Determination of Pesticide Residues in curry leaf in local markets of Hyderabad, India

Swarupa Rani, S Shashi Vemuri, V.Venkateswara Reddy*

AINP on Pesticide Residues, PJTSAU, Hyderabad,Telangana state, India
*Jawaharlal Nehru Technological University,Hyderabad, India

Abstract— *Studies were conducted for determining the residues of commonly used pesticides in curry leaf samples collected periodically from different markets of Hyderabad, India during 2013 - 2014. A total of 120 samples were collected from five selected markets of Hyderabad every month and analysed using QuEChERS method on LC-MS/MS. The most commonly detected pesticide residues were of Profenophos (22.5%), Ethion (20%), Cyfluthrin (16.67%), Bifenthrin (8.33%), Chlorpyriphos (7.5%), Dimethoate(7.5%), Triazophos (5.83%), Phorate (4.17%), Methyl parathion (3.33%) , cypermethrin (2.5%), Fenpropathrin (2.5%), Monocrotophos (2.5%), Acetamaprid(1.67%), Methamidophos(1.67%). Acephate, Allethrin, alpha cypermethrin ,Fipronil, Carbendazim, Deltamethrin, Malathion, Quinalphos all (0.83%) indicating that, curry leaf samples contained detectable level of the pesticides residues for which Maximum Residue Limits (MRL) are not fixed. As there are no MRLs for curry leaves, it should be considered as most important to fix MRLs to ensure food safety and consumer health and to create awareness among the farmers about the application dose, method of application and Pre Harvest Intervals. The mismanagement or non-availability of proper information about the pesticide application can lead to contamination of pesticide residues in curry leaf. The findings of this study provided important data about contamination of pesticide residues in curry leaf sold in the local markets of Hyderabad and hence, it is essential to conduct monitoring studies in other curry leaf growing agro climatic regions, which may serve as basis for future policy about the standards and quality control of pesticides.*

Keywords—Curry leaf, QuEChERS method, Chlorpyriphos, Cypermethrin, Monocrotophos and LC-MS/MS.

I. INTRODUCTION

Murraya koenigii L. (curry leaf) belonging to family Rutaceae is a leafy spice characterizing authentic Asian-Indian cuisine and it is used in small quantities for its distinct aroma as well as for preservation purposes. Curry leaf oil an volatile oil , produced from the plant has uses in the soap industry. (Salikutty Joseph and Peter, 2008). Recent studies have shown that carbazole alkaloids have several biological activities such as anti carcinogenic effects in dimethyl hydrazine (DMH) treated rats (Khanum et al., 2000), anti platelet activity and vaso relaxing effects (Wu et al., 1998). Chevalier (1996) also reported that curry leaf has medicinal value as traditionally used in Eastern Asia. Interest in greater use of curry leaf has been stimulated since its high antioxidant potency was reported and this antioxidant activity is attributed due to maha nimbine, murrayanol and mahanine from *M. koenigii* (Tachibana et al., 2003; Ningappa et al.,2008). Chowdhury et al. (2001) reported that these alkaloids have antimicrobial activity against gram positive and negative bacteria, and fungi. Lee et al. (2002) noted that enrichment of phenolic compounds within the plant extract is correlated with their enhanced antioxidant activity, It is reported to have antioxidant, anti-diabetic, anti carcinogenic,anti dysenteric stimulant, hypo glycaemic and anti microbial activities (Khanum et al , 2000). Biologically active carbazole alkaloids are reported to have anti microbial properties (Ramsewak et al ,1999). Curry leaves have been reported to contain tocopherol, b-carotene, lutein and alkaloids (Khanum et al., 2000). But it is observed that curry leaves have received red alert message from the European Union, who are the major importers, where the pesticide residue limits were found much beyond the permissible levels. This created a panic among the mass as curry leaves constitute a major spice exported from India.

Uncontrolled use of pesticides and non-adoption of safe waiting periods has led to pesticide accumulation in curry leaf crop. The residues being persistent in nature infiltrate crops, contaminate water, pollute complete food chain and enter our body through diet. Pesticide exposure may produce biochemical alterations in the body long before adverse clinical health effects are manifested (Khan et al., 2008).

