changes in its physico-chemical and biological properties, turning it into a eutrophic water body. The proprietors of houseboats on Dal Lake in Srinagar City deal with the increasingly challenging problem of incorrect solid waste management. This is caused by a number of factors, including poor environmental awareness among the populace, growing urbanization and tourism, and inadequate services provided by the Lake Conservation Management Authority (LCMA).

#### Description of the sites:

- 1) Lakut Dal: According to Fazal and Amin (2012), the location of the site is 34°03'N & 74.48°E. There are around 250 houseboats in the vicinity of this spot.
- 2) Bod Dal: As stated by Fazal and Amin (2012), the location of the site is 34.13°N and 74.48°E. There are more than 250 houseboats in the vicinity of this area.
- 3) Gagribal 34° 5' 10" N, 74° 50' 40" E are the geographic coordinates of the location.(maps, 2023) and has over 270 houseboats in it.
- 4) Nigeen: 34.11°N 74.83°E are the geographic coordinates of the location. (Google Maps, 2023) There are about 250 houseboats in the vicinity of this spot.

## II. METHODOLOGY

Study area: Dal Lake in Srinagar, Jammu & Kashmir, served as the site of the inquiry. The lake is home to about 38,000 people, and houseboats may accommodate up to 10,000 people (Majeed et al., 2021). Particularly during the summer months, the people living around the lake adds to the solid waste, which either directly or indirectly pollutes the lake by disposing of waste materials carelessly into the water. The fact that so many people reside in hamlets inside Dal Lake makes it special.

#### Research Design:

Research methodology: To gain a thorough grasp of the difficulties in managing solid waste in Dal Lake, this study used a mixed-methods research methodology that included qualitative and quantitative methodologies.

#### Data Collection:

##### Primary Data:

- a) Surveys: A pre-testing survey was carried out to gain an understanding of the difficulties experienced by Dal Lake houseboat operators. The interview was designed with the goals and the data gathered from the pre-test survey in mind. It was also translated into Urdu for improved

understanding. Structured questionnaires were distributed to houseboat owners in order to gather data on their experiences, opinions, and present problems with solid waste management in Dal Lake.

- b) Interviews: To acquire a greater understanding of the difficulties encountered, in-depth interviews with important informants and community leaders were undertaken.

#### Secondary Data:

- a) Literature Review: To comprehend the larger context and pinpoint best practices, existing literature, research papers, reports, and policy documents pertaining to solid waste management in comparable ecological environments were studied.
- b) Official documents: Information on trash creation, collection, transportation, and disposal procedures was obtained by accessing pertinent official documents from waste management organizations like LCMA.

#### Sampling:

- a) sample Strategy: A mix of random and selective sample methods was used. Owners of houseboats residing in Dal Lake houseboats were among the selection criteria for responders.
- b) Sample Size: After obtaining a list of registered houseboats from the LCMA, it was found that 950 of them were located in Dal Lake, which includes Lakutt Dal, Bod Dal, Gagribal, and Nigeen Lake. Stratified random selection was used to pick a sample of 100 houseboats for the study, 25 from each site.

#### Data Analysis:

- a) Quantitative Analysis: In order to obtain significant insights and spot trends and patterns, survey data were analyzed using the appropriate statistical techniques, such as descriptive statistics and inferential tests.
- b) Qualitative Analysis: To find reoccurring themes and significant difficulties in solid waste management, a thematic analysis was used to interview transcripts. Coding, classification, and interpretation of qualitative data were performed in this research to provide a comprehensive understanding of the relevant issues.

## III. RESULT AND DISCUSSION

This essay goes into great detail on the creation of solid trash, how it is managed, and the difficulties faced by Dal Lake houseboat owners. The LCMA hired G-active Services, one of the firms, to handle the solid waste collection in Dal. The agency was chosen via an appropriate tendering procedure.

**Site 1 (Lokut Dal).**

The vast majority of house boat owners at the location (92%) said that neither the LCMA nor they had made any efforts to get color-coded trash cans for collection of waste. It was found that the respondents had bought little, non-color-coded bins to collect rubbish from the houseboats but were unaware of the need of colored-coded bins. They didn't separate their garbage since they didn't understand how important it was. Majeed et al. (2012) reported comparable results, emphasizing the need for distinct trash receptacles for on-site segregation, such that the amount of solid waste that ends up in the disposal unit is minimal. According to 76% of respondents, the Lake Conservation Management Authority (LCMA) and houseboat owners do not collaborate. The houseboat owners felt that the LCMA officials should establish a close relationship with them in order to enable them to function at the ground level and help the houseboat owners overcome the problems or difficulties encountered. They ought to help them recognize the requirements and difficulties experienced by houseboat owners. According to 72% of the respondents, the large number of tourists that stay on houseboats during the March–April tourism season generates a significant quantity of garbage. Similar conclusions were reached by Shah & Wani (2013), who found that the amount of solid waste generated is directly correlated with the number of rooms occupied by residents and visitors. However, they expressed concern about the small size and inadequate capacity of the carts used for solid waste collection, as well as the poor quality of the cart service. They also mentioned that the collectors' harsh behaviour and poor sanitation were issues with waste pickup. When asked to clean up gently, the trash collectors would verbally dispute and mistreat the people who had left waste in wooden boxes that occasionally had unpleasant smells. The garbage collectors also failed to properly remove the rubbish that had been placed outside their houseboats. 32% of respondents said they would be interested in learning more about solid waste management. They expressed a wish to get knowledgeable about solid waste management strategies in order to turn trash into money. Twenty percent of houseboat owners said that their lack of experience in this area prevented them from advising visitors and residents of Dal Lake on how to properly dispose of their trash. On the other hand, houseboat owners who receive training and education may contribute to solid waste management by giving tips on how to handle rubbish better. Sixteen percent of respondents said that the trash collected was dumped close by, which both houseboat owners and visitors found inconvenient due to the offensive stench.

**Site 2 (Bod Dal)**

Table 2 enumerates the challenges that the houseboat owners at Site 2 confront. 84% of those surveyed said that the LCMA's rubbish collection service was inconsistent. Ganie & Hashia also discovered similar results, pointing out that enormous piles of solid trash are present both inside and outside houseboats and are frequently overlooked by the department. Due to the fact that garbage collection was only done once every 20 days, there was a substantial amount of trash accumulation in the houseboats, which resulted in a foul smell. They disclosed that the offensive stench emanating from the trash was a source of annoyance for both houseboat occupants and visitors. 80% of those surveyed stated that although LCMA had provided boats for garbage collection, another challenge with solid waste management was that the boats were non-operational. They could not carry all of the waste from the houseboats to the disposal site because they were very small damaged, and unreliable. Sixty-eight percent of the houseboat owners deduced that they were obliged to bring their own garbage to the disposal site due to the LCMA's inconsistent services. They are so overworked since they are required to finish duties that LCMA workers would typically perform. At site 2, the houseboat owners also showed that they were prepared to impose stringent regulations on the locals and visitors. There is a noticeable annual inflow of tourists in and around Dal Lake. Even though LCMA has supplied dustbins, people still buy delicacies from shops close to the lake and discard their rubbish in the water and surrounding areas. on a manner similar to this, people who go boating on the lake and live there purchase meals from merchants. After devouring the treats, the majority of them discard the rubbish or leftovers into the lake. Both locals and tourists patronize Mina Bazar, a lakeside market place. Additionally, the throwaway plates that are used to feed guests wind up in the lake. The houseboat owners believed that the LCMA need to establish stringent guidelines and policies for disposing of garbage. They said the LCMA need to employ security personnel who would keep an eye out for litterers and take swift action against them. At site 2, color-coded dustbins were favored by 72% of respondents for efficient trash disposal and rubbish segregation. Furthermore, as reported by 28% of respondents at this location, there was little cooperation between LCMA representatives and houseboat owners, leading to a notable discrepancy between the real and ideal circumstances. Four percent of the participants said that the growing number of houseboats in Dal Lake was negatively impacting the lake's health and suggested reducing the houseboat population. In addition to preventing congestion, this would help maintain the lake's elegance. Twenty percent of

those surveyed said that they were required to tip the garbage collectors, who only visited the site once a month to pick up the rubbish. On the other hand, many stated that they would prefer Consistent services and a fixed price rather than gratuities and sporadic assistance.

### Site 3 (Gagribal)

Table 3 lists the difficulties houseboat owners at Site 3 (Gagribal) are attempting to overcome. The majority of responders at this location reported similar problems to those at site 2. Nonetheless, 16% of respondents at this location desired that the garbage collectors wear uniforms so that houseboat owners could quickly identify them and get in touch with them to remove the rubbish off their boats. They thought that doing this would also aid in teaching trash collectors discipline. In order to keep the area around the lake clean, 44% of respondents said there wasn't enough labor to gather rubbish, and they consequently indicated the wish to hire more personnel.

### Site 4 (Nigeen)

The houseboat owners at site 4 had comparatively less obstacles than those faced by their counterparts at sites 1, 2, and 3. The investigator found that the participants expressed more satisfaction with the services provided by LCMA. Nonetheless, the houseboat owners brought to light some concerns, such as the fact that the LCMA employees were only picking up garbage twice a week when they would have expected it to happen at least four times. They also thought that strong regulations, such fines for litterers, should be put into place.

Unlike other sites, houseboat owners here have colored-coded trash cans. Merely 24% of participants reported that their garbage cans lacked color coding. It was also positive that 36% of the respondents said they had no problems with managing solid trash.

Table 1: Difficulties encountered by houseboat owners at site 1 (Lakut Dal)

n1=25

s.no	CHALLENGES	f	%	MS	RANK
1	Absence of Color-coded bins	23	92	9.2	I
2	Insufficient collaboration between LCMA and houseboat owners	19	76	6.84	II
3	Insufficient vehicles for door-to-door pickup	18	72	5.76	III
4	Insufficiently competent and trained labor force for collecting solid trash	13	52	3.64	IV
5	irregular services for the collection of solid garbage	11	44	2.64	V
6	Absence of knowledge on solid waste management	8	32	1.6	VI
7	Manpower shortage	7	28	1.12	VII
8	Carts' inadequate capacity	6	24	0.72	VIII
9	Inadequate instructions for residents and visitors on how to properly dispose of solid trash	5	20	0.4	IX
10	Absence of good manners in trash collectors	4	16	0.16	X

MS=Mean score

Table 2: Difficulties encountered by houseboat owners at site2 (Lakut Dal)

n2=25

s.no	CHALLENGES	f	%	MS	RANK
1	irregular services for the collection of solid garbage	21	84	8.4	I
2	Absence of newly constructed or renovated LCMA boats with large carrying capacities for collecting solid garbage	20	80	7.2	II
3	Manpower shortage	17	68	4.76	IV
4	Inadequate instructions for residents and visitors on how to properly dispose of solid trash	8	32	1.92	V
5	Absence of Color-coded bins	18	72	5.76	III
6	Insufficient collaboration between LCMA and houseboat owners	7	28	1.4	VI

7	houseboat odors	4	16	0.48	VIII
8	Absence of restrictions on the number of visitors on Dal Lake houseboats	1	4	0.04	X
9	Absence of a monthly charging structure	5	20	0.8	VII
10	Inability to restrict the number of tourists on houseboats	3	12	0.24	IX

Table 3: Difficulties encountered by houseboat owners at site 3 (Gagribal)

n3=25

s.no	CHALLENGES	f	%	MS	RANK
1	irregular services for the collection of solid garbage	18	72	6.48	II
2	Insufficient collaboration between LCMA and houseboat owners	23	92	9.2	I
3	Absence of surveys from LCMA	5	20	0.8	VII
4	houseboat odors	3	12	0.24	VIII
5	Absence of Color-coded bins	16	64	4.48	IV
6	Absence of uniform for trash pickers	4	16	0.48	IX
7	Inadequate instructions for residents and visitors on how to properly dispose of solid trash	8	32	1.6	VI
8	Absence of newly constructed or renovated LCMA boats with large carrying capacities for collecting solid garbage	17	68	5.44	III
9	Absence of a monthly charging structure	2	8	0.08	X
10	Manpower shortage	11	44	2.64	V

Table 4: Difficulties encountered by houseboat owners at site 4 (Nigeen)

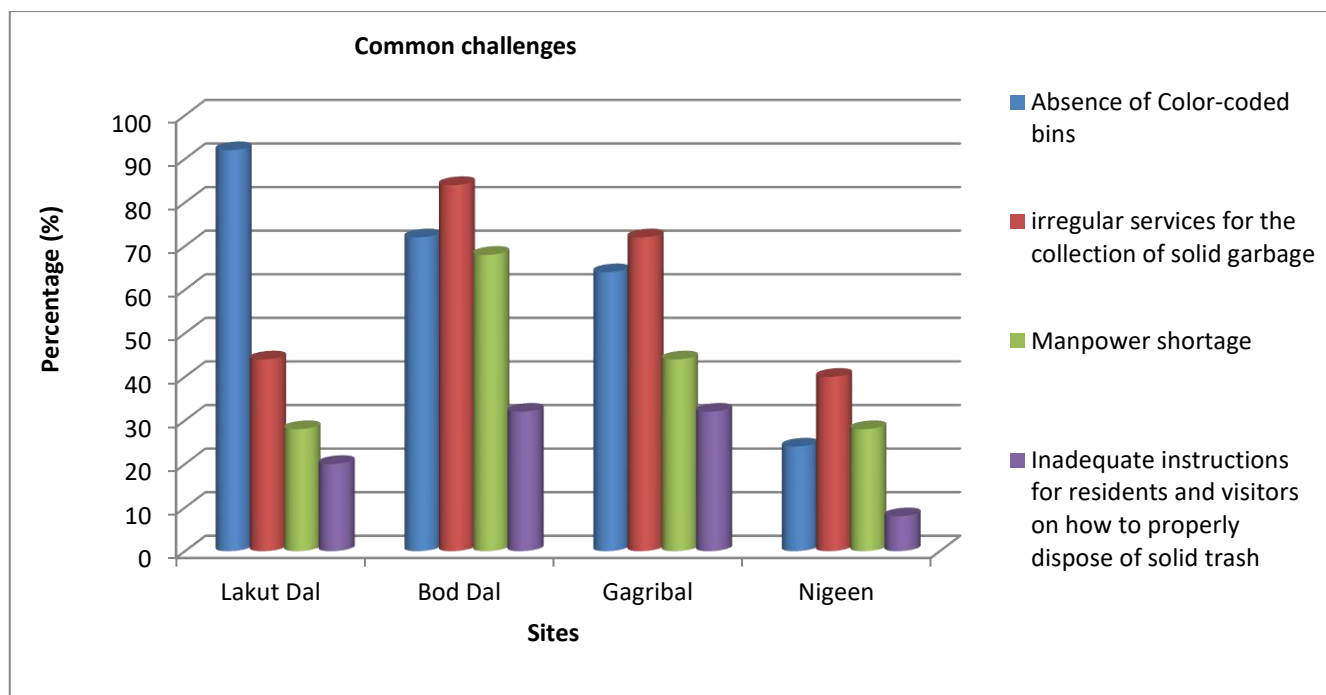
n4=25

s.no	CHALLENGES	f	%	MS	RANK
1	Absence of daily trash collection offerings	10	40	2.0	I
2	Absence of Color-coded bins	6	24	0.96	III
3	Inadequate road infrastructure	1	4	0.04	V
5	Inadequate instructions for residents and visitors on how to properly dispose of solid trash	2	8	0.24	IV
6	Manpower shortage	7	28	1.12	II

Table 5: common issues from Dal Lake's four locations

n5=100

s.no	CHALLENGES	%	MS	RANK
1	Absence of Color-coded bins	63	2.52	I
2	Inadequate instructions for residents and visitors on how to properly dispose of solid trash	25	0.25	IV
3	Manpower shortage	42	0.84	III
4	irregular services for the collection of solid garbage	60	1.8	II



#### IV. CONCLUSION

The investigation's findings support the notion that managing solid waste presented a number of difficulties for houseboat owners. They lack the proper infrastructure for disposing of solid waste, such as collection systems or facilities. Houseboat owners find it difficult to dispose of their garbage because of this lack of infrastructure. Houseboat occupancy fluctuates throughout the year, with peak season producing large amounts of garbage. As a result, the LCMA has to have effective plans in place to deal with the extra waste generated during peak season. It's possible that visitors and houseboat owners are unaware of the necessary waste management techniques that have a detrimental influence on the ecosystem. As Houseboats are not affable by LCMA trucks for the collection of garbage, hence the provision of special boats for this purpose is essential.

#### V. RECCOMENDATIONS

##### IMPARTING GENDER INCLUSIVE TRAININGS

It is required that the houseboat owners of Lakut Dal and Nigeen Lake get on-campus instruction about solid waste management in cooperation with the Faculty of Fisheries SKUAST-K's extension division (Social Sciences). Houseboat owners in Bod Dal and Gagribal did not show a willingness to participate in the trainings because of their busy schedules, but their female population can receive solid waste management training through on-campus or off-campus programs, which can help lessen the impact of solid waste on Dal Lake in the long run.

#### CONTRIBUTORY APPROACH

Since the LCMA has been tasked with overseeing and protecting the lake. In order to manage solid waste effectively, conservation policies must now be implemented in a collaborative rather than an authoritarian fashion, including all stakeholders and making incorrect disposal of solid waste punishable by appropriate laws or rules. Considering the outcome, the majority of houseboat owners acknowledged that they didn't get along with LCMA representatives, supporting the earlier claim. The success of the LCMA's efforts to manage solid waste in Dal Lake depends on its ability to guarantee that all parties involved, including houseboat owners participate in all stages of the initiatives created to guarantee secure disposal of garbage. A participatory strategy will provide two-way contact between the LCMA authorities and important stakeholders, as well as help the LCMA develop stronger programs and ideas for managing solid waste.

#### GENERATING MONEY FROM WASTE

Training in waste management and segregation must be offered. If the responders are taught how to convert the enormous amount of trash created by houseboats across all four research locations into organic manure, it may be utilized for plants as well as Dal Lake's floating garden. Promoting the use of organic manures rather than fertilizers may also be advantageous for the environment and help the LCMA develop better plans and initiatives for the management of solid waste.

### ORDINARY SERVICES

Regular services must be provided by LCMA at least four times a week. This would help houseboat owners preserve Dal Lake's general health as well as the cleanliness of their community. It is recommended that the LCMA charge houseboat owners a monthly fee in exchange for their regular services in order to establish an effective garbage collection system. The LCMA should fix the damaged boats that were used to remove waste from houseboats. It would be necessary to bring new, spacious boats in order to make sure that all of the garbage from the houseboats gets collected. Promoting boats for rubbish collection is necessary since they are the best means of accessing a lake's interior..

### SETTING UP A SUFFICIENT NUMBER OF WASTE COLLECTORS

Because Dal Lake is a well-liked tourist site and produces a significant amount of rubbish, LCMA needs to hire a large number of waste collectors. Garbage collectors must to be required to wear uniforms and complete solid waste management training in order to establish discipline.

### TEAMWORK PROGRAMME WITH FACULTY OF FISHERIES, SKUAST-K

The Faculty of Fisheries SKUAST-K and the LCMA should work together on any training and research pertaining to Dal Lake preservation.

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# Amino Acid Profile and Physicochemical Properties of African Locust Bean (*Parkia biglobosa*) Seeds as affected by Combined Irradiation and Cooking

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**Abstract**— This study examined the impact of cooking, Co-60 gamma irradiation (5 kGy and 10 kGy) and combined irradiation (10 kGy) and cooking treatments on the amino acid profile and physicochemical properties of African locust bean (*Parkia biglobosa*) seeds, as well as the quality of the seed oil. The treated and untreated (control) seeds were mechanically dehulled and milled into flour using an attrition mill, and subsequently analysed for chemical composition, amino acid profile, functional properties and quality of the seed oil. The results showed a significant increase in the fat content ( $p \leq 0.05$ ) after cooking and post-irradiation cooking treatments. The cooked seed samples recorded a 3.84% increase in total mineral content although there was a loss in the total percentage minerals after combined irradiation and cooking which could be linked to leaching of soluble minerals into cooking water. The 10 kGy irradiation dose improved the total essential amino acids present in the seed possibly due to the lyses of adherent microflora. Percentage cysteine was highest in the 5 kGy irradiated seed sample with about 4.8% increase compared to control. There was a 92.6% loss in foaming capacity of the seed flour as a result of processing; however, 5 kGy irradiated sample recorded only about 26% loss in the foaming capacity. The anti-nutrients were significantly reduced by cooking, irradiation, post-irradiation cooking up to about 60%, 40%, 26.7% and 60% for cooked, 5 kGy irradiated, 10 kGy irradiated and post-irradiated cooked samples, respectively. Tannin content was not significantly affected ( $p < 0.05$ ) by the irradiation treatments; it was however reduced significantly by post-irradiation cooking. The 5 kGy and 10 kGy doses did not significantly affect iodine value of the oil sample. There was however an increase in iodine value as a result of cooking and post-irradiation cooking with values as high as 163.53 g I<sub>2</sub> and 182.77 g I<sub>2</sub> per 100g, respectively. Overall, it could be concluded that medium dose gamma irradiation did not negatively affect the quality of the African locust bean seed, which is desirable for elongation of storage life of the seed.



**Keywords**— Gamma irradiation, Cooking, Locust bean seed, Physicochemical properties, Oil quality.

## I. INTRODUCTION

The quest for greater value-addition, improved aesthetics and elongation of shelf-life of unconventional and wild uncultivated forest seeds with high nutrient potentials has

led to wider exploration and upgrading of traditional foods which hitherto were consumed at the basic subsistence level (Enujiugha, 2000; Oguntimehin *et al.*, 2023). Different techniques are employed to preserve such seeds, one of which is food irradiation that involves exposure of food to



a controlled source of ionizing radiation with a view to reducing microbial load, extending product shelf life, and disinfecting agricultural produce (Enujiugha, *et al.*, 2012; 2023a; Oyinloye *et al.*, 2023). Food irradiation is a more reliable and safer technique of preserving food and improving the nutritional value (Diehl, 2002; Al-Kaisey *et al.*, 2002). Three types of radiation are applicable for use in food preservation: gamma rays, X-rays and rays from high energy electron beams (James, 2006). Gamma irradiation, also known as cold pasteurization, is a technique of food preservation that has been proven to prevent insect infestation in food products during storage and also deter food contamination by microorganisms (Farkas, 1990). Combining low-dose gamma irradiation with cooking in food processing and preservation is a hurdle strategy that allows effective processing while minimizing the severity of treatment (Enujiugha *et al.*, 2012). To maximize the effectiveness of combined treatment, mild irradiation between 1 and 10 kGy is proposed as best (Campbell-Platt and Grandson, 1990). The use of cooking and  $\gamma$ -irradiation as forms of preservation are both proven technologies and the effectiveness of both could be maximized if they are combined in a hurdle effect (Olotu *et al.*, 2014a,b).

The main drive in the application of hurdle technology, and in this case, combination of low dose gamma irradiation with cooking, has been the prevalent food safety concerns and low shelf life of our indigenous foods. Fagbemi *et al.* (2023) observed the high microbial density in selected indigenous foods available for direct human consumption in a typical urban neighbourhood in sub-Saharan Africa, which is a public health concern when the huge numbers of consuming populations are considered. It is widely believed that irradiation of agricultural produce at low doses, before storage and processing, could reduce the normally-high incidence of microbial infestation and curtail the occurrence of disease-causing pathogenic micro-organisms. Combination of this preservation technique with other treatments like cooking in a hurdle effect would ensure effectiveness without adversely affecting the innate nutrients.

Low income earners in developing countries like Nigeria can hardly afford animal products, such as milk, meat, fish and eggs, which are rich sources of protein. In these areas, staple diets consist mainly of cereal grains or starchy root and tuber crops thus leading to various health problems associated with protein and vitamin/mineral deficiencies (Enujiugha, 2020). This has led to various researches being directed towards the utilization of plant protein sources in the form of nuts, oilseeds and legumes to tackle the prevailing protein-energy malnutrition ravaging many parts of less-developed nations (Enujiugha and Ayodele-Oni, 2003; Enujiugha *et al.*, 2023b; Talabi *et al.*, 2023). In the

exploration of plant protein and vitamin substitutes, the African locust bean (*Parkia biglobosa*) has become very popular especially in the fermented 'Dawadawa' and 'iru' forms, which are commonly-derived products from the seeds (Enujiugha, 2009; Gernah *et al.*, 2007). Dawadawa or iru (fermented *Parkia biglobosa* seeds) is used for seasoning of traditional soups in all parts of Nigeria and indeed the West Coast of Africa (Enujiugha *et al.*, 2006). The sticky, sour solid contains sufficient amounts of protein, vitamins, energy (Oyedokun *et al.*, 2016; 2020) and has appreciable shelf life even without refrigeration. Combined irradiation and cooking treatments could further extend the shelf life but have also been shown to effect various changes in the physical, chemical and functional properties of foods (Olotu *et al.*, 2014a). This study examined the effect of combined low-dose gamma irradiation and post-irradiation cooking on the functional and physicochemical properties of African locust bean seed and its oil quality characteristics, in comparison to single irradiation and cooking treatments. This was with a view to highlighting the potentials of such hurdle treatments in the preservation of the nutrient potentials of the seeds while at the same time achieving the twin functions of disinfection and processing.

## II. MATERIALS AND METHODS

### Material Procurement and Preparation

Wholesome African locust bean seeds (*Parkia biglobosa*) were obtained from a local market (*Oja Oba*), in Akure, Ondo State of Nigeria. Upon reception, the seeds were visually inspected and defective ones were discarded. The seeds were then kept in air tight polyethylene containers in a dry and cool environment until ready for use. All the chemicals and reagents used in the study were of analytical grade. The raw grains were treated at room temperature with gamma rays at doses of 5 and 10 kGy using a multipurpose gamma irradiator with a cobalt 60 source (compact-type commercial radiator) at the Shedan Science and Technology Complex (SHESTCO), Abuja Nigeria (Oyinloye *et al.*, 2023). Raw untreated locust bean seeds served as control in the experiment. The 5 kGy sample was milled immediately while the 10 kGy irradiated sample was divided into two parts; a part was milled while the other part and half part of the raw (uncooked and un-irradiated) samples were subjected to hydrothermal treatment in the proportion of 1:3 (seed to water ratio) for 6 h at 100 °C. The samples were placed in aluminum trays and dried with forced circulation of air at 50-55 °C until constant weight (approximately 24 h).

### Determination of Proximate chemical composition

Quantitative composition was determined on each of the flour samples using the following analytical methods: Moisture content using the air oven method at 105 °C until constant weight was achieved (AOAC, 2012); crude protein using the micro-Kjeldahl apparatus (AOAC, 2012); crude fat extracted overnight in a Soxhlet extractor with n-hexane and quantified gravimetrically; crude ash via exhaustive combustion in a Muffle furnace at 550 °C for 8 h (AOAC, 2012); crude fibre estimated after digesting known weights of fat-free samples in refluxing 1.25% sulphuric acid and 1.25% sodium hydroxide; and carbohydrate determined by difference method (subtracting the percent crude protein, crude fibre, crude fat, and ash from 100% dry matter). All analyses were carried out in triplicates. The gross energy was calculated based on the formula reported by Enujiugha and Ayodele-Oni (2003).

### Mineral analysis

Analysis of sodium and potassium contents of the samples was carried out using flame photometer, while phosphorus was determined colourimetrically by the phosphovanadomolybdate (yellow) method (AOAC, 2012). The other elemental concentrations were determined after wet digestion of sample ash with a mixture of nitric and perchloric acids (1:1 v/v), using atomic absorption Spectrophotometer (AAS, Buck Model 20A, Buck Scientific, East Norwalk, CT06855, USA). All the determinations were carried out in triplicates.

### Determination of Amino Acids Profile

The amino acids profile in the sample was determined using the method of Olotu *et al.* (2014b), with slight modifications. The samples were dried to constant weight, exhaustively defatted, acid-hydrolyzed (or alkaline hydrolyzed, in the case of tryptophan), evaporated in a rotatory evaporator and loaded into the technicon sequential multi sample amino acid analyzer (TSM). Briefly, a known weight of sample powder was weighed into extraction thimble and any remaining fat was extracted with chloroform/methanol (2:1) using Soxhlet extraction apparatus as described by AOAC (2012); the extraction lasted for 15 hours. A known weight of the defatted sample was then weighed into glass ampoule. Exactly 7 ml of 6N HCL (or 6N KOH) was added and oxygen was expelled by passing nitrogen into the ampoule in order to avoid possible oxidation of some amino acids during hydrolysis e.g. methionine and cysteine. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 ± 5 °C for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the remains. The filtrate was evaporated to dryness at 40 °C under vacuum in a rotator evaporator. The residue

was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer. The amount of hydrolysate loaded into the TSM analyser was between 5 to 10 microliters. This was dispensed into the cartridge of the analyzer. The TSM analyser is designed to separate and analyze free acidic, neutral and basic amino acid of the hydrolysate. The period of an analysis lasted for 76 minutes. All determinations were carried out in triplicates.

### Determination of functional properties

The determination of water and oil absorption capacities followed a modification of the method of Prinyawiwatkul *et al.* (1999). Each flour sample (5 g) was thoroughly mixed, without pH adjustment with 25 ml of deionized water or oil in 50 ml centrifuge tubes. Suspensions were stirred intermittently over a 30 min period at room temperature (28±2 °C) and then centrifuged at 12,000 x g for 30 min at 25 °C. The volume of decanted supernatant was centrifuged and the water and oil absorption capacities were calculated. Triplicate samples were analyzed for each flour sample category.

Least gelation concentration was carried out as described by Enujiugha and Akanbi (2005). Triplicate suspension of 1-20% seed flour sample (dry w/v at 1% increment) were prepared in 10 ml of deionized water and mixed thoroughly without pH adjustment. The slurries were heated in 125 x 20 mm screw-capped test tubes in a water bath with in-built magnetic stirrer (Julabo Model SW22, Julabo Labortechnik GMBH, Seelbach, Germany) at 95 ± 2 °C. After 1 h of heating, tubes were immediately cooled in tap water for 30 s and then in ice water for 5mins to accelerate gel formation. All tubes were then held at 4 °C for 3 h. The least gelation concentration (%) was determined as the concentration above which the sample remained in the bottom of the inverted tube.

The foaming properties of the samples were determined using the method of Coffmann and Garcia (1977), with slight modifications. Two grams (2 g) of the sample was weighed into 60 ml distilled water in a 100 ml cylinder. Solid material was dispersed with spatula and the suspension was whipped for 5 min using ultra-Turax T25 mixer at high speed. Volumes before and after whipping were noted and volume increase due to whipping was calculated. The volume of foam in the standing cylinder was also recorded for foam stability at 1, 5, 10, 20, 30, 60, 90, 120 and 180 min after whipping. The results were expressed in percentages (g/g basis).

Emulsifying properties were determined with a slight modification of the method described by Ige *et al.*, (1984). A known quantity (1.8 g) of sample was dispersed in 25 ml distilled water and 25ml vegetable oil (pure groundnut oil)

was added. The 50 ml mixture was emulsified at high speed using ultra-Turax T25 mixer for 1 min. emulsion was filled into centrifuge tubes and centrifuged for 5 min at 1,300 x 6 rpm. Percentage emulsion was then expressed as:

$$\% \text{ Emulsion} = 100 \times \frac{x}{y}$$

Where  $x$  = height of emulsified layer

$y$  = height of whole solution in centrifuge tube.

The results were expressed in percentages (g/g basis).

#### Determination of anti-nutritional factors

The modified method of Reddy *et al.*, (1982) was used for phytic acid and phytate-phosphorus determinations. Phytic acid was extracted from each 3 g flour sample with 3% trichloroacetic acid by shaking at room temperature followed by high-speed centrifugation (30,000 x g for 5 min), the phytic acid in the supernatant was precipitated as ferric phytate and iron in the sample was estimated. Phytate-phosphorus (phytate-P) was calculated from the iron results assuming a 4:6 iron: phosphorous molecular ratio (AOAC, 2012). The phytic acid was estimated by multiplying the amount of phytate-phosphorous by the factor 3.55 based on the empirical formula  $C_6P_6O_{24}H_{18}$  (Enujiugha and Olagundoye, 2001).

Tannin contents were determined by the modified vanillin-HCl method (Price *et al.*, 1978). A 2 g sample was extracted with 50 ml 99.9% methanol for 20 min at room temperature with constant agitation. After centrifugation for 10 min at 653 x g, 5 ml of vanillin-HCl (2% vanillin, 1% HCl) reagent was added to 1 ml aliquots and the colour developed after 20 min at room temperature was read at 500 nm. Correction for interference by natural pigments in the sample was achieved by subjecting the extract to the conditions of the reaction but without vanillin reagent. A standard curve was prepared using catechin (Sigma Chemical, St. Louis, MO) after correcting for blank, and tannin concentration was expressed in mg/g.

Determination of oxalate was by the AOAC (2012) method. One gram (1 g) of finely ground sample was dissolved in 75 ml of 1.5 N  $H_2SO_4$ . The solution was carefully stirred intermittently with a magnetic stirrer for about 1hr and filtered using Whatman no. filter paper. A 25 ml sample of the filtrate (extract) was collected and titrated hot (80-90 °C) against 0.1 N  $KMnO_4$  solution to the point when a faint pink colour appeared that persisted for at least 30 s. The concentration of the oxalate in each sample got from the calculation: 1 ml 0.1 N permanganate = 0.006303 g oxalate. All procedures were carried out in triplicates.

#### Determination of seed oil characteristics

The seed oils of the samples were extracted using Soxhlet apparatus (Talabi and Enujiugha, 2014) and the rancidity indices (peroxide value, saponification value, iodine value, free fatty acids content and acid value) were determined according to the standard methods of AOAC (2012). The peroxide values were expressed as miliequivalents of peroxide oxygen per kg of sample (mEq/kg) while the free fatty acids were expressed as g oleic acid per 100 g of sample (g/100 g). The acid value was expressed as mg NaOH per g of sample (mg NaOH/g). The saponification value was expressed as mg KOH per g of sample (mg KOH/g). Iodine value was determined by the AOAC (2012) method using Wij's iodine solution.

#### Statistical analysis

Data collected from the study were subjected to one-way analysis of variance (ANOVA). Differences among means were separated using Duncan's new multiple range test, and significances were accepted at 5% confidence level ( $P \leq 0.05$ ). The statistical software used was SPSS 16.0 for windows.