II. MATERIALS AND METHODS

Market study

For the evaluation of pesticide residues, a total of 120 curry leaf samples were collected from five local markets of Hyderabad, India at monthly intervals for a period of two years from January 2013 - December 2014. Each sample was processed and analyzed for determination of pesticides. Samples were analyzed within 24 hrs.

Sample extraction procedure

curry leaf samples were analyzed for pesticide residues following the AOAC official method 2007.01 (QuEChERS) after validation of the method in the laboratory. The samples were collected randomly from 5 locations of the market in polythene bags. Each sample was homogenized separately with robot coupe blixer and homogenized 15 ±0.1g sample was taken in 50 ml centrifuge tube and 30±0.1 ml acetonitrile was added to sample tube. The sample was homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher. 3±0.1 g sodium chloride was added to sample, mixed thoroughly by shaking gently followed by centrifugation for 3 min at 2500-3000 rpm to separate the organic layer. The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube and added with 9±0.1 g anhydrous sodium sulphate to remove the moisture content. 8 ml of extract was taken in to 15 ml tube, containing 0.4±0.01 g PSA sorbent (for dispersive solid phase d-SPE cleanup),1.2±0.01 g anhydrous magnesium sulphate and 0.05 g of GCB(Graphatised Carbon Black).The sample tube was vortexed for 30 sec then followed by centrifugation for 5 min at 2500-3000rpm. The extract of about 1 ml (0.5 g sample) was taken for analysis on LCMS/MS under standard operational conditions.(Table-1).

Certified Reference Materials (CRM) of different pesticides having purity ranging from 95.10to 99.99 per cent were stored in a freezer at low temperature, with light and moisture excluded. Solvents used in the study were all glass distilled before use. Sodium sulphate, sodium chloride and magnesium sulphate were activated in hot air oven at 450 °C for 5 h. A weighed amount of analytical grade material of each pesticide was dissolved in a minimum quantity of distilled acetone and diluted with methanol to obtain a stock solution of 1000 mg kg-1 The intermediate standards and working standards of 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 mg kg-1 were prepared by suitably diluting the stock solution in methanol and used as standard check in analysis, linearity and recovery studies (Table-2).

Table.1: LC MS/MS Operating Parameters

LC-MS/MS	SHIMADZU LCMS/MS - 8040.
Detector	Mass Spectrophotometer
Column	Kinetex, 2.6µ, C18 Column, 100 x 3.0.
Column oven temperature	40°C
Nebulizing gas	Nitrogen
Nebulizing gas flow	2.0 litres/min
Pump mode/ flow	Gradient / 0.4 ml/ min
Solvents	A:Ammonium FormateInWater(10Mm) B: Ammonium Formate In Methanol(10Mm)
LC programme	**Time solvent Conc** 0.01 B Conc 35% 2.00 B Conc 35% 7.00 B Conc 60% 9.00 B Conc 60% 14.00 B Conc 95% 17.00 B Conc 85% 19.00 B Conc 70% 21.00 B Conc 35% 24.00 B Conc 35%
Total Time Programme	24 min

III. METHOD VALIDATION

The analytical method for estimation of residues of pesticides in curry leaves has been validated by conducting recovery studies using control samples. 15g of sample was taken in 50 ml centrifuge tubes in three replicates, each were spiked with pesticide mixture at the required fortification levels ie.LOQ, 5x LOQ and 10x LOQ, adding an appropriate volume of working standard. This mixture was then shaken to attain a proper homogeneity of pesticides in the samples. The tubes containing fortified samples were left open for a while, just to allow the evaporation of excess solvent. Sample extraction procedure was followed as given above.

Levels of pesticides present in commodity was estimated using the formula: = (Peak area of sample × Volume of sample injected x Concentration of standard injected × Dilution Factor) / Peak area of standard x Volume of standard injected

Table.2: Average recoveries and R.S.Ds % of different insecticides from curry leaf samples fortified at 10, 50 and 100ppb Levels.