### III. RESULTS AND DISCUSSION

#### Proximate Composition of *Parkia biglobosa*

Table 1 shows the effects of gamma radiation on the proximate compositions of raw and cooked *Parkia biglobosa* seeds. There was an increase in the fat content of the cooked seeds after irradiation. This may be due to the rupture of oil cells in the seed skin. Badiani *et al.* (2002) reported that most nutrients increased their concentration as a consequence of moisture loss through cooking. The protein content of the cooked seeds was reduced by 2.9% from the raw. This is in line with the report of Attia *et al.* (1994) who observed a reduction in protein contents after cooking chickpea. There was also a slight decrease in the protein content of seed samples upon irradiation. This might be due to damage on sulphur-containing amino acids. The most radiation sensitive amino acids are those that contain sulphur, notably cysteine, methionine and tryptophan. Desulphurization is one of the principal effects of ionizing radiation on amino acids and proteins (Singh *et al.*, 1991). The single and combined irradiation and cooking treatments did not significantly ( $p \geq 0.05$ ) affect the ash, fibre and carbohydrate contents of the locust bean seeds.

Table 1: Proximate composition of raw and treated *Parkia biglobosa* seeds

Parameters (g/100g)	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
Moisture Content	4.59 <sup>a</sup> ±0.13	4.50 <sup>a</sup> ±0.24	4.53 <sup>a</sup> ±0.09	4.57 <sup>a</sup> ±0.10	4.62 <sup>a</sup> ±0.12
Ash content	6.18 <sup>a</sup> ±0.02	5.97 <sup>a</sup> ±0.03	5.89 <sup>a</sup> ±0.03	5.75 <sup>a</sup> ±0.02	5.76 <sup>a</sup> ±0.04
Total fat/oil	33.92 <sup>b</sup> ±0.12	34.06 <sup>ab</sup> ±0.10	33.55 <sup>b</sup> ±0.11	33.87 <sup>b</sup> ±0.09	34.04 <sup>a</sup> ±0.12
Crude Fibre content	5.57 <sup>a</sup> ±0.02	5.24 <sup>a</sup> ±0.01	5.82 <sup>a</sup> ±0.02	5.60 <sup>a</sup> ±0.02	5.71 <sup>a</sup> ±0.02
Crude protein	32.82 <sup>a</sup> ±0.14	31.88 <sup>b</sup> ±0.12	32.73 <sup>a</sup> ±0.13	32.48 <sup>a</sup> ±0.13	32.22 <sup>ab</sup> ±0.12
Carbohydrate content	20.45 <sup>a</sup> ±0.32	20.56 <sup>a</sup> ±0.24	21.57 <sup>a</sup> ±0.29	21.47 <sup>a</sup> ±0.26	21.41 <sup>a</sup> ±0.42
Gross Energy (KJ 100g <sup>-1</sup> DM)	2168.393	2212.59	2171.645	2165.8	2250.559

\*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different (p≥0.05).

Table 2: Amino acid composition of raw and treated *P. biglobosa* seeds

Parameters	Raw	Cooked	Irradiated(10kGy)	Irradiated+Cooked
Lysine*	3.54 <sup>c</sup> ±0.01	3.60 <sup>b</sup> ±0.02	3.76 <sup>a</sup> ±0.01	3.19 <sup>d</sup> ±0.04
Histidine*	2.29 <sup>c</sup> ±0.12	2.54 <sup>b</sup> ±0.02	2.57 <sup>a</sup> ±0.03	2.07 <sup>d</sup> ±0.02
Arginine*	10.03 <sup>b</sup> ±0.04	10.04 <sup>b</sup> ±0.03	10.30 <sup>a</sup> ±0.03	9.36 <sup>c</sup> ±0.22
Asparagine	10.60 <sup>a</sup> ±0.03	10.15 <sup>c</sup> ±0.04	10.59 <sup>a</sup> ±0.02	10.28 <sup>b</sup> ±0.14
Threonine*	3.27 <sup>a</sup> ±0.02	3.00 <sup>b</sup> ±0.02	3.27 <sup>a</sup> ±0.01	3.27 <sup>a</sup> ±0.02
Serine	2.91 <sup>b</sup> ±0.12	2.55 <sup>c</sup> ±0.03	3.05 <sup>a</sup> ±0.02	2.54 <sup>c</sup> ±0.03
Gluthamine	16.20 <sup>a</sup> ±0.04	14.14 <sup>c</sup> ±0.21	15.14 <sup>b</sup> ±0.30	13.24 <sup>d</sup> ±0.02
Proline	3.29 <sup>c</sup> ±0.02	4.14 <sup>b</sup> ±0.02	4.46 <sup>a</sup> ±0.01	2.97 <sup>d</sup> ±0.01
Glycine	4.69 <sup>b</sup> ±1.02	4.52 <sup>c</sup> ±0.13	5.01 <sup>a</sup> ±0.13	4.17 <sup>d</sup> ±0.20
Alanine	3.94 <sup>b</sup> ±0.03	3.90 <sup>c</sup> ±0.72	4.05 <sup>a</sup> ±0.42	3.59 <sup>d</sup> ±1.33
Cysteine*	1.65 <sup>c</sup> ±0.22	1.69 <sup>b</sup> ±0.66	1.72 <sup>a</sup> ±0.92	1.39 <sup>d</sup> ±0.12
Valine*	3.83 <sup>c</sup> ±0.14	5.08 <sup>a</sup> ±0.13	5.00 <sup>b</sup> ±0.34	3.60 <sup>d</sup> ±0.87
Methionine*	1.41 <sup>a</sup> ±0.26	1.30 <sup>c</sup> ±0.01	1.38 <sup>b</sup> ±0.12	1.20 <sup>d</sup> ±0.01
Isoleucine*	3.95 <sup>b</sup> ±0.03	3.83 <sup>c</sup> ±0.05	4.14 <sup>a</sup> ±0.03	3.39 <sup>d</sup> ±0.18
Leucine*	6.05 <sup>a</sup> ±0.01	5.66 <sup>c</sup> ±0.01	5.85 <sup>b</sup> ±0.01	5.02 <sup>d</sup> ±0.01
Tyrosine*	1.61 <sup>d</sup> ±0.00	2.74 <sup>b</sup> ±0.02	2.90 <sup>a</sup> ±0.04	2.25 <sup>c</sup> ±0.02
Phenylalanine*	4.56 <sup>b</sup> ±0.02	4.31 <sup>c</sup> ±0.18	4.65 <sup>a</sup> ±0.14	3.97 <sup>d</sup> ±0.06
Tryptophan	2.10 <sup>a</sup> ±0.05	1.97 <sup>a</sup> ±0.02	2.08 <sup>a</sup> ±0.07	1.95 <sup>a</sup> ±0.01

\*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different (p≥0.05).

### Effects of processing on the amino acid profile of *Parkia biglobosa*

Table 2 presents the results of the effect of processing (cooking, irradiation and a combination of both) on the amino acids composition of *Parkia biglobosa* seeds. Amino acids are needed for the synthesis of most body tissues,

enzymes, hormones and other metabolic molecules (Olotu et al., 2014b). Glutamate and Aspartate accounted for about 30% of the amino acids in the sample which is similar to amino acid profiles reported previously in some oil seeds (Olotu et al., 2014b; Igwe et al., 2012). Sulphur-containing amino acids (methionine and cysteine) were the least

concentrated with values ranging from 1.20 g to 1.41 g and 1.39 g to 1.72 g, respectively. The reduced concentration of methionine could be attributed to the simple amino acids undergoing reductive deamination and decarboxylation during irradiation (Enujiugha *et al.*, 2023a). Cooking significantly ( $p \leq 0.05$ ) reduced the amino acid contents except for valine which is heat stable. The reductive effects of cooking on protein and amino acid compositions of the seeds may be attributed to Amadori rearrangements that may go beyond the deoxy-ketosyl stage. It may also be due to the formation of D-amino acids which results from high and prolonged heat treatment (Olaofe *et al.*, 1994). This was likely the case because the method used for amino acid analysis will only detect L-amino acids from animal and plant proteins that do not produce racemisation (Adeyeye *et al.*, 2010). Increase in the amino acid concentration of the 10 kGy irradiated sample was observed with arginine, histidine, tyrosine, glycine, lysine, proline, threonine, phenylalanine and serine. The increase of alanine, glutamic acid, valine, methionine, isoleucine and cystine were significant ( $p > 0.05$ ). The changes in the concentration of amino acids induced by irradiation may be due to free radicals of the peptide bonds, deamination and decarboxylation reactions of amino acids followed by chains of chemical reactions forming other new radicals

(Bamidele, 2015). This finding is in line with the report of Abdel-Ghaffar (2013), who reported an increase in some amino acids of soy flour.

#### Effect of processing on the mineral composition of *Parkia biglobosa*

The results of the mineral analysis as shown in Table 3 indicate that the locust bean seeds were richer in iron and potassium after cooking, which could be interpreted as a consequence of the probable inactivation of anti-nutrients. Sodium was lost in the cooked as well as post-irradiated cooked samples. This may be due to its solubility and its ability to leach-off in cooking water. It can however be stated from the findings that mineral bioavailability can be increased with cooking with little impact on the mineral constituent of the seed. Zinc was significantly increased in cooked as well as 10 kGy irradiated samples

These minerals act as stabilizers of the structures of membranes and cellular components. Zinc is an important component of several enzymes and their biochemical functions, especially in the synthesis and degradation of macromolecules such as carbohydrates, proteins, lipids and nucleic acids as well as wound healing (Frossard *et al.*, 2000).

Table 3: Mineral composition of raw and treated *Parkia biglobosa* seeds

Parameters (mg/100g)	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
Potassium	18.21 <sup>a</sup> ±1.65	14.47 <sup>b</sup> ±0.65	15.25 <sup>b</sup> ±0.15	17.30 <sup>a</sup> ±0.65	15.57 <sup>b</sup> ±0.15
Sodium	2.88 <sup>a</sup> ±0.10	2.68 <sup>ab</sup> ±0.07	2.57 <sup>b</sup> ±0.18	2.54 <sup>b</sup> ±0.10	2.18 <sup>c</sup> ±0.13
Calcium	14.68 <sup>c</sup> ±0.55	18.30 <sup>a</sup> ±1.08	18.17 <sup>b</sup> ±0.21	15.86 <sup>c</sup> ±1.91	11.18 <sup>d</sup> ±0.19
Magnesium	4.61 <sup>a</sup> ±0.82	3.49 <sup>b</sup> ±0.24	3.40 <sup>b</sup> ±0.10	3.60 <sup>b</sup> ±0.14	2.85 <sup>b</sup> ±0.25
Zinc	4.19 <sup>c</sup> ±0.02	5.19 <sup>b</sup> ±0.03	4.59 <sup>b</sup> ±0.75	6.34 <sup>a</sup> ±0.06	4.19 <sup>c</sup> ±0.59
Iron	1.46 <sup>b</sup> ±0.02	1.73 <sup>a</sup> ±0.04	1.42 <sup>bc</sup> ±0.11	1.24 <sup>cd</sup> ±0.20	1.09 <sup>d</sup> ±0.03
Phosphorus	3.67 <sup>bc</sup> ±0.39	5.75 <sup>a</sup> ±0.03	4.25 <sup>b</sup> ±0.06	4.00 <sup>b</sup> ±0.55	3.36 <sup>c</sup> ±0.18

\*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different ( $p \geq 0.05$ ).

#### Effect of processing on Functional properties

The effects of cooking, irradiation and combined treatment of both on the functional properties of locust bean seeds are presented in Table 4. Water absorption capacity (WAC) is an important functional property of proteins and is a measure of the quality (juiciness, texture, binding of structure, appearance and mouth feel) of flour. There was a significant decrease in the water absorption capacities of the cooked (as a single treatment) sample and the irradiated cooked sample compared to the raw. Cooking led to significant increase in oil absorption capacity (OAC) of the

seed flour. Dissociation and denaturation results in increased oil absorption of treated proteins compared to native proteins (Siddharaju *et al.*, 2002). This observation is consistent with the results of Sosulski *et al.* (1976) who reported similar results using sunflower seeds.

Cooking significantly reduced the foaming capacity by about 92.6%. This finding is in agreement with that reported by Yusuf *et al.* (2008) for Bambara groundnut which was attributed to protein denaturation. Irradiation at 10 kGy decreased the foaming capacity of the seed flour significantly by 25.9%, probably owing to extensive

denaturation and protein cross-linking. However, given the foaming capacity values in the present study, African locust bean seed flour has its foaming capacity compromised by all the treatments. The 5 kGy irradiated sample has the highest foam stability while the cooked sample has the lowest foam stability. This agrees with the findings of Lin *et al.* (1974) which stated that native proteins give higher foam stability than denatured protein.

Significant reduction in least gelation concentration (LGC) was observed at 5 kGy gamma irradiation dose ( $p < 0.05$ ). Such a decrease in LGC might be attributed to increased interaction of proteins with water (Adebowale and Lawal, 2004). Improvement in gelation property is beneficial as it allows the utility of seed flour in preparation of food products like custards, ice creams, sausages and other bakery products (Bhat and Sridhar, 2008). The current findings are in agreement with results obtained for

*Pentaclethra macrophylla* (Enujiugha *et al.*, 2012) and groundnut (Enujiugha *et al.*, 2023a).

Emulsion capacity denotes the maximum amount of oil that can be emulsified by protein dispersion. The high emulsion capacity in the present study could be as a result of high content of free fatty acids which leads to increased oil absorption (Ihekoronye and Ngoddy, 1985). Irradiation at 5 kGy caused a significant decrease in the emulsion capacity of the seed flour accounting for more than 10% loss of emulsion capacity. Irradiation at 10 kGy had no significant ( $p < 0.05$ ) effect on emulsion capacity of the oil seed flour when compared with the non-irradiated (control) samples. The decrease in emulsion capacity has been speculated to have resulted from changes, such as protein aggregation as well as surface hydrophobicity and charge characteristics (Cheftel *et al.*, 1985).

Table 4: Functional properties of raw and processed *Parkia biglobosa* seeds

Parameters	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
WAC (ml/g)	380.00 <sup>a</sup> ±0.21	360.00 <sup>ab</sup> ±0.35	360.00 <sup>b</sup> ±0.08	380.00 <sup>a</sup> ±0.02	360.00 <sup>ab</sup> ±0.03
LGC (m/v)	8.00 <sup>a</sup> ±0.01	6.00 <sup>a</sup> ±0.02	2.00 <sup>b</sup> ±0.03	4.00 <sup>b</sup> ±0.01	2.00 <sup>b</sup> ±0.02
OAC(ml/g)	190.00 <sup>ab</sup> ±0.03	210.00 <sup>a</sup> ±0.02	175.00 <sup>b</sup> ±0.02	180.00 <sup>b</sup> ±0.01	200.00 <sup>ab</sup> ±0.02
FC (%)	45.00 <sup>a</sup> ±0.03	3.33 <sup>e</sup> ±0.12	33.33 <sup>b</sup> ±0.02	25.00 <sup>c</sup> ±0.04	6.67 <sup>d</sup> ±1.23
EC (%)	380.00 <sup>a</sup> ±0.09	360.00 <sup>b</sup> ±0.01	340.00 <sup>c</sup> ±0.31	380.00 <sup>a</sup> ±0.03	360.00 <sup>b</sup> ±0.01

\*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different ( $p \geq 0.05$ ).

WAC = Water absorption capacity; OAC = Oil absorption capacity; LGC = Least gelation concentration; FC = Foaming capacity; EC = Emulsion capacity

### Effect of processing on anti-nutritional factors

The levels of some anti-nutritional components in the raw and processed seed flour samples are presented in Table 5. Anti-nutritional factors are generally reported to have the effect of lowering digestibility and absorption of important dietary nutrients.

Oxalate can have deleterious effects on human nutrition and health, particularly by decreasing calcium absorption and aiding the formation of kidney stones (Noonan and Savage, 1999). Cooking has been found effective in the reduction of oxalate content in oil seeds (Chai and Liebman, 2005). A decrease in the oxalate content was observed in both cooked samples. The higher percentage of oxalate reduction occurs during boiling and may be due to its solubility in water. Boiling may cause skin rupture and facilitate the leakage of soluble oxalate from seeds into cooking water (Albihn and Savage, 2001). Comparing the different treatments, cooking and post-irradiation cooking are to be preferred to

irradiation alone in order to keep oxalate intake as low as possible. Observations were comparable to the reports of Albihn and Savage (2001) and Wanasundera and Ravindran (1992). The reduction in oxalate levels on cooking is to enhance the bioavailability of essential dietary nutrients of the African locust bean seeds

The results obtained indicate that phytate content decreased slightly with treatment. Irradiation alone effectively reduced the phytate level in the oil seed by 4.8% compared to the control. Decrease in the phytate content in the irradiated and cooked sample was as high 24.8%. The reduction in phytate content during cooking may be partly due to the formation of insoluble complexes between phytates and other components, such as phytate-protein and phytate-protein-mineral complexes, or to the inositol hexaphosphate being hydrolyzed (Siddhuraju and Becker, 2001). Cooking has been reported to lower the phytate levels in several plant foodstuffs (Badifu, 2001). Taghinejad-Roudbaneh, (2010) in a study where canola

meal was irradiated using electron beams up to 45 kGy dose observed a total disappearance of phytic acid at 30 kGy and a reduction of up to 89.66% at 15 kGy irradiation dose. Currently there is evidence that dietary phytate at low levels may have beneficial effect as an antioxidant, anticarcinogen and may likely play an important role in the control of hypercholesterolemia and atherosclerosis (Kumar *et al.*, 2010; Phillippy *et al.*, 2004).

The high tannin contents of the seed might have been a major cause of the astringency observed when tasted in its unprocessed form. Tannins usually form insoluble

complexes with proteins thereby interfering with their bioavailability (Enujiugha and Agbede, 2000). Reduction of tannins might have resulted in a significant increase in Zinc content at 10kGy irradiation dose.

#### Rancidity indices of oil from *Parkia biglobosa*

The products of rancidity are known to be hazardous to health since they are associated with aging, membrane damage, heart disease and cancer (Cosgrove *et al.*, 1987). The rancidity indices of oil as affected by processing are presented in Table 6.

Table 5: Anti-nutritional factors in raw and treated *Parkia biglobosa* seeds

Parameters	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
Oxalate(mg/g)	0.15 <sup>a</sup> ±0.02	0.06 <sup>d</sup> ±0.13	0.09 <sup>c</sup> ±1.04	0.11 <sup>b</sup> ±0.18	0.06 <sup>d</sup> ±0.05
Phytate(mg/g)	1.25 <sup>b</sup> ±0.02	1.19 <sup>a</sup> ±0.02	1.57 <sup>a</sup> ±0.02	1.11 <sup>b</sup> ±0.02	0.94 <sup>b</sup> ±0.03
Tannin(mg/g)	0.02 <sup>a</sup> ±0.01	0.02 <sup>a</sup> ±0.01	0.02 <sup>a</sup> ±0.01	0.02 <sup>a</sup> ±0.02	0.02 <sup>b</sup> ±0.01
Phytate-P	0.35 <sup>b</sup> ±0.12	0.34 <sup>bc</sup> ±0.05	0.44 <sup>a</sup> ±0.04	0.31 <sup>bc</sup> ±0.01	0.33 <sup>c</sup> ±0.26

\*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different (p≥0.05).

Table 6: Rancidity indices of the raw and treated seed oil extract

Parameters	Raw	Cooked	5 kGy Irradiated	10 kGy Irradiated	10 kGy Irradiated and cooked
Peroxide value (mEq./kg)	0.71 <sup>c</sup> ±0.05	0.73 <sup>c</sup> ±0.10	0.73 <sup>c</sup> ±0.28	0.79 <sup>b</sup> ±0.05	0.86 <sup>a</sup> ±0.06
Iodine Value (mg/100g)	140.13 <sup>a</sup> ±1.80	133.53 <sup>b</sup> ±2.82	142.48 <sup>a</sup> ±0.17	144.40 <sup>a</sup> ±2.05	122.77 <sup>b</sup> ±8.85
Acid Value (mgNaOH/g)	1.07 <sup>c</sup> ±0.03	1.11 <sup>ab</sup> ±0.01	1.07 <sup>c</sup> ±0.01	1.09 <sup>bc</sup> ±0.02	1.14 <sup>a</sup> ±0.02
Saponification Value (mgKOH/g)	176.33 <sup>c</sup> ±0.21	184.00 <sup>b</sup> ±0.10	173.33 <sup>b</sup> ±0.01	180.67 <sup>b</sup> ±0.02	186.67 <sup>a</sup> ±0.09
Free Fatty Acid (% Oleic acid)	1.31 <sup>cd</sup> ±5.51	1.62 <sup>ab</sup> ±2.00	1.63 <sup>d</sup> ±2.08	1.69 <sup>c</sup> ±1.15	1.85 <sup>a</sup> ±1.53

\*Values are means of triplicate determinations (n=3) ± standard deviation. Values along same row with the same letters in superscript are not significantly different (p≥0.05).

The peroxide value is an indicator of oxidative rancidity. As oxidation takes place, the double bonds in the unsaturated fatty acids are attacked leading to the formation of peroxides. Fresh oils have been shown to have peroxide values lower than 10 mg/g and oils become rancid with peroxide value ranging from 20 to 40 mg/g (Oyinloye and Enujiugha, 2017). The highest peroxide value was observed in the oil from the seeds with combined treatments of irradiation and cooking. These results are generally in agreement with those of Mexis *et al.* (2009) for gamma irradiated almonds where the peroxide values increased with dosage. Cooking alone accounted for 3.6% increase in

peroxide value of the oil while 5kGy irradiation dose resulted in 2.82% increase in the peroxide value. This increase conforms to the work of Farag *et al.* (1992) who reported acceleration of cottonseed oil oxidation during irradiation heating which as indicated by an increase in peroxide value. Increases in peroxide values are expected as irradiation produces large amounts of free radicals that enhances lipid peroxidation (Sajilata and Singhal, 2006).

Iodine value is a measure of the unsaturation of fats and oils. It is based on the ability of an unsaturated carbon to carbon bond to add halogen atoms. Irradiation treatments alone (5 kGy and 10 kGy) did not significantly affect the iodine

value of the resultant oil. However, significant decreases in iodine value were observed for the cooked and post-irradiated cooked samples (133.53 g I<sub>2</sub> per 100 g and 122.77 g I<sub>2</sub> per 100 g respectively). These were in agreement with the reports of Omafuvbe *et al.*, (2004) who observed a decrease in iodine value in African Oil Bean seeds as a result of cooking.

The acid value of oils indicates the total acidity as estimated by the fatty acids in the sample. The acid value obtained from the raw sample (1.07 mgKOH/g) correlates with the report of Omafuvbe *et al.*, (2004) for African Locust bean seed oil extract. Cooking of African locust bean seeds during processing increased acid value significantly (1.11 mgKOH/g for cooked and 1.14 mgKOH/g for irradiated and cooked).

Saponification value (SV) is a measure of the alkali-reactive groups in fats and oils which is useful in predicting the type of glycerides in a sample. Glycerides containing short-chain fatty acids have higher SV than those with longer chain fatty acid. The saponification values of the locust bean seed oils as shown in Table 5 ranged from 173.33 mg KOH/g for 5 kGy irradiated sample to 186.67 mg KOH/g for post-irradiated cooked samples. These results conform with the ranges reported in literature for vegetable oils: olive oil (184-196 mg KOH/g), rapeseed oil (168-181 mg KOH/g), sunflower seed oil (188-194 mg KOH/g) and pumpkin seed oil (174-197 mg KOH/g) (Nichols and Sanderson, 2003). This is an indication that there was formation of short chain fatty acid from the breakage in the longer chain length as a result of radiolysis.

#### IV. CONCLUSION

Gamma irradiation at doses up to 10 kGy has been shown to potentially reduce anti-nutritional factors in African locust bean seeds. Combined gamma irradiation and post-irradiation cooking however, can be concluded to be viable hurdle technique for the treatment of African locust bean (*Parkia biglobosa*) seed as it effectively enhances the nutritional component of the oil seed by inactivating anti-nutrients and allowing for bioavailability of minerals needed for body development. The findings highlight the great potentials of low dose gamma irradiation in the preservation of neglected and underutilized forest seeds that commonly dot the African agro-ecological landscape.

#### DATA AVAILABILITY

The collated data presented in this work is available for whatever scientific purpose that is required.

#### ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This work is part of a wider research which was approved by the Ethics Committee of the School of Agriculture and Agricultural Technology, Federal University of Technology with assigned number FUTA/SAAT/ETH/2011/14

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# Trichoderma-fortified compost in controlling diseases and increasing yield of tomato

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**Abstract**— An attempt was made to reduce the tomato disease severity caused by several fungal pathogens in the field conditions and increase the growth and yield of tomato through the application of different concentrations of Trichoderma-fortified compost. The antagonism of the tested stock culture isolate of TH 7 of *T. harzianum* was found to be stable in its antagonistic character and observed more than 90% growth inhibition of all the tested pathogens. Based on the confirmation of the repeated antagonism test, the highly antagonist isolate of *T. harzianum* TH 7 was selected to prepare the Trichoderma-fortified compost. In the field experiment, post-emergence seedling mortality was completely free whereas Trichoderma-fortified compost at 300 g colonized Trichoderma was used in the treatment T<sub>6</sub>. All other doses of Trichoderma-fortified compost reduced the seedling mortality 60 to 80% and 20% reduction of seedling mortality was observed in the treatment T<sub>1</sub> where compost was used without Trichoderma. Early blight, collar rot and southern blight, root rot and Fusarium wilt are also recorded in the field from the growing stage to the last harvesting period. All the diseases were completely controlled with the treatment T<sub>6</sub> except early blight, where the highest reduction was 75% with the treatment T<sub>6</sub> and T<sub>5</sub>. A similar trend of reduction of diseases was observed in the case of other treatments. Although, anthracnose was not observed in the tomato plants but at the ripening stage of the crop 2.5% tomato fruits were infected with anthracnose disease. Tomato fruits in the field were completely free from anthracnose fruit rot in the treatment T<sub>6</sub>. The treatments T<sub>4</sub> and T<sub>5</sub> reduced 75% and 80% anthracnose fruit rot in the field condition. All the treatments significantly increased the growth-promoting components in comparison to the untreated control in the treatment T<sub>8</sub> where no supplements were added. Significantly the highest 28.08% yield was recorded in treatment T<sub>6</sub> followed by 21.67% increased yield in Treatment T<sub>5</sub>. The highest quantity of tomato was harvested at the 3rd harvest in the case of all the treatments.



**Keywords**— Anthracnose, Collar rot, Early blight, Fusarium wilt, Root rot, Trichoderma, Tomato

## I. INTRODUCTION

Tomato (*Solanum lycopersicum*), a member of the nightshade family (Solanaceae) is one of the popular vegetables in Bangladesh. Tomato and its products are gaining popularity day by day for various uses including

salad, soup, sauce, juice, flavour in crackers and biscuits. Over the centuries, however, humans realized the great versatility of the fruit in the culinary realm, as well as the potential health benefits from the fruit's antioxidants. Tomatoes are now grown all over the world throughout the year. A ripe tomato contains around 94% water with higher

contents of vitamin A, B, C including calcium and carotene (Bose and Som, 1990). Tomato has ranked 4<sup>th</sup> in terms of production among major vegetables grown in the country (BBS, 2022). Although the total cultivated area and production of tomato in our country have increased gradually over the last few years but the productivity is still very low 6.46 ton/ha compared to the average of the world yield 40.84 ton/ha (FAOSTAT, 2022). Every year about 10-30% crop loss occurs due to diseases in Bangladesh that causes annual losses of about \$120 million but it may be as high as 100% if control measures are not taken properly (Ahmed, 1994).

Disease is a major limiting factor for tomato production. It can be classified into two groups. The first are those caused by infectious microorganisms that include fungi, bacteria, viruses, and nematodes. These diseases are contagious and can spread from plant to plant in a field, often very rapidly when environmental conditions are favorable. Among various diseases of tomato, soil-borne diseases caused by *Sclerotium rolfii*, *Rhizoctonia solani*, *Fusarium oxysporum* f. s. *lycopersici*, *Pythium* spp. are very serious both in the seedling stage and mature stage in the field. In Bangladesh, other severe tomato plants and fruits damage diseases are anthracnose (*Colletotrichum coccodes*), early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*). Growers can be much benefitted if they can reduce the losses due to diseases and minimize the production cost for chemical pesticides and fertilizers.

Loss of soil organic matter and associated loss of soil microbial activity has contributed to an increase in soil-borne plant diseases. Pests and diseases cause significant economic crop losses to agricultural crops each year, and management of pests and diseases is identified as one of the top farm management issues by growers across the country. Management of tomato disease is very difficult as the causal pathogens are mostly soil-borne as well as seed-borne and survive in the soil for longer time either in the form of sclerotia, chlamydospores or as fragmented mycelium associated with crop residues and also can survive in the seed as dormant stage over a period of time.

Soil-borne fungal and oomycete plant pathogens, among the major factors limiting the productivity of agroecosystems, are often difficult to control with conventional strategies such as the use of resistant host cultivars and synthetic fungicides. The lack of reliable chemical controls, the occurrence of fungicide resistance in pathogens, and the breakdown or circumvention of host resistance by pathogen populations (McDonald and Linde, 2002) are some of the reasons underlying efforts to develop new disease control measures. The ban of methyl bromide, the most effective fumigant used worldwide for soil disinfestations, has further increased the need for

alternative control methods (Martin, 2003). In this context, the search for alternatives with high efficiency, low cost and limited environmental impact is a challenge for eco-sustainable modern agriculture. The use of organic amendments such as animal manure, green manure (the incorporation of crop residues into the soil), composts and peats has been proposed, both for conventional and biological systems of agriculture, to improve soil structure and fertility (Cavigelli and Thien, 2003), and decrease the incidence of disease caused by soil-borne pathogens (Noble and Coventry, 2005). Available control measures including cultural and chemical methods are not satisfactory to control several diseases. During past years, pesticides and chemical fertilizers have become the foundation of highly productive forms of agriculture and their indiscriminate use causes the risk of pollution, serious changes in ecological symmetry and poisoning (Danielle and Rai, 2006). However, the high frequency of chemical use, non-target effects, development of resistance to many chemicals, pathogens which remain viable for many years and risk to human health and the surrounding environment have stimulated the development of alternative methods for disease management. Moreover, pesticides are not available for some diseases, and generally pesticides are more effective against aerial plant pathogens than their soil-borne counterparts. It is also technically difficult to treat large amount of soil, and the range of approved chemicals is declining as active compounds are withdrawn for toxicological and environmental. The current trend to near-zero market tolerance for pesticide residues in fresh leafy vegetables provides an additional motivation to search for non-chemical means to control pests and diseases (Reuveni et al., 2002). These issues are motivating increased interest in reduced chemical use and in the disease suppression benefits of organic products including composts.

As a result, sustainable alternatives are being sought to replace or complement these strategies. In recent years the use of microbial systems for nutrient mobilization, or as biofertilizers are getting popular and new systems are being introduced to cater to different cropping systems. Biological control agents are now being used in developed countries to control disease. The dual role of antagonistic activity against plant pathogens and promotion of soil fertility makes the biological control agents including antagonist fungi and bacteria appealing alternatives to hazardous fumigants and fungicides.

*Trichoderma* is a cosmopolitan soil and compost-borne saprophytic fungus used widely as a biological control agent in the field against plant diseases caused by economically important plant pathogens. Considerable efforts, both in the academic and commercial sectors, have

been made to promote this group of fungi as a credible alternative to synthetic chemicals for combating against plant disease (Rubayet and Bhuiyan, 2023).

Composts or compost extracts used as an organic fertilizer have beneficial effects on plant growth and considered as a valuable soil amendment (Gharib et al., 2008). A water-based compost extract containing high population of beneficial microbes, is attracting the attention of growers and researchers for its apparent disease-suppressive activity and improvement of soil fertility. Composting is the biological decomposition of organic waste under controlled conditions. The use of *Trichoderma* spp., a cellulose decomposer fungus, to hasten the decomposition of agricultural wastes that is ready for application is referred to as compost fungus activator (CFA) reduces decomposition time of agricultural wastes from the normal 5–6 months to 3–5 weeks. Research on the application of compost has demonstrated a variety of disease-suppressive effects. The results indicate that suppressive effects can result from a combination of physiochemical and biological characteristics (Boulter et al., 2002). The activities of beneficial microorganisms within the total microbial community and their response to energy reserves available from the composts are the basis of disease control (Hoitink and Boehm, 1999).

The mechanism of biological control involves the production of antibiotic compounds by beneficial microorganisms that are effective in controlling various plant pathogens such as sclerotia of pathogenic fungus *Rhizoctonia solani* and *Sclerotium rolfsii* (Hoitink and Boehm, 1999). Composts can provide natural biological control of diseases of roots as well as of plant foliage. Beneficial fungi *Trichoderma* spp. are considered the predominant and aggressive parasites of *Rhizoctonia* and *Sclerotium* (Kuter et al., 1983). Not only have composts prepared from different raw materials/feedstock varied in disease suppressiveness, but those prepared from different batches of the same raw materials have varied in their effect (Nelson and Craft, 1992). Common beneficial microbes compete pathogens for food and space around plant roots. This mechanism (direct competition) is very effective against *Fusarium*, *Pythium*, *Phytophthora*, *Rhizoctonia* and *Sclerotium* causing damping off and seedling mortality of tomato crop. Among antagonistic microbes, *Trichoderma* is easy to culture and mass multiplication and mainly used for the prevention and control of soil-borne fungal diseases in plants such as *Fusarium*, *Verticillium* wilt, damping-off disease, Blight, *Gaeumannomyces* diseases, sheath blight, all kinds of vegetables and other seedling diseases. *Trichoderma* is

also popular because it is good at combating a range of diseases on vitally important crops.

*Trichoderma* inhabits naturally in nearly all soils, so it is easy to find. It is also easy to cultivate. Producers can mix it with compost and sell it in bags or it may be produced in liquid form to be sprayed on leaves for the treatment of foliar fungal diseases. The beauty of *Trichoderma* is that it can be used to combat almost every pathogenic fungus that people want to control. There are constraints in using *Trichoderma* as biocontrol agents. *Trichoderma* colonizes in the spermosphere effectively but they do not survive well in the rhizosphere (Deacon, 1994). The same author observed that *Trichoderma* spp. are active only in some types of soil and season thus achieving only transitory localized dominance of the rhizosphere. For this reason, another constraint is the quiescent and inactive nature of *Trichoderma* spores in the soil and because of this, *Trichoderma* cannot be added as spores (Vidhyasekaran, 2004).

Furthermore, the application of compost fortified with *Trichoderma* is most effective not only control the pathogenic diseases or enhancing plant growth but also removal of atmospheric carbon through soil carbon sequestration, achieved directly through storage of compost carbon, and indirectly through enhanced plant growth, which in turn contributes also to increased soil carbon levels.

Research on the application of biocontrol agents began in the mid- 1990s but results from documented field trials on the application of *Trichoderma*-fortified compost in controlling vegetable diseases are scarce. Considering the above-mentioned facts, the study was undertaken to optimize the dose of *Trichoderma* in the *Trichoderma*-fortified compost in controlling the major field diseases of tomato and increase the growth promotion and yield of tomato.