S.no	Pesticide	RT	"Mean recovery% (Spiking Level 0.01 mg/Kg)"	RSD% (n = 3)	"Mean recovery% (Spiking Level 0.050 mg/Kg)"	RSD% (n = 3)	"Mean recovery% (Spiking Level 0.100 mg/Kg)"	RSD% (n = 3)	R^2
1	Abamectin	18.1	112	3.6	97	15.1	111	1.9	0.9941
2	Acephate	1.3	82	1.2	97	15.0	100	1.4	0.997
3	Acetamprid	2.9	101	1.3	90	11.3	101	1.8	0.999
4	Alachlor	14.0	96	0.8	91	2.3	112	2.2	0.997
5	Allethrin	16.8	96	2.3	91	11.6	105	2.3	0.992
6	Anilophos	15.1	89	17.4	101	14.1	98	1.4	0.992
7	Atrazine	10.0	96	1.9	91	3.6	103	2.1	0.998
8	Azinophos ethyl	13.9	89	0.7	92	5.4	114	1.9	0.997
9	Bifenthrin	18.8	119	1.4	88	10.6	97	1.4	0.990
10	Carbaryl	8.7	99	2.8	91	2.7	99	2.2	0.999
11	Carbendazim	4.0	96	2.0	90	9.2	91	2.1	0.998
12	Carbofuran	7.9	112	2.0	94	3.1	107	2.4	0.998
13	Chlorfenviphos	15.3	91	0.9	99	15.8	92	0.5	0.997
14	Chlorpyriphos	17.0	112	5.3	94	17.9	87	1.4	0.978
15	Cypermethrin	17.7	98	2.9	99	16.7	104	2.6	0.966
16	Demeton-s-methylsulfone	1.6	92	12.6	100	7.0	105	4.6	0.997
17	Diazonin	15.3	93	0.6	96	19.3	113	3.0	0.996
18	Dimethoate	2.9	104	0.5	88	3.6	100	1.7	0.998
19	Ethion	16.9	111	0.6	92	17.4	103	1.1	0.993
20	Fenamidone	12.2	97	1.2	96	15.0	103	1.9	0.998
21	Fenpropathrin	17.0	101	6.2	97	13.1	102	2.2	0.984
22	Hexaconazole	15.3	95	2.2	99	1.8	99	1.4	0.995
23	Imidacloprid	2.2	116	3.9	97	5.7	105	1.9	0.996
24	Indoxycarb	16.1	88	7.5	88	3.1	94	1.5	0.986
25	L-cyhalothrin	17.6	105	6.2	103	18.8	108	9.7	0.975
26	Malathion	13.0	114	3.5	98	18.5	99	2.3	0.999
27	Malaxon	8.5	103	0.6	91	7.5	107	1.3	0.998
28	Metalaxyl	10.4	104	0.7	104	10.8	106	1.7	0.998
29	Methamidophos	1.3	102	1.3	99	17.2	99	1.6	0.998
30	Methomyl	1.7	81	12.9	101	1.5	85	4.1	0.994
31	Myclobutanil	13.2	90	16.9	93	12.4	96	4.4	0.985
32	Nitenpyram	14.0	93	1.4	89	8.3	103	1.8	0.996
33	Penconazole	14.9	107	0.6	92	12.9	91	1.7	0.992
34	Pendimethalin	17.1	88	3.8	102	13.8	94	1.5	0.997

35	Phorate	15.5	99	2.6	95	16.8	110	2.1	0.993
36	Phosalone	15.1	89	3.5	88	6.1	117	2.3	0.987
37	Phosphomidan	7.0	90	1.2	89	2.6	102	1.5	0.999
38	Profenophos	16.4	109	1.7	99	15.9	106	1.7	0.994
39	Quinolphos	14.8	91	0.8	94	16.2	102	1.1	0.996
40	Simazine	7.8	97	7.5	97	16.1	98	2.0	0.998
41	Spinosad-a	17.7	116	2.3	93	2.3	106	2.3	0.994
42	Spinosad-d	18.1	98	1.2	90	8.5	99	1.9	0.993
43	Spiromesifen	17.3	113	1.3	87	7.5	95	1.6	0.991
44	Spirotetramate	14.0	91	16.9	97	9.3	103	1.7	0.986
45	Tebuconazole	15.0	88	0.5	90	16.2	94	1.6	0.991
46	Thiacloprid	3.9	112	3.1	102	12.9	96	1.8	0.998
47	Thiamethoxam	1.7	97	2.0	90	2.7	103	1.5	0.993
48	Tricyclozole	4.8	83	0.7	93	8.1	96	1.8	0.997
49	Trifloxy	16.1	97	1.6	91	9.4	90	2.7	0.997