## II. MATERIALS AND METHODS

The experiment was conducted in the laboratory and research field of the department of Plant Pathology Department at the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur 1706, Bangladesh. The experimental site is located at the centre of Madhupur tract (24°09' N latitude and 90°26' E longitude) having an elevation of 8.2 m from sea level. The soil type of the experimental field belongs to the Shallow red brown terrace type under Salna series of Madhupur tract (Saheed, 1984) of Agro ecological zone (AEZ) 28 which is characterized by silty clay with pH value of 6.5, less rainfall, almost clear sunshine and moderate temperature.

### Preparation of compost

Before setting the experiment in the field, Trichoderma-fortified compost at different concentration was prepared by mixing saw dust, kitchen garbage, rice straw and cowdung on equal proportion in a 1.0 m x 1.0 m x 1.5 m composting pit and covered with polythene sheet allowed for 90 days for decomposition and degradation following the procedure of the preparation of standard quality compost as described by James (2008). To determine the suitability of the compost to be used for the suppression of diseases and production of tomato, percent seed germination, root growth and germination index (GI, a factor determined by both germination & root growth) were carried out based on the following formula as stated by Zucconi et al. (1981):

### Selection of *Trichoderma* isolate for compost

The highly antagonist isolate of *Trichoderma harzianum* TH 7 was collected from the stock culture of Plant Pathology Laboratory of BSMRAU, Gazipur and checked the antagonism against the highly virulent isolates of *Alternaria solani*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Colletotrichum coccodes* in dual culture plate technique to reconfirm the antagonism of *T. harzianum* isolate TH 7. After reconfirmation of the antagonism wheat grain colonized inoculums of *T. harzianum* isolate TH 7 was prepared following the procedure as described by Raihan et al. (2003) and the requisite amount of inoculum was mixed with the compost at five different doses.

### Preparation of *Trichoderma* inoculum

Inoculum of the selected isolate of *T. harzianum* isolate TH 7 was prepared on wheat grains in 1000 ml Erlenmeyer flask. Wheat grains were soaked in water for 12 hours. The water was drained off and 500 g of water-soaked grains were poured into 1000 ml Erlenmeyer flask. Five-millimeter diameter discs of the fungal mycelium were cut from the edge of three days old PDA cultures of *T. harzianum* isolate TH 7 in petridishes. Twelve to fifteen mycelial discs of PDA grown *T. harzianum* isolate TH 7 were added to the flasks containing autoclaved wheat grain and incubated at 25 C for 21 days (Rubayet and Bhuiyan, 2016). They were shaken by hand at 2-3 days intervals for even colonization. The colonized wheat grain with *T. harzianum* was air-dried for 1 week and stored at 10 C for using as inocula.

### Mixture of wheat grain colonized *Trichoderma*

A total of six compost pits was prepared where each compost pit contains 40 kg well mixed saw dust, kitchen garbage, rice straw and cowdung in equal proportions. After 45 days of decomposition wheat grain colonized

*Trichoderma* inoculum at 1.6, 2.4, 3.2, 4.0 and 4.8 kg were mixed in five different compost pits. A compost pit without wheat grain colonized *Trichoderma* inoculum was maintained as control treatment. Compost pits were allowed a total of 90 days for complete decomposition before application in the field.

### Treatments maintained in the experiment

From prepared compost pits, a total of 2.5 kg Trichoderma-fortified compost was applied per plot at five different doses and maintained one control treatment of only compost without wheat grain colonized *Trichoderma*. The amount of wheat grain colonized *Trichoderma* for each treatment per plot is given below:

### Treatments

T<sub>1</sub>= only compost without wheat grain colonized *Trichoderma*, T<sub>2</sub>= compost with 100 g wheat grain colonized *Trichoderma*, T<sub>3</sub>= compost with 150 g wheat grain colonized *Trichoderma*, T<sub>4</sub>= compost with 200 g wheat grain colonized *Trichoderma*, T<sub>5</sub>= compost with 250 g wheat grain colonized *Trichoderma*, T<sub>6</sub>= compost with 300 g wheat grain colonized *Trichoderma*, T<sub>7</sub> = without compost only 200 g wheat grain colonized *Trichoderma*, T<sub>8</sub> (Control) = no compost and no wheat grain colonized *Trichoderma*.

### Land preparation and design of experiment

Land was prepared with well tillth using a tractor driven disc plough, rotavator and harrow. The experiment was laid out in the Randomized Complete Block Design (RCBD) with three replications. After land preparation the whole experimental area was divided into three blocks, representing three replications. The unit plot size was 2.5 m x 1.5 m, distance between block to block was 1.0 m and that of plot to plot in a block was 0.50 m. Drains were made surrounding each unit plots and the excavated soil from the drains were used for raising plots 15 cm high from the general soil surface. Eight different treatments were allotted randomly to eight-unit plots in each block.

### Plantation of Seedlings

Twenty-five days aged healthy tomato seedlings variety 'Ratan' was collected from the Horticulture Research Centre of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur for plantation where row to row and plant to plant distance was 50 cm. A total of 24 seedlings were planted in each plot. Weeding, irrigation and other intercultural operations were done as and when necessary, until the maturity of plants.

### Observation of disease development

Tomato plants were observed regularly immediately after transplantation to record the incidence of post

transplanting seedling mortality and different diseases at different stages of plant growth both on plant parts & fruits. Infected tomato plants were identified based on characteristic symptoms of the diseases. The causal agents of the recorded diseases were identified by isolation of the pathogen from the infected leaves, plants and fruits. The disease incidence was recorded continuously at 3 days intervals from transplanting to final harvest. Observations were made by selecting six plants randomly from each plot. Diseases of the crop were expressed as percentage.

#### Observation of growth promoting factors at maturity

Growth promoting factors including root length, root diameter, fresh root weight, dry root weight, dry shoot weight, plant height, no. of leaves, no. of branches, fresh shoot weight and dry shoot weight were recorded randomly taken three plants from each replication of all the

### III. RESULTS AND DISCUSSION

#### Potential *Trichoderma* isolate TH 7

The highly antagonist isolate of *Trichoderma harzianum* TH 7 was collected from the stock culture of Plant Pathology Laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University and checked the antagonism against the highly virulent isolates of test pathogens. In the present study, more than 90% growth inhibition of all the tested pathogens was observed. Based on the confirmation of the present study the highly antagonist isolate of *T. harzianum* TH 7 was selected to prepare the *Trichoderma*-fortified compost. The antagonism of *T. harzianum* TH 7 against *F. oxysporum*, *S. rolfisii*, *R. solani* and *C. coccodes* of the present study are in highly agreement with previous work (Sen, 2010; Bhuiyan and Rubayet, 2023). Many researchers reported the antagonistic behavior of the *Trichoderma* spp. against several types of seed and soil-borne as well as air-borne fungal pathogens (Ahmed et al., Arefin et al., Das et al., Liton et al., Simi et al., 2019; Rahman et al., 2020ab; Rubayet et al., 2020; Rahman et al., 2021, Roy et al., 2022).

#### Control of disease development in the field

A total of five diseases were recorded during the production of the crop in the field. Immediately after the plantation of tomato seedlings, post-transplanting seedling mortality caused by *R. solani*, *S. rolfisii* and *F. oxysporum* was recorded until two weeks of the field growth (Fig. 1). Post-transplanting seedling mortality was completely free where *Trichoderma*-fortified compost at the rate of 300 g colonized *Trichoderma* was used in the treatment T<sub>6</sub>. All other doses of *Trichoderma*-fortified compost reduced the seedling mortality 60 to 80% and 20% reduction of seedling

treatments attained after fruit ripening stage. Fruits were harvested through four different stages depending on growth, maturity and ripening of the fruits. Because of bulk production, fruit were weighed by truss rather than individually. A cluster was harvested whenever the fruit had reached the breaker stage. Cluster weight and fruit number were recorded.

#### Collection of data and analysis

The experiment had eight treatments with three replications following a Randomized Complete Block Design (RCBD). Data were analyzed for ANOVA using MSTAT-C program. Duncan's Multiple Range Test (DMRT) was used to compare the means. Whenever necessary, data were transformed before statistical analysis following the appropriate method.

mortality was observed in the treatment T<sub>1</sub> where compost was used without *Trichoderma*. In treatment T<sub>7</sub> where only wheat grain colonized *Trichoderma* without compost was applied also reduced 60% seedling mortality.

The major diseases observed in the field were early blight and Fusarium wilt in the tomato plants and anthracnose in the fruits at the ripening stage. The highest incidence of early blight caused by *A. solani* was recorded at 44.44% in the untreated plot in treatment T<sub>8</sub>. The highest 75% reduction of early blight was recorded in the treatment T<sub>5</sub> and T<sub>6</sub> where *Trichoderma*-fortified compost at 250 and 300 g colonized *Trichoderma* were applied. At the lower three doses 100, 150 and 200 g *Trichoderma*-fortified compost in the treatments T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> reduced early blight disease at 25% while only wheat grain colonized *Trichoderma* without compost in the treatment T<sub>7</sub> reduced 50% incidence of early blight disease. The lowest 12.12% early blight reduction was recorded with the application of only compost (Table 1).

Collar rot and Southern blight caused by *Sclerotium rolfisii*, Root rot caused by *Rhizoctonia solani* and Fusarium wilt caused by *F. oxysporum* are also recorded in the field from growing stage to the last harvesting stage. Among the three diseases, Fusarium wilt, fatal disease even at the mature stage of the crop was the most predominant and recorded 22.22% while collar rot and southern blight and root rot were observed at 22.22 and 12.12%, respectively in the untreated treatment T<sub>8</sub>. All these three diseases were completely controlled with the treatment T<sub>6</sub>. A similar trend of reduction of diseases was observed in the case of the other treatments. Although anthracnose was not observed in the tomato plants, 2.5% tomato fruits were infected with anthracnose disease at the ripening stage of the crop.



Tomato fruits in the field were completely free from anthracnose fruit rot in the treatment T<sub>6</sub>. In the treatments T<sub>4</sub> and T<sub>5</sub> reduced 75% and 80% anthracnose fruit rot in the field condition (Table 1). Application of only compost and wheat grain colonized *Trichoderma* without compost also considerably reduced the appearance of anthracnose fruit rot in the field. The Recycled Organics Unit of The University of New South Wales (NSW, 1966) demonstrated the application of compost in controlling soil-borne diseases of tomato which are in partial agreement with the findings of the present study. Controlling of Fusarium wilt of tomato

was successfully achieved with application of *Trichoderma* induced suppressive soil in Malaysia in accordance to Ingham (2005). Hoitink and Boehm (1999) stated that the natural disease-suppressive effects of composts are due to an increase in microbial biomass of *Trichoderma* is an ideal food base for biocontrol agents, it aids in their introduction and establishment into the soil for sustained biocontrol activities of soil microbiota supports the present findings of the study of controlling diseases of tomato by *Trichoderma*-fortified compost.

Table 1. Effect of *Trichoderma*-fortified compost in controlling field diseases of tomato

Treatments	% disease reduction					
	Transplanting seedling mortality	Early blight disease	Collar rot and southern blight	Root rot	Fusarium wilt	Anthracnose fruit rot
T <sub>1</sub>	20.01 e	12.12 e	25.00 d	25.00 e	25.00 d	31.25 e
T <sub>2</sub>	60.00 c	25.00 d	50.00 c	50.00 d	75.00 b	43.75 d
T <sub>3</sub>	80.0 b	25.00 d	50.00 c	50.00 d	75.00 b	43.75 d
T <sub>4</sub>	80.0 b	25.00 d	75.00 b	62.50 c	75.00 b	75.00 c
T <sub>5</sub>	80.0 b	75.00 a	75.00 b	62.50 c	100.00 a	80.00 b
T <sub>6</sub>	100.00 a	75.00 a	100.00 a	100.00 a	100.00 a	100.00 a
T <sub>7</sub>	60.00 c	50.00 b	75.00 b	75.00 b	50.00 c	43.75 d
T <sub>8</sub>	27.78 <sup>#</sup> d	34.44 <sup>#</sup> c	22.22 <sup>#</sup> e	12.12 <sup>#</sup> f	22.22 <sup>#</sup> e	2.50 <sup>#</sup> f

<sup>#</sup> % Disease incidence. T<sub>1</sub>= only compost without wheat grain colonized *Trichoderma*, T<sub>2</sub>= compost with 100 g wheat grain colonized *Trichoderma*, T<sub>3</sub>= compost with 150 g wheat grain colonized *Trichoderma*, T<sub>4</sub>= compost with 200 g wheat grain colonized *Trichoderma*, T<sub>5</sub>= compost with 250 g wheat grain colonized *Trichoderma*, T<sub>6</sub>= compost with 300 g wheat grain colonized *Trichoderma*, T<sub>7</sub>= without compost only 200 g wheat grain colonized *Trichoderma*, T<sub>8</sub> (Control) = no compost and no wheat grain colonized *Trichoderma*.

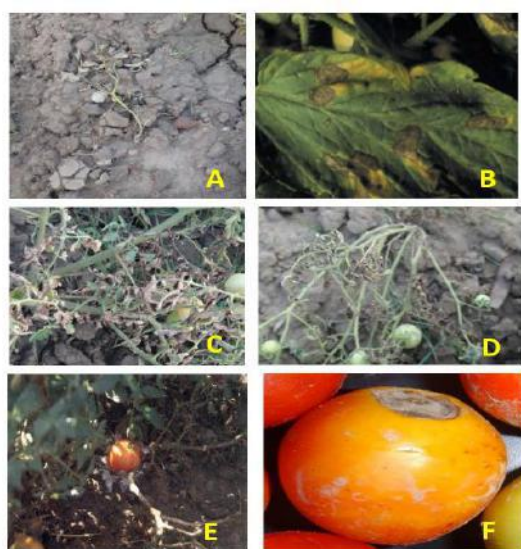


Fig 1. Different disease symptoms in the field

A. Post-emergence seedling mortality by *R. solani* B. Early blight disease Symptom caused by *Alternaria solani* C. Severe early blight disease symptom D. Fusarium wilt at mature stage E. Southern blight at mature stage by *S. rolfsii* F. Anthracnose fruit rot in the field by *C. coccodes*.

#### Effect of *Trichoderma*-fortified compost on growth promotion components of tomato

Growth promoting factors including root length, root diameter, fresh root weight, dry root weight, dry shoot weight, plant height, no. of leaves, no. of branches, fresh shoot weight and dry shoot weight were recorded randomly taken three plants from each replication of all the treatments attain after certain maturity (Table 2 and 3).

All the treatments significantly increased the growth-promoting components in comparison to the untreated control in the treatment T<sub>8</sub> where no supplements were added. Significantly increased growth parameter including

root length, root diameter, fresh root weight, dry root weight, dry shoot weight, plant height, no. of leaves, no. of branches, fresh shoot weight and dry shoot weight were recorded in the treatment T<sub>6</sub> where the highest amount of wheat grain substrate colonized *Trichoderma* was mixed in the compost and identical in increasing growth promoting components with the treatment T<sub>5</sub>, T<sub>4</sub> and T<sub>3</sub> except dry root weight. Growth promoting characters of the treatment T<sub>1</sub>, T<sub>2</sub> and T<sub>7</sub> are identical but significantly inferior to the treatments T<sub>5</sub>, T<sub>4</sub> and T<sub>3</sub>. The treatments T<sub>1</sub> and T<sub>2</sub> are identical in increasing root dry weight but significantly inferior to T<sub>7</sub>.

The result of the current study suggests the superiority of *Trichoderma*-fortified compost in comparison to the single application of compost or wheat grain colonized *Trichoderma* in the treatments T<sub>1</sub> and T<sub>7</sub> but significantly increased all the growth parameters. The results of the present study support the observation of De Brito et al. (1995), who observed the enhancement of the growth of carrot by *T. harzianum*. Increased growth promotion of different vegetable crops through the application of compost and *Trichoderma* was also reported by Gharib et al. (2008); Chowdhury et al. (2024) supports the findings of the current study.

Table 2. Effect of *Trichoderma*-fortified compost on growth parameters of tomato

Treatments	Root length (cm)	Root diameter (cm)	Fresh Root weight (g)	Dry Root weight (g)	Fresh Shoot weight (g)	Dry Shoot Weight (g)
T <sub>1</sub>	46.67 c	3.48 c	41.67 c	10.00 e	319.3 c	100.0 c
T <sub>2</sub>	48.33 bc	3.93 bc	44.00 bc	10.60 de	333.3 bc	105.0 bc
T <sub>3</sub>	52.00 ab	4.23 ab	47.33 ab	11.67 c	360.3 ab	113.7 ab
T <sub>4</sub>	54.00 a	4.87 a	49.00 a	12.67 b	374.3 a	118.0 a
T <sub>5</sub>	55.00 a	4.97 a	50.33 a	13.33 ab	380.7 a	120.7 a
T <sub>6</sub>	56.00 a	5.67 a	51.33 a	13.67 a	388.0 a	122.7 a
T <sub>7</sub>	49.00 bc	3.19 bc	44.00 bc	11.00 cd	333.3 bc	105.7 bc
T <sub>8</sub>	37.6 d	2.98 d	34.00 d	9.00 f	254.0 d	80.7 d

T<sub>1</sub>= only compost without wheat grain colonized *Trichoderma*, T<sub>2</sub>= compost with 100 g wheat grain colonized *Trichoderma*, T<sub>3</sub>= compost with 150 g wheat grain colonized *Trichoderma*, T<sub>4</sub>= compost with 200 g wheat grain colonized *Trichoderma*, T<sub>5</sub>= compost with 250 g wheat grain colonized *Trichoderma*, T<sub>6</sub>= compost with 300 g wheat grain colonized *Trichoderma*, T<sub>7</sub>= without compost only 200 g wheat grain colonized *Trichoderma*, T<sub>8</sub> (Control) = no compost and no wheat grain colonized *Trichoderma*.

Table 3. Effect of *Trichoderma*-fortified compost on plant height, number of leaves and number of branches of tomato

Treatments	Plant height (cm)	No. of branches	No. of leaves
T <sub>1</sub>	80.67 c	16.33 d	5.333 a
T <sub>2</sub>	87.00 c	17.67 c	5.667 a
T <sub>3</sub>	94.67 b	19.33 b	6.000 a
T <sub>4</sub>	98.00 ab	20.33 ab	5.667 a
T <sub>5</sub>	100.3 ab	20.67 a	6.000 a
T <sub>6</sub>	102.0 a	21.00 a	5.667 a
T <sub>7</sub>	87.33 c	18.00 c	5.667 a
T <sub>8</sub>	67.00 d	14.00 e	4.333 b

T<sub>1</sub>= only compost without wheat grain colonized *Trichoderma*, T<sub>2</sub>= compost with 100 g wheat grain colonized *Trichoderma*, T<sub>3</sub>= compost with 150 g wheat grain colonized *Trichoderma*, T<sub>4</sub>= compost with 200 g wheat grain colonized *Trichoderma*, T<sub>5</sub>= compost with 250 g wheat grain colonized *Trichoderma*, T<sub>6</sub>= compost with 300 g wheat grain colonized *Trichoderma*, T<sub>7</sub>= without compost only 200 g wheat grain colonized *Trichoderma*, T<sub>8</sub> (Control) = no compost and no wheat grain colonized *Trichoderma*.

**Effect of Trichoderma-fortified compost on the yield of tomato**

The highest 28.08% yield was increased in treatment T<sub>6</sub> followed by 21.67% in treatment T<sub>5</sub> (Table 4). The yield in the treatments T<sub>4</sub>, T<sub>3</sub> and T<sub>2</sub> were identical and significantly inferior to the treatment T<sub>5</sub>. The application of only compost in T<sub>1</sub> appeared to be superior in comparison to the application of only wheat grain colonized *Trichoderma* in the treatment T<sub>7</sub>. On the other hand, the yield of tomato was increased with increasing the amount of *Trichoderma* in the compost (Fig. 2 & 3).

However, in all the supplement added treatments increased yields were significantly higher in comparison the untreated control treatment T<sub>8</sub>. Harvesting was done at for different times as tomato fruits were not mature and ripen at the same time. The highest quantity of tomato was harvested at the 3<sup>rd</sup> harvest in the case of all the treatments. The size, uniformity and colour of tomatoes were also varied depending on the treatment. Tomatoes produced under the treatments T<sub>6</sub> and T<sub>5</sub> were larger size, uniform

and mostly desirable reddish colour while tomatoes under the untreated control treatment were smaller and variable in size with variable colour yellowish to reddish.

The results of the present study of the application of Trichoderma-fortified compost in increasing as total yield supports the increased yield of vegetable crops reported by other investigators who applied *Trichoderma* mixed with compost (Hoitink and Keener, 1993). Beneficial effects of composts include increased plant yield and vigor, improved food quality, and improved soil fertility including suppression of diseases caused by plant pathogens was also reported by Hoitink and Keener (1993) which are in partial agreement with findings of the present study. Similar observations are also reported in the case of rice in the Philippines where composting with the fungus *Trichoderma* as an activator is mainly utilized as organic fertilizer (Cuevas, 1997). Composts produced from biosolids are used widely as peat substitutes to reduce production costs in horticulture which might also be applicable for crop production in Bangladesh.

Table 4. Effect of Trichoderma-fortified compost on the yield of tomato

Treatments	Fruit wt. (g) per plot				Total yield (Kg/plot)	Yield (ton/h)	% increased yield
	1 <sup>st</sup> harvest	2 <sup>nd</sup> harvest	3 <sup>rd</sup> harvest	4 <sup>th</sup> harvest			
T <sub>1</sub>	1950	2150	7100	2600	13.80	36.80 d	13.30
T <sub>2</sub>	2100	2200	7160	2100	13.96	37.23 cd	14.61
T <sub>3</sub>	1900	2450	7650	2050	14.05	37.47 c	15.35
T <sub>4</sub>	1900	2300	7750	2150	14.10	37.60 c	15.76
T <sub>5</sub>	2000	2600	7600	2620	14.82	39.52 b	21.67
T <sub>6</sub>	2100	3050	8550	2200	15.60	41.60 a	28.08
T <sub>7</sub>	1750	2350	6650	2150	12.90	34.40 e	5.91
T <sub>8</sub>	1700	2035	6450	1995	12.18	32.48 f	-

T<sub>1</sub>= only compost without wheat grain colonized *Trichoderma*, T<sub>2</sub>= compost with 100 g wheat grain colonized *Trichoderma*, T<sub>3</sub>= compost with 150 g wheat grain colonized *Trichoderma*, T<sub>4</sub>= compost with 200 g wheat grain colonized *Trichoderma*, T<sub>5</sub>= compost with 250 g wheat grain colonized *Trichoderma*, T<sub>6</sub>= compost with 300 g wheat grain colonized *Trichoderma*, T<sub>7</sub> = without compost only 200 g wheat grain colonized *Trichoderma*, T<sub>8</sub> (Control) = no compost and no wheat grain colonized *Trichoderma*.

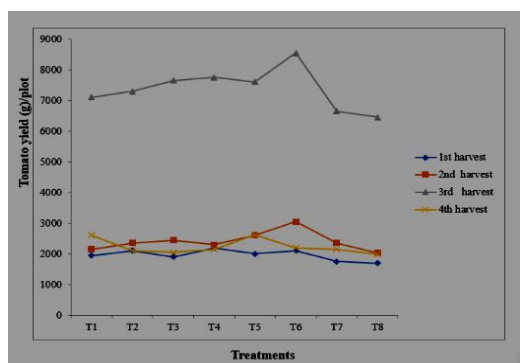


Fig. 2. Yield of tomato at different stages of harvesting

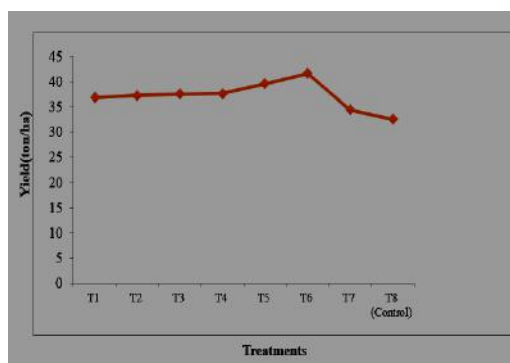


Fig. 3. Yield of tomato under different treatments

#### IV. CONCLUSION

Based on the current study, it could be concluded that Trichoderma-fortified compost appeared to be excellent in controlling different diseases of tomato with the significant increase of growth and yield. Farmers can adopt Trichoderma-fortified compost, an eco-friendly control measure for different diseases of vegetables with the lower cost in comparison to chemical pesticides.

#### ACKNOWLEDGEMENTS

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# The benefits of market gardening in a context of food insecurity in the commune of Djirataoua in Niger

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**Abstract**— Market gardening plays an important role in household food security and is an income-generating activity for market gardeners in the rural commune of Djirataoua. The objective of this study is to study the advantages of market gardening in the district of Djirataoua. To achieve this objective, the data collection method mainly involved individual surveys and focus groups on each of the six (6) sites, namely Djirataoua North, Djirataoua South, Kodrewa-Maradou, Radi-Adrewa, RPC (Rural Promotion Center) and Bakaoua. A total of 209 producers representing 5% of the producer workforce. Focus groups were held with the members of the cooperative. The data were processed by the Excel spreadsheet, IBM SPSS statistics 25 and Minitab 18 software for percentage calculations and subjected to the multivariate PCR and AFC statistical tests. The results show that thirteen (13) market gardening crops are grown in the district of Djirataoua. . The most widely grown crops are anise, red pepper, green pepper, onion and tomato. The least commonly grown crops are watermelon, sweet potato, carrot, potato and pepper. Thus, for phytosanitary treatment, chemicals are generally the most used. Thus, this activity has significant socio-economic impacts. The average annual income generated by this activity ranges from less than 200,000 CFA francs to more than 1,000,000 CFA francs. However, market gardeners encounter problems in the practice of this activity. To ensure the smooth running of this activity, solutions such as reducing the cost of the fee, support for agricultural inputs, technical support and the search for outlets for the products are proposed by market gardeners. There is no doubt that market gardening is an alternative to the chronic food insecurity problem experienced by the people of Djirataoua in particular and, in general, the problem of chronic food insecurity.

**Keywords**— Food insecurity, Practice, market gardening, Advantage and Djirataoua



## I. INTRODUCTION

In Niger, the main activities of rural populations are agriculture and livestock, which employ more than 85% of rural households and contribute 40% to the country's Gross Domestic Product (GDP) (Yacouba et al., 2021). Despite its importance, the agricultural sector is struggling to modernize and remains largely dependent on climatic and edaphic hazards and unmodern techniques (Bouchetara, 2021; Yacouba, 2021). This sector does not cover the food needs of both rural and urban populations, plunging them into a situation of chronic food insecurity. According to a study conducted by Recca-Niger in 2011, food insecurity

affects 17.3% of the rural population globally and remains similar to that in urban areas in this country. Although the last century has seen enormous scientific and technological triumphs, overcoming food insecurity is still one of the major challenges for the countries of the South, including those that are relatively advantaged in terms of climatic conditions (Salissou, 2006). Due to the structural and cyclical nature of food insecurity in Niger, assistance options must meet the short-, medium- and long-term needs of vulnerable populations. Priority should be given to severely food insecure households, severely affected urban

poor households, children, and pregnant and lactating women (Reca-Niger, 2011).

Faced with this deterioration in agricultural production conditions, farmers have adopted several options, including rural exodus, emigration to more distant destinations and the adoption of market gardening practices in and around the villages, cities and the surrounding area, thus ensuring or increasing agricultural productivity. Thus, in order to cope with this situation of food insecurity, the populations have introduced the practice of market gardening, which, according to the WFO (World Food Organization) (2012), occupies an important place for human consumption and constitutes one of the most productive agricultural systems in Africa. Market gardening plays a key role in most nutrition and poverty alleviation programs and contributes significantly to family incomes (James et al., 2010 ; Yolou et al., 2015, Rabo, 2 019 ; Rabo et al., 2022). As a result, vegetable crops represent an important and varied food source that completes the dietary needs of populations whose basic diet consists mainly of carbohydrates, the main energy elements (Yolou et al., 2015). Market gardening is marked by specific and varietal diversification and is an activity that can be found in almost all regions of the country, but its extent varies greatly between producers and between regions (Koc et al., 2006; Kanda et al., 2014). Also, by selling market garden products, market gardening provides income to the population (Rabo, 2019; Rabo et al., 2022; Rabo et al., 2024; Ndiaye et al., 2021). In addition, the development of both urban and peri-urban market gardening is encouraged by the growth of local demand.

Market gardening is practiced near water sources such as dams, rivers, lakes, wells, boreholes, but is only intensively exploited when the conditions for transport and preservation of the products are ensured. However, the Djirataoua site is characterized by boreholes, which are a real source of irrigation water. Indeed, apart from agriculture and livestock, the collection of market garden products is an important source of income and food for the community of Djirataoua. The contribution of market gardening products to food security is appreciable because they play an important role in food security and provide income to populations (Ndjeikornom, 2015; Rabo et al., 2022). According to Madjigoto et al. (2018), the introduction of market gardening aims to address cereal deficiencies, fight poverty, hunger and food insecurity. Market gardening therefore plays a major role in the lives of the population. It would therefore be imperative to study the practice of market gardening in the district of Djirataoua.

## II. MATERIALS AND METHODS

### Presentation and choice of study site

This study was conducted in the rural district of Djirataoua, located at 13 km from the city of Maradi (Figure 1). Large-scale market gardening was the main criterion for the choice of the study area. Thus, the perimeter is subdivided into six (6) sites, namely: Djirataoua North, Djirataoua South, Kodrewa-Maradou, Radi-Adrewa, RPC (Rural Promotion Center) and Bakaoua.

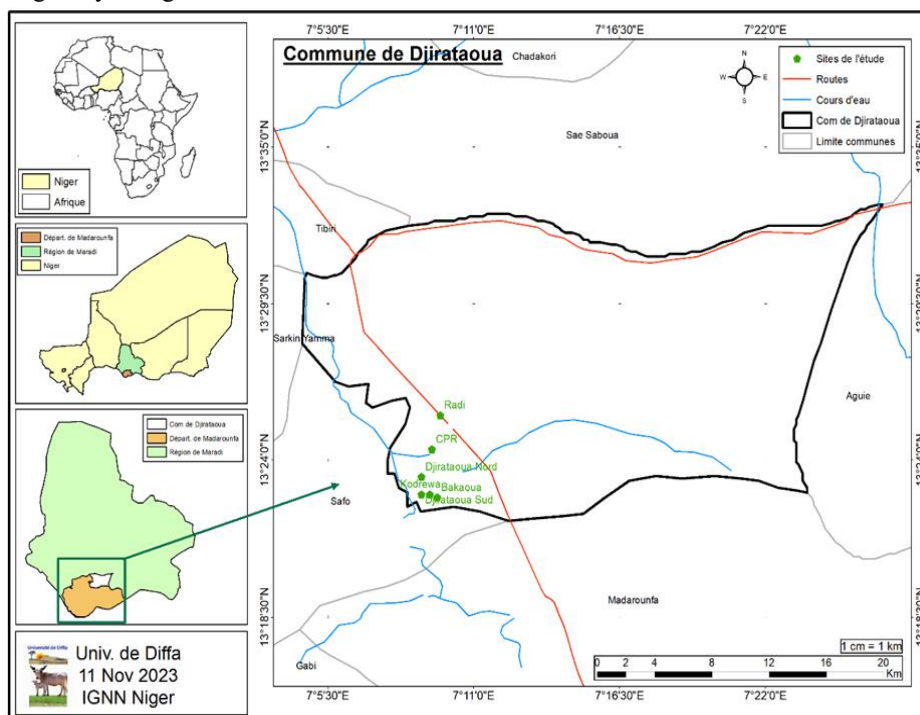


Fig.2: Location map of the rural district of Djirataoua

### Sampling and data collection

This step was carried out as a result of two stages. First of all, it is about making contact with the technical services and the farmers' leaders. The help of these two experienced agricultural actors, who know the area well, made it possible to know the total number of sites and the number of producers per site who exploit the irrigated perimeter of Djirataoua.

Data on the productivity and socio-economic roles of vegetable crops were collected on the six (6) sites using a questionnaire as a basic tool. The objective is to survey at

least 5% of producers at the level of each site, the data collection concerned market gardeners aged about 18 years and over in order to obtain better information. A total of 209 producers have been selected on all sites. It consists of going to the level of market gardeners with the help of a pre-established survey sheet. Individual interviews and focus groups were conducted. A total of 209 cards were administered. The only criterion used for the choice of respondents is that they are market gardeners. Among these producers, the gender aspect has been respected. Thus, 29 questionnaires, or 14%, were administered to women vegetable producers.

Table 1: Number of Producers by Site

Sites	Number of producers	Sample (5%)
Djirataoua Nord	692	34
Djirataoua Sud	925	46
Kodrewa-Maradou	1016	50
Radi	756	37
CPR	680	34
Bakaoua	158	8
<b>Total</b>	<b>4227</b>	<b>209</b>

### Data processing and analysis.

The collected data were captured, coded, processed and analyzed using the Excel spreadsheet and Minitab 18 and IBM SPSS V25 software. To relate the information collected on the crops cultivated, the incomes of market gardeners and the study sites, a Correspondence Factor Analysis (CFA) was carried out.

In addition, principal component analysis (PCA) was performed on quantitative data to identify the relationship between certain parameters and sites. Also, ANOVA tests were also carried out with the software after checking the normal distribution of the data as well as the equality of the variances.

## III. RESULTS

### Socio-professional characteristics of respondents

Socio-occupational characteristics relate to gender, ethnicity, age group, marital status, size of household and agricultural assets, level of education and main activity of the head of household, method of land acquisition and respondents' experience in market gardening. The results of the survey show that men are more represented in the practice of market gardening, as they represent 86% of respondents (Figure 2 A). In addition, the Hausa are in the majority in market gardening, with 97% of respondents representing them (Figure 2 B). Figure 2C gives us the age groups of the respondents on the site. According to this figure, young people (aged about 18 to 40 years) represent more than 50% of respondents, with market gardeners aged of 40 and over accounting for 42% of respondents. Figure 2D provides information on the marital status of the respondents. This figure shows that 80% of respondents are married.