IV. RESULTS AND DISCUSSIONS

A multi residue method was used to monitor 49 pesticide residues by LC MS-MS. The targeted 22 pesticides were detected (Table -3) and quantified based on calibration standard at 0.1mg kg-1 and of the 120 curry leaf samples analysed 71 samples were detected with more than one pesticide . 27 of them were contaminated with Profenophos, 24 with ethion, 20 with cyfluthrin, 10 with bifenthrin, 9 with chlorpyrifos,7 with triazophos, 5 with phorate, 4 with methyl parathion, 3 with monocrotophos, 2 samples with methamidophos and one each with quinalphos, malathion, acephate, allethrin, alphacypermethrin, carbendazim, deltamethrin and fipronil residues. Residue levels of Profenophos were high (25.69 mg kg-1) followed by B- Cyfluthrin and monocrotophos (12.65mg kg-1 and 12.23 mg kg-1), cypermethrin (10.81mg kg-1), dimethoate (4.90 mg kg-1), ethion (4.79 mg kg-1), bifenthrin (2.87 mg kg-1), chlorpyrifos(1.86 mg kg-1),while allethrin was the least (0.046 mg kg-1)(Fig-1). Most of the pesticides detected were Organo Phosphates and Synthetic Pyrethroids This exhibits the shift from Organochlorines to OPs and SP insecticides and the restricted use of OC insecticides. The results obtained in the present study are in agreement with those of Fytianos et al.1985, and Gupta et al,1998. The pesticides detected during the three seasons (Rainy, winter and summer seasons and year wise in 2013 and 2014(Figs 2 to 4 and tables 4 -5) shows different pesticides in different seasons at different levels. During rainy season the residues of cyfluthrin 2.16 mg kg -1 and bifenthrin at 1.15 mg kg -1 and the lowest of 0.05 mg kg -1 of allethrin were detected. However in winter season Methmidophos residues were the highest (12.33 mg kg -1) followed by cyfluthrin (4.03 mg kg -1) and bifenthrin (0.93 mg kg -1).In summer season among the pesticide residues detected alphamethrin was the highest (6.29 mg kg -1) followed by cypermethrin (5.64 mg kg -1) where as bifenthrin residues detected were 1.03 mg kg -1 . Lowest of 0.09 mg kg -1 of phorate was detected.. Cyfluthrin, bifenthrin and ethion residues were the commonly detected pesticides in all 3 seasons . Intensive cultivation technologies produce high infestation of crops by some pests and diseases, trigger off major losses of quality crops and initiate the use of more pesticides. The increase in frequency and magnitude of residues in the curry leaf samples could be attributed to indiscriminate and over use of pesticides by farmers despite efforts by various concerned agencies. It has been found that the farmers are neither following recommended waiting periods nor abide by good agricultural practices (GAP). (Bhanti et al., 2004). Therefore an effective way of educating the farmers via training and electronic media is advised particularly in view of the export potential of the crop. A periodical monitoring studies of pesticide residues may be extended to different agro climatic regions to know actual status of contamination and to strengthen the confidence of consumer in quality of food as well as food quality control authorities for future policies.

Table.3: Retention time, MRL values and pesticide residues (mg/kg) detected in curry leaf samples during 2013 to 2014.

S.No	Pesticide	RT (min)	No of samples contaminated with each pesticide(n=120)	% of samples contaminated	Residue range (mg/kg)		
					Min	Max	Mean
1.	Acephate	1.3	1	0.83	0.285	0.286	0.286
2.	Acetamaprid	2.9	2	1.67	0.071	0.095	0.083
3.	Allethrin	16.8	1	0.83	0.046	0.046	0.046
4.	cypermethrin	17.48	1	0.83	0.050	0.050	0.050
5.	Bifenithrin	18.8	10	8.33	0.131	2.874	1.503
6.	Carbendazim	4	1	0.83	0.080	0.080	0.080
7.	Chlorpyrifos	17	9	7.50	0.184	1.860	1.022
8.	Cyfluthrin		20	16.67	1.065	12.654	6.860
9.	Cypermethrin	17.7	3	2.50	0.345	10.810	5.578
10.	Deltamethrin	17.55	1	0.83	0.369	0.369	0.369
11.	Dimethoate	2.9	9	7.50	0.075	4.902	2.489
12.	Ethion	16.9	24	20.00	0.070	4.790	2.430
13.	Fenpropathrin	17	3	2.50	0.262	1.095	0.679
14.	Fipronil		1	0.83	0.091	0.091	0.091
15.	Malathion	13	1	0.83	1.739	1.739	1.739
16.	Methamidophos	1.3	2	1.67	0.120	0.256	0.188
17.	Methyl parathion		4	3.33	0.050	0.187	0.119
18.	Monocrotophos	1.67	3	2.50	0.478	12.231	6.355
19.	Phorate	15.5	5	4.17	0.055	0.548	0.302
20.	Profenofos	16.4	27	22.50	0.063	25.690	12.877
21.	Quinalphos	14.8	1	0.83	1.017	1.017	1.017
22.	Triazophos		7	5.83	4.330	0.110	2.220