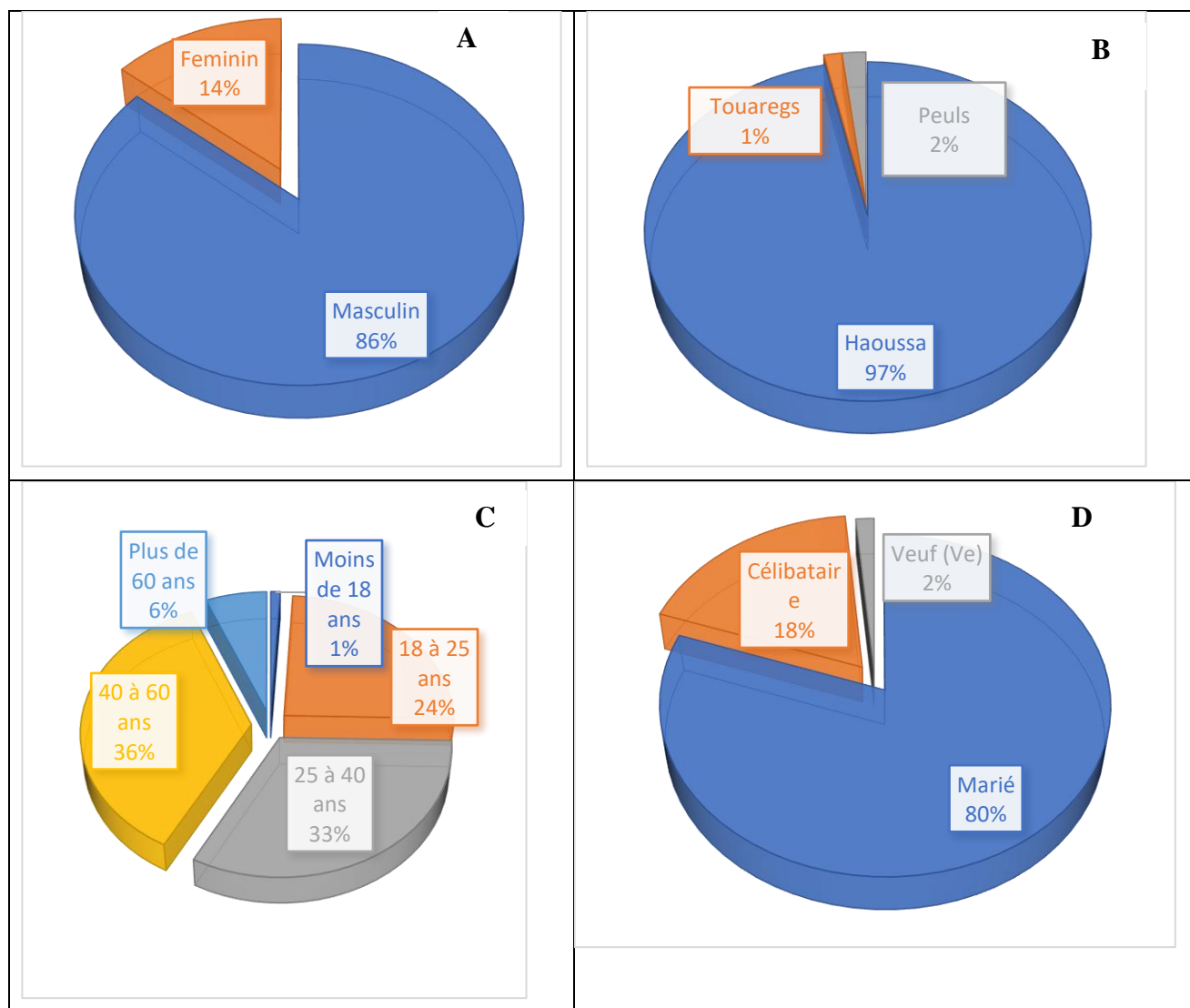


Fig.2: Proportion of respondents in (A) by sex, (B) by ethnicity, (C) by age group and (D) by marital status

Table 2 shows the average household size and the average number of agricultural workers. Analysis of this table shows that there is no significant difference between household sizes at the 5% threshold ( $P = 0.287$ ). On the other hand, there is a statistically significant difference between the numbers of agricultural workers at the same 5% threshold ( $p = 0.001$ ).

Table 2: Household Size and Number of Farm Workers

Parameters	Average Value	Probability
Household size	9,7±5,68	0,287
Farm assets	5,25±3,75	0,001

Figure 3A shows the level of education of the respondents at the site. The analysis of this figure shows that nearly 60% of the respondents attended Koranic school and more than

38% went to modern school, of which less than 1% have a university degree. Figure 3B shows the main activities by the respondents. The analysis of this figure shows that 95% of the respondents are engaged in agriculture as their main activity. On the other hand, land acquisition can be done through inheritance, purchase, lease, gift and loan (Figure 3C). This figure shows that more than 50% of respondents have acquired their land by inheritance and more than 40% farm their own acquired land in various ways. Also, the 3D figure illustrates the years of experience of market gardeners. The analysis of this figure shows that 30% of the respondents have 20 to 30 years' experience in market gardening, while 37% have 10 to 20 years of experience, while the youngest in practice, with less than 5 years of experience, representing 8%.

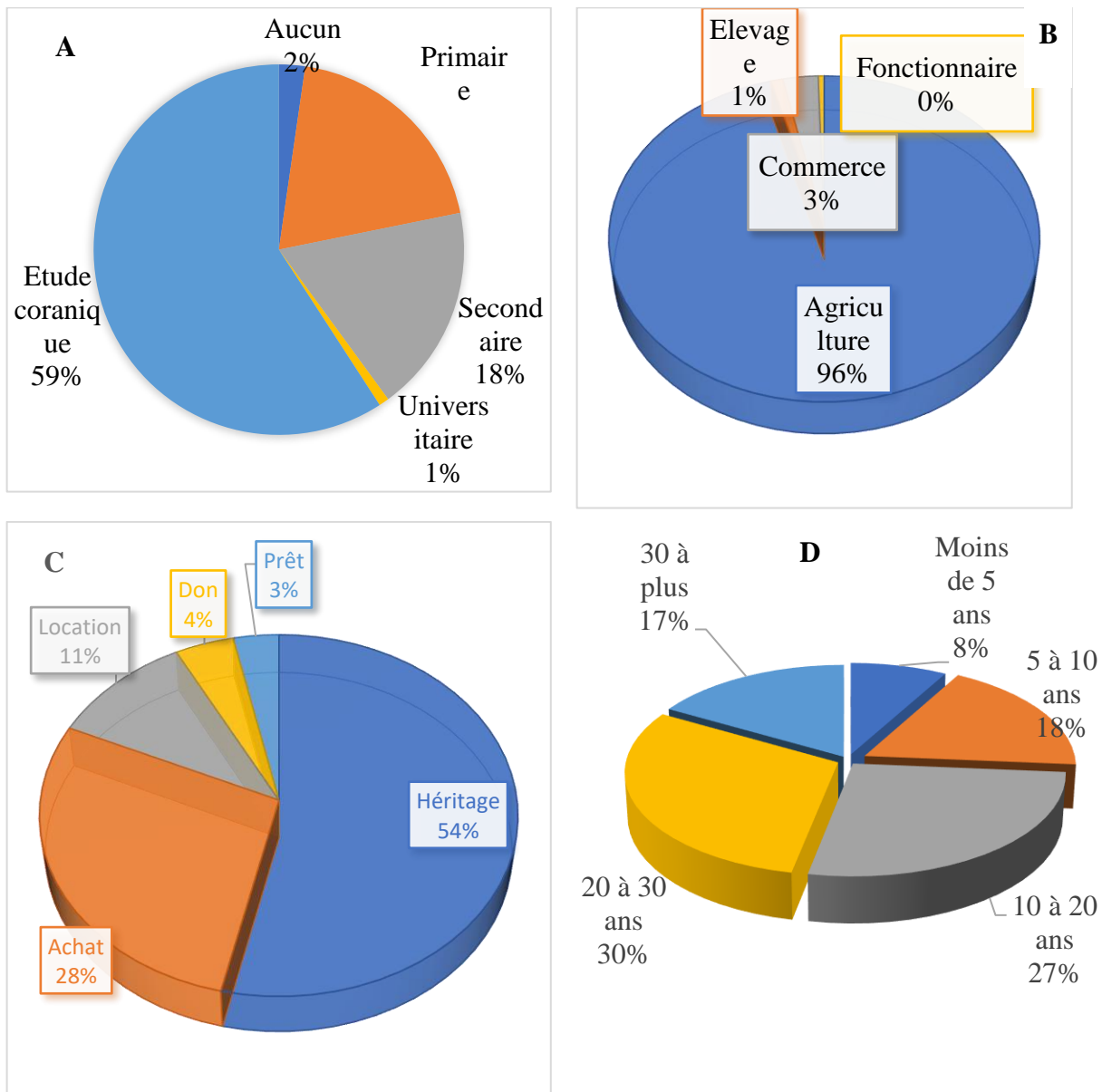


Fig.3: Frequency of respondents in (A) : Level of education (B) : Activities practiced in (C) : Land acquisition method and (D) : Number of years of experience

### Market gardening grown on the site

Table 3 shows the different market gardening practices in the different districts of Djirataoua. An analysis of this table shows that a diversity of market gardening crops is practiced. In fact, thirteen (13) market gardening crops are grown on all the sites. The crops present on all sites are anise

and chili peppers and represent respectively 30.63% and 12.86% of the vegetable crops cultivated. On the other hand, the least cultivated crops are watermelon (0.32%), sweet potatoes (1%) and carrots (1%), potatoes (1%) and to a lesser extent peppers (2%). On the Bakaoua site, only two (2) crops, anise and chili, are grown.

Table 3: Representativeness of vegetable crops grown on the site

<b>Frequency (%)</b>
----------------------

Crops	Scientific Names	Bakaou a	CPR	Djrt N	Djrt S	Kodrewa	Radi	Total
Anise	<i>Pimpinella anisum</i>	72,73	33,73	22,37	31,72	32,47	31,76	30,63
Eggplant	<i>Solanum melangena</i>	0,00	9,64	13,16	12,41	2,60	3,53	8,41
Cabbage	<i>Brassica oleracea</i>	0,00	0,00	5,26	4,14	9,09	3,53	4,92
Carrot	<i>Solanum melangena</i>	0,00	0,00	1,32	4,14	0,00	0,00	1,27
Lettuce	<i>Lactuca sativa</i>	0,00	8,43	3,95	8,28	2,60	0,00	4,60
Onion	<i>Allium cepa</i>	0,00	4,82	9,87	8,97	16,23	22,35	12,06
Chili pepper	<i>Capsicum frutescens</i>	27,27	16,87	9,87	6,21	18,18	14,12	12,86
Potato	<i>Solanum tuberosum</i>	0,00	0,00	0,00	1,38	0,00	2,35	0,63
Chili pepper	<i>Capsicum frutescens</i>	0,00	0,00	4,61	0,00	1,30	2,35	1,75
Watermelon	<i>Citrillus vulgaris</i>	0,00	0,00	1,32	0,00	0,00	0,00	0,32
Sweet potato	<i>Ipomoea batatas</i>	0,00	0,00	6,58	0,00	0,00	0,00	1,59
Green Chili Pepper	<i>Capsicum annuum</i>	0,00	14,46	11,84	15,86	12,34	12,94	13,17
Tomato	<i>Lycopersicum esculentum</i>	0,00	12,05	9,87	6,90	5,19	7,06	7,78
Total		100,00	100,00	100,00	100,00	100,00	100,00	100,00

Legend: CPR: Centre for Rural Promotion; Djrt N: North Djirataoua; Djrt S: South Djirataoua

In addition, Figure 4 illustrates the results of correspondence factor analysis (CFA) whose axes account for 70% of the inertia. Thus, the results of the analysis revealed that crops such as green chili, eggplant, lettuce, pepper, watermelon, tomato are the most practiced in

Djirataoua North, Djirataoua South, and CPR. On the other hand, crops such as anise, onion, cabbage, sweet potato and chili pepper are more widely grown in Bakawa, Kodrewa and Radi.

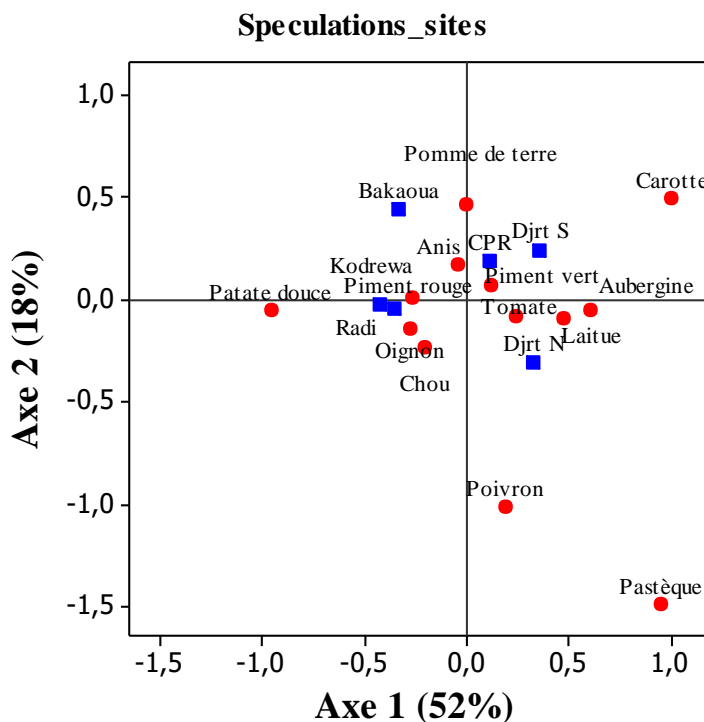


Fig.4: Relationship between sites and crops grown in the Factorial axis system after a Correspondence Factorial Analysis

**Areas under cultivation**

Figure 5 shows the areas occupied by vegetable crops. Indeed, the areas vary from less than 0.1 to 1 ha. Thus, areas between 0.25 and 0.5 ha are the most represented at the level of the CPR site (22.86%) and Djirataoua North (21.43%)

while areas of less than 0.1 ha are the most represented at the level of Djirataoua South (27.03%) and Radi (32.43%). Areas between 0.5 and 1 ha are the most represented in Kodrewa (52.94%) and for the Bakawa site, areas between 0.1 and 0.25 ha are the most represented.

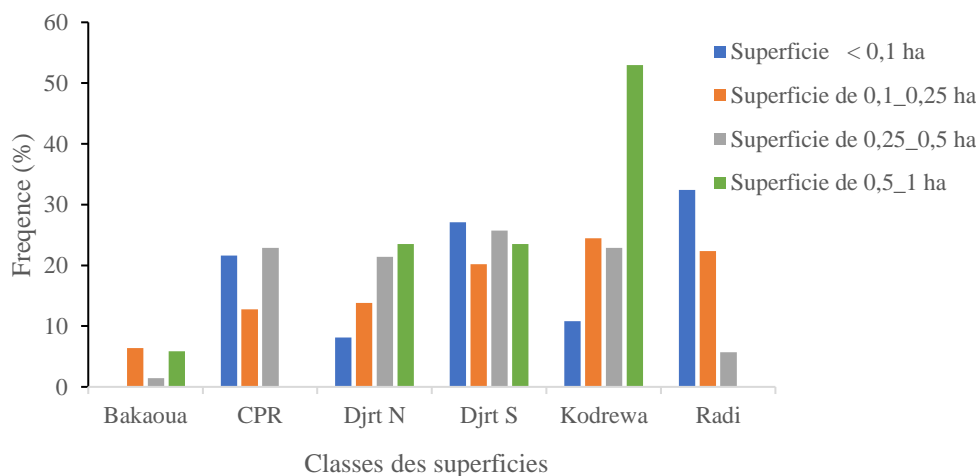


Table 4 shows the labour force employed at the different study sites with their respective costs. An analysis of this table shows that the number of men per day varies on average between 3.5±1.195 and 5.182±3.45 per head of household. Analysis of variance shows that there is no statistically significant difference at the 5% level. Also, market gardeners invest on average, in labour, 5406±2866

FCFA on the Bakaoua site, 7125±6781 FCFA, 7417±4500 FCFA, 7791±5409 FCFA, 7333±5355 FCFA and 4250±201 FCFA respectively on the CPR sites Djirataoua North, Djirataoua South, Kodrewa and Radi.

Analysis of variance shows that there is a statistically significant difference between average labour costs. Thus,

market gardeners in South Djirataoua are the ones who have invested the most in labour, unlike those in Radi.

Table 4: Average cost of labour employed at the different sites

Sites	Man/day	Labor Cost (FCFA)
Bakaoua	3,5±1,195a	5406±2866ab
CPR	3,813±1,401a	7125±6781ab
Djrt N	5,182±3,45a	7417±4500ab
Djrt S	4,907±2,486a	7791±5409a
Kodrewa	4,359±3,208a	7333±5355ab
Radi	3,824±1,834a	4250±2012b
<b>Probability</b>	<b>0,149</b>	<b>0,036</b>

Means followed by the same letter on the column do not have a statistical difference

Legend: CPR: Centre for Rural Promotion; Djrt N: North Djirataoua; Djrt S: South Djirataoua

#### Yield of main crops

Table 5 shows the yield of four (4) main crops: anise, green pepper, red pepper and onion. Thus, it appears from the analysis of this table that the production of anise and green pepper varies from 7,658 kg/ha and 7,875 kg/ha (Djirataoua north) to 456.75 kg/ha and 247.5 kg/ha (Djirataoua south)

respectively. For red pepper and onion, the best yields were recorded at the Djirataoua site with 8,507.25 kg/ha and 14,950 kg/ha respectively. On the other hand, the lowest yield of red pepper is observed at Kodrewa with 1,247.625 kg/ha. Analysis of variance shows that there is no statistically significant difference between crop yields at the 5% level.

Table 5: Main Crops Yields

Sites	Yield of main crops(Kg/ha)			
	Anise	Green Chili Pepper	Chili Pepper	Onion
Bakaoua	2 625a	2 587,5a	3 411,9a	–
CPR	3 886,4a	1 162,12a	3 712,5a	10 114a
Djrt N	7 658a	7 875a	1 462,5a	11 267,1a
Djrt S	456,75a	247,5a	8 507,25a	14 950a
Kodrewa	7 140a	4 279,5a	1 247,625a	10 240a
Radi	1 467,2a	3 882,37a	4 455a	10 075a
<b>p-value</b>	<b>0,285</b>	<b>0,256</b>	<b>0,4506</b>	<b>0,850</b>

Legend: CPR: Centre for Rural Promotion; Djrt N: North Djirataoua; Djrt S: South Djirataoua

Means followed by the same letters in the same column are not statistically different

#### Income from market gardening

In this study, the income of vegetable producers was estimated (Figure 6). Thus, four (4) income classes have been defined. The results show that 24.71%, 4.71% and 35.29% of respondents have an income of less than 200,000 CFA francs respectively at the sites of Radi, Bakaoua and

Kodrewa. Income between 500,000 FCFA and 1,000,000 FCFA is better represented at the level of the RPC site (21.43%). For the Djirataoua North site, the income between 200,000 and 500,000 CFA francs is more represented. And finally, the income of 1,000,000 FCFA at more is better represented at the level of South Djirataoua (50%).

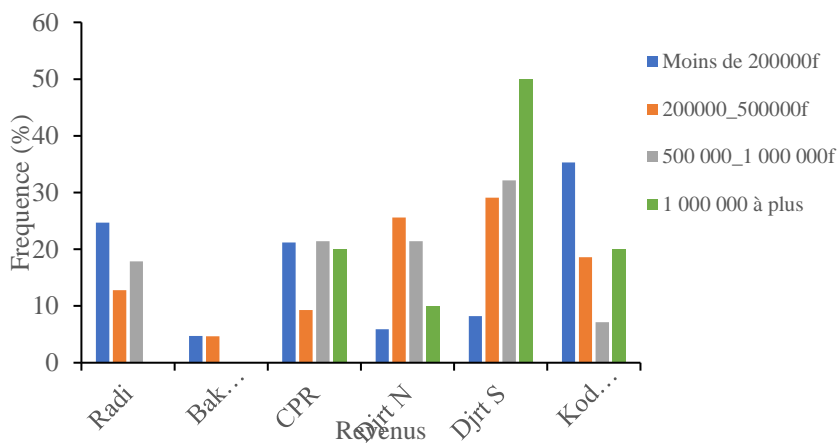


Fig.6: Income from market gardening

The results of the principal component analysis (Figure 7) show that the first two axes concentrate 66% of the inertia, which seems sufficient for the interpretation of the data. The analysis of this figure shows that all crops are more widely grown in Djirataoua North, Djirataoua South and Kodrewa and the highest incomes (500,000 to more than 1,000,000

CFA francs) are more likely to be found at these sites. The vegetable crops grown are red pepper, sweet potato, cabbage and onion. However, low incomes, of less than 200,000 CFA francs, are more common in Bakawa, Radi and CPR.

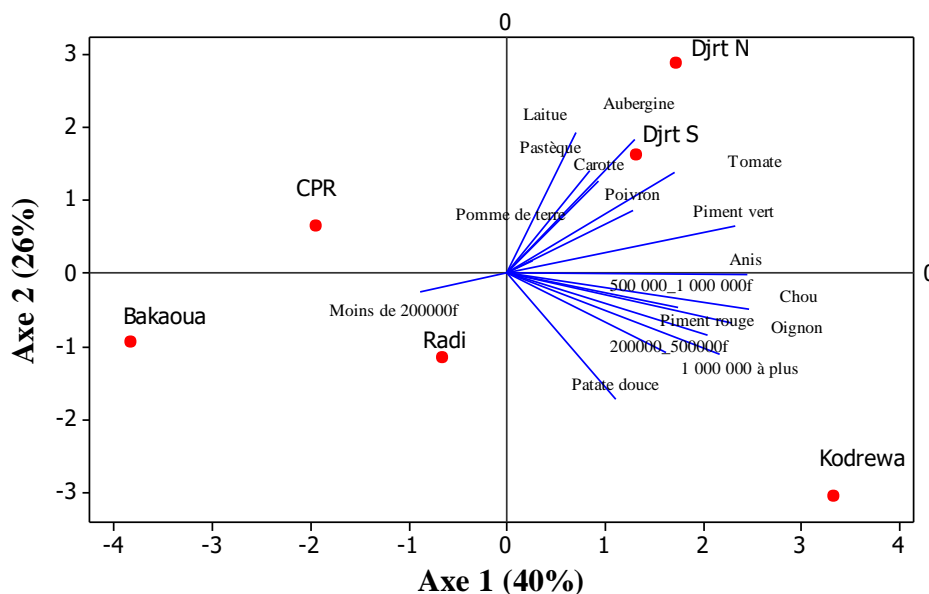


Fig.7: PCA linking crops, income by site

Legend: CPR: Centre for Rural Promotion; Djirt N: North Djirataoua; Djirt S: South Djirataoua

**Use of income from market gardening**

The income from market gardening contributes to the satisfaction of family needs. Thus, Figure 8 shows the proportions of the different uses of market gardening income. It appears from this figure that 22% of households

allocate their income from market gardening to marriage/naming ceremony, 18% to health and children's schooling; Only 11% of respondents use their income from market gardening to buy food. Some respondents invest in small businesses (14% of respondents) and others in livestock fattening (15% of respondents).

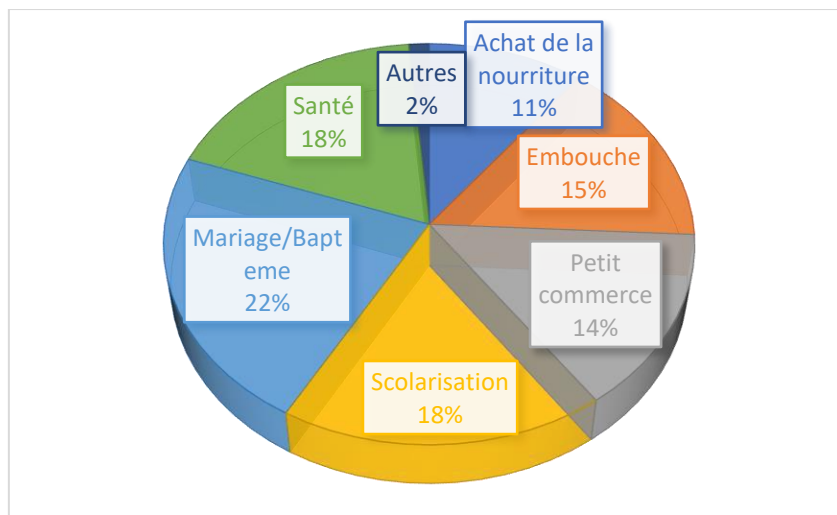


Figure 8: Use of income from market gardening

**Constraints related to the practice of market gardening**

Market gardeners face difficulties in this activity, both technically and financially. Table 6 summarizes the problems faced by producers. The analysis of variance shows that there is a statistically significant difference between the mean proportions of the stresses, at the 5% level. Thus, it appears from the analysis of this table that the

constraints such as pest attack and cost of the fee are much greater at the sites of Djirataoua South and Kodrewa. On the other hand, theft, the problem of flow and flow are more encountered in Kodrewa. As for the cost of fertilizers, it is much more cited by respondents at the Djirataoua South site. The Bakaoua site is the one where respondents encounter fewer constraints.

Table 6: Representativeness of constraints related to vegetable crops

Constraints	Bakaoua	CPR	Djrt N	Djrt S	Kodrewa	Radi	P-value
Pest Attack	4,68b	15,79ab	16,96ab	20,47a	25,73a	16,37ab	0,001
Water Fee Cost	4,76b	18,10ab	13,33ab	25,71a	25,71a	12,38ab	0,03
High Fertilizer Cost	3,45b	10,34ab	18,97ab	36,21a	20,69ab	10,34ab	0,0137
Theft	5,26b	21,05ab	20,00a	10,53b	36,84a	26,32ab	0,04
Flow problem	3,23b	12,90ab	16,13ab	35,48a	22,58a	9,68ab	0,001
Water Problem	6,82b	18,18ab	18,18ab	9,09b	20,45a	27,27a	0,02

Legend: CPR: Centre for Rural Promotion; Djrt N: North Djirataoua; Djrt S: South Djirataoua

Means followed by the same letters on the same line are not statistically different. CPR: Central

**Possible solutions to problems**

To overcome the various constraints faced by market gardeners in the rural commune of Djirataoua, solutions were proposed by the respondents (Figure 9). The analysis in Figure 9 shows that among the solutions envisaged by the

respondents, are the reduction of the cost of the fee, support for agricultural inputs, technical support and the search for outlets for the products. Thus, nearly 35% of respondents are asking for support in terms of agricultural inputs and a reduction in the cost of the fee at the same time, and 25% are asking for technical support.

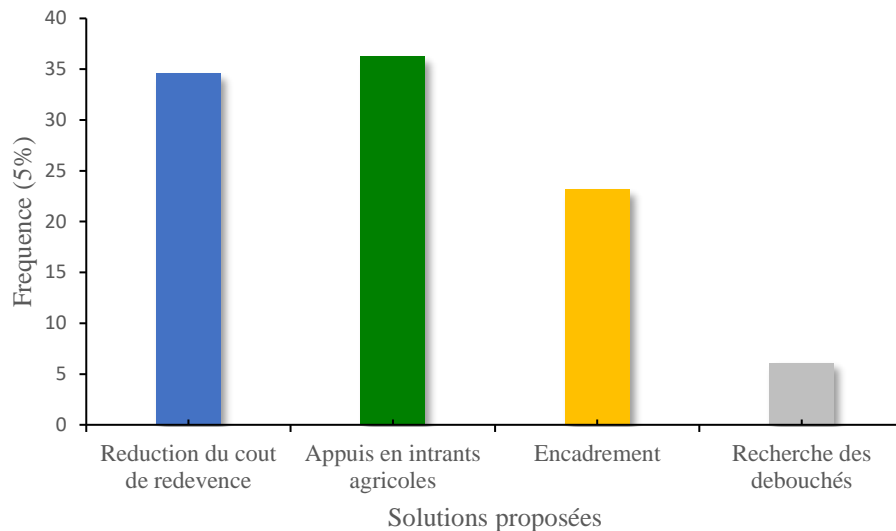


Fig.9: Representativeness of Proposed Solutions

#### IV. DISCUSSION

Market gardening is a practice that takes an important place for both men and women, as this study shows. In the selected sites, 209 market gardeners were interviewed on the basis of their availability, of which 86% are men and only 14% are women. This high proportion of men can be explained by the fact that field work in general, and market gardening in particular, requires a great deal of physical effort, which men are most capable of exercising. Similar results were reported by Rabo et al. (2022) where men account for 93% and 77% of market gardeners respectively at the sites of Tibiri Gobir and Madarounfa. These results corroborate those of Rabo et al. (2024). Indeed, by studying the socio-economic effects of insecurity on pepper producers and adaptation strategies in Diffa region of Niger, these authors reported that men represent 88.3% of the respondents and thus dominate pepper production. Similar results have been reported by Saley et al. (2022), who state that pepper cultivation is a predominantly male activity with a low percentage of women (12%) involved in pepper production activities. According to these authors, the low involvement of women in pepper production could be explained by land tenure status, which traditionally gives primacy to men.

In addition, the sites are characterized by a diversity of cultivated species. Indeed, thirteen (13) species have been recorded on the irrigated perimeter of Djirataoua. This diversity is lower than that reported by Rabo et al. (2022) who identified eighteen (18) and seventeen (17) in Tibiri gobir and Madarounfa, respectively. It is also higher than that reported by Rabo et al. (2019). These authors have identified a total of nine (9) species of vegetable crops

associated with *M. oleifera* in the Niger River valley and seven (7) in the Goulbi of Maradi with a specialization of the sites in certain crops.

The most widely grown crops are anise, green pepper, red pepper and onion. Speculations such as peppers, sweet potatoes, carrots, potatoes and watermelons are the least practiced. These results are similar to those of Rabo et al. (2022). According to these authors, the crops most commonly grown by growers are onions, tomatoes and cabbage, regardless of the site. In addition, the soils are fertilized with organic manure and/or mineral fertilizers. The combination of the two (2) types of fertilizer is dominant in our study area with a rate of 85 to 100%. These results corroborate those of Ouikoun et al. (2019) in Southern Benin, who stipulate that the application of organic and mineral fertilizer is observed by 90% to 98% of respondents. The results on phytosanitary treatment show that 99.52% of producers use only chemical pesticides for crop treatment compared to only 0.48% who combine two types of products (pesticides and natural products). This could be explained by the fact that producers do not master the manufacturing process of natural bio-pesticides. These results are high, compared to those obtained by Sani (2018) who reports that 70% of the people surveyed are not familiar with natural pesticides in the irrigated perimeter of Djirataoua.

This study also examined the yields of vegetable crops, including anise, green pepper, red pepper and onion. Indeed, anise yields vary from 7,658 to 7,875 kg/ha. These results are out of step with those of Manzo (2018) who reported an anise yield of 2519.33 kg/ha in the rural district of Djirataoua. This could be explained by the fact that this



author has limited himself to a single site. Similarly, the results of this onion yield study (10,075 to 14,950 kg/ha) are not consistent with those reported by Rabo et al. (2020) who obtained a yield of  $157886 \pm 26706$  kg/ha on the one hand, but with those reported by Abdoul Habou et al. (2015), on the other hand. By studying the effect of nitrogen on the shelf life of onion bulbs, the authors found yields ranging from 17 to 34 t/ha.

From an economic point of view, the sale of market garden products is an income-generating activity. Thus, on the different production sites, the average annual income of producers varies from less than 200,000 CFA francs to more than 1,000,000 CFA francs. These results are similar to those obtained by Zeinadou (2019) who reports that the average annual income of producers is 434328.75 FCFA or 36194.7 FCFA per month, which testifies to the profitability of market gardening and that onions, tomatoes and cabbage are the most popular crops in the commune of Malbaza. Market gardening is an important source of product supply for the population. In the rural district of Djirataoua, market gardening contributes not only to social cohesion but also to their economic development. From a social point of view, market gardening creates jobs. The cultivation of market garden products occupies a large number of the population and makes it possible to meet their basic needs. The results of this study showed that 20.02% of households allocated the income from market gardening to marriage/baptism, 19.40% bought food, 16.31% to health and schooling of children, 12.90% to livestock fattening, 1.24% to small businesses. These results are also close to those of Zeinadou (2019) who states that "100% of producers say they buy food with income generated by market gardening, 42.5% of producers provide health care for their families and 28% ensure their children's schooling. These results corroborate those of Ndiaye et al. (2021) who state that given the multiple benefits of market gardening, this activity constitutes a supplementary activity. The largest incomes were obtained from crops such as red pepper, sweet potato, cabbage and onion. Similar results were reported by Rabo et al. (2022). According to these authors, the most profitable vegetable crops are eggplant and, to a lesser extent, melon, pumpkin, watermelon, cassava, onion and pepper can and can provide up to 500,000 CFA francs/ha/cycle. By studying the structures functioning and prospects for improvement of Moringa oleifera-based agroforestry systems in the Niger River and Maradi Goulbi valleys in Niger, similar results have been reported by Rabo (2019) who states that average financial productivity of crops is 304,688 to 10565167 FCFA in year 1 and 912670 to 2433571 FCFA in year 2, but that the systems of the Maradi goulbi are individually more cost-effective. This can be explained by the fact that farmers in Goulbi spend less than those in the river valley on the one

hand, and that vegetable crops have been combined with moringa on the other. Indeed, according to this author, the most profitable river valley systems are, only, those including sorrel, eggplant, squash and cucumber while all the Maradi goulbi systems have proven to be profitable.

This study also revealed several forms of constraints faced by market gardeners in the rural district of Djirataoua. The constraints most noted by the respondents were pest attacks, the cost of water fees, theft, the cost of fertilizers and the problem of drainage. The results of this study are in line with those of Plea (2016). According to this author, water insufficiency is cited by 85.75% of respondents, he also states that the lack of means to access equipment and inputs was reported by 60% and 62% of respondents. The problem of equipment and input was also reported by NDAO (2009) in the rural community of Ndiob. According to Plea (2016), 51.43% of respondents mentioned difficulties related to marketing. Doing a prospecting, surveying, and collecting study of pepper (*Capsicum Annuum*) accessions grown in the Diffa region, Saley et al. (2022) reported that pepper cultivation faces a number of biotic and abiotic constraints. According to the surveys carried out by these authors, insecurity and flooding are the major constraints faced by producers. They add that constraints such as lack of financial means, crop diseases and insects, lack of inputs, and weeds have been highlighted by producers with varying proportions from one department to another.

## V. CONCLUSION

This study focused on the benefits of market gardening in the rural district of Djirataoua. The results of the producer survey identified thirteen (13) crops grown in the study area. The role of this activity is no longer to be demonstrated today given to its socio-economic impact through its contribution to the dietary diversification of populations and to the income of the population. However, the market gardening and marketing sector faces many problems that jeopardize its development and limit its participation in the local economy.

However, the promotion of local consumption, the construction of infrastructure, and the provision of technical and financial support to producers could make the production and the marketing of market garden products profitable and less arduous. This situation requires effective solutions in order to make market gardening profitable and less arduous.

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