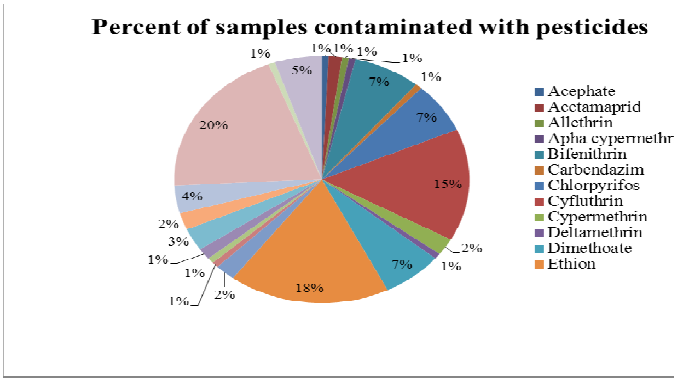


Fig.1: Percent Samples contaminated with pesticides

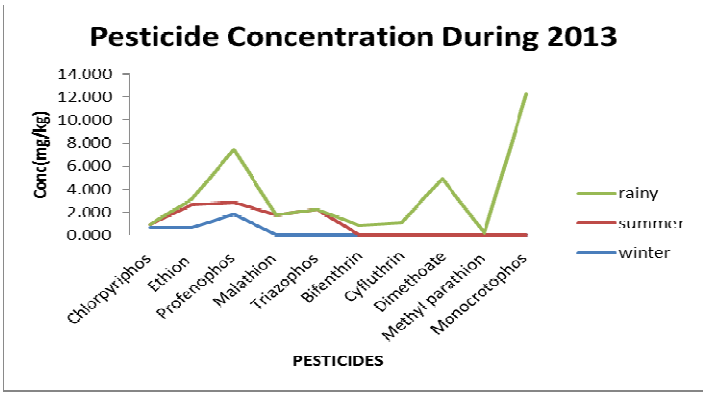


Fig.2: Pesticide Concentration during 2013

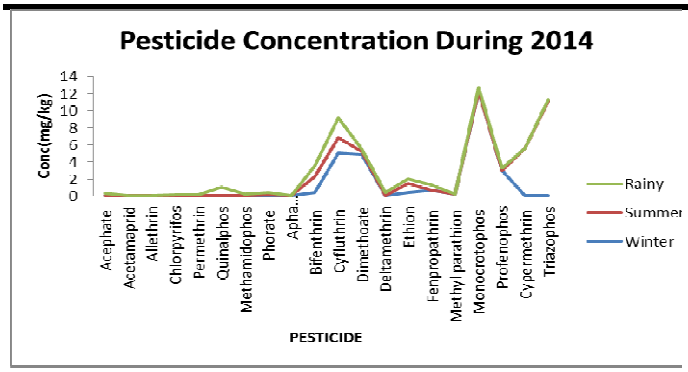


Fig.3: Pesticide concentration during 2014

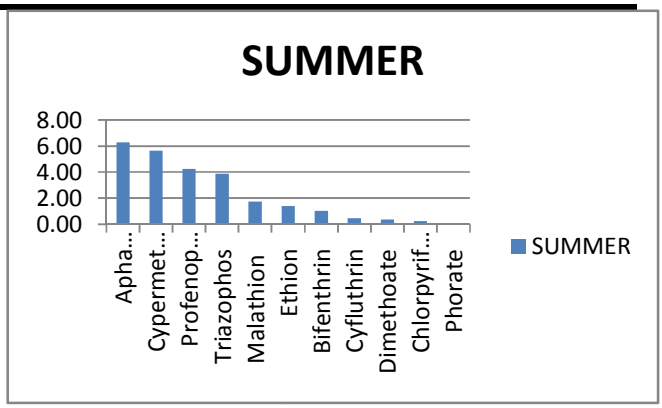
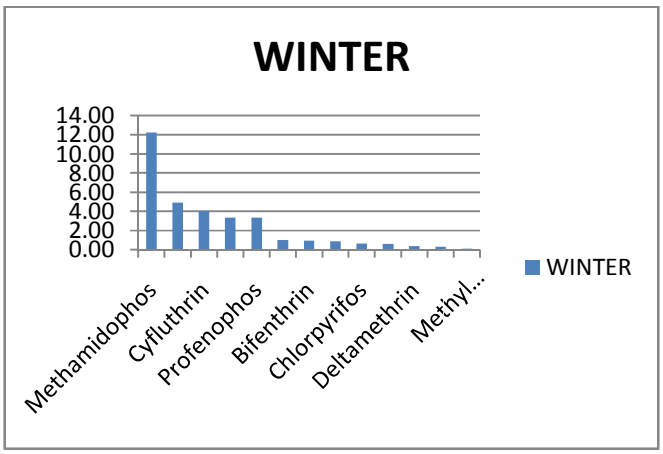


Fig.4: Pesticides detected in different seasons on curry leaf



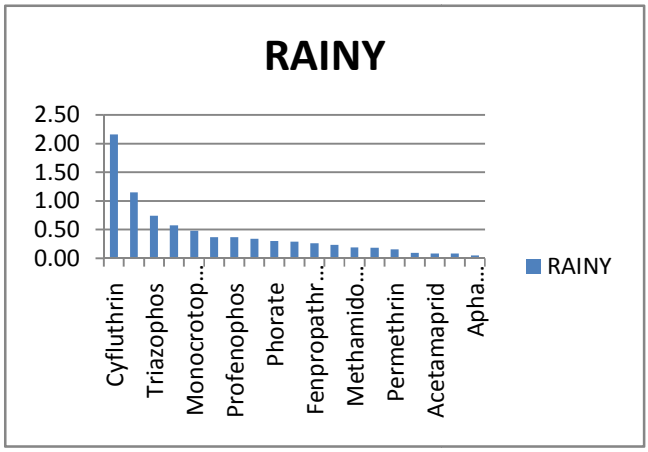


Table.4: PESTICIDE CONCENTRATION DURING 2013

Pesticide	Winter	Summer	Rainy
Chlorpyriphos	0.664	0.240	-
Ethion	0.685	1.930	0.493
Profenophos	1.791	1.030	4.617
Malathion	-	1.739	-
Triazophos	-	2.237	-
Bifenthrin	-	-	0.850
Cyfluthrin	-	-	1.065
Dimethoate	-	-	4.902
Methyl parathion	-	-	0.187
Monocrotophos	-	-	12.231

Table.5: Pesticide Concentration During 2014

Pesticide	Winter	Summer	Rainy
Acephate	-	-	0.286
Acetamaprid	-	-	0.083
Allethrin	-	-	0.046
Chlorpyrifos	-	-	0.121
Permethrin	-	-	0.156
Quinalphos	-	-	1.017
Methamidophos	-	-	0.188
Phorate	-	0.091	0.305
Aphacypermethrin	-	0.050	-
Bifenthrin	0.381	1.930	1.187
Cyfluthrin	5.053	1.804	2.325
Dimethoate	4.902	0.362	0.233
Deltamethrin	-	-	0.369
Ethion	0.404	1.044	0.590

Fenpropathrin	0.645	-	0.679
Methyl parathion	0.187	-	0.052
Monocrotophos	12.231	-	0.478
Profenophos	2.971	-	0.293
Cypermethrin	-	5.555	-
Triazophos	-	11.124	0.139

REFERENCES

[1] Bhanti, M., Shukla, G. and Taneja, A. (2004) Contamination levels of organochlorine pesticides and farmers' knowledge, perception, practices in rural India–A case study,Bull EnvironContam Toxicol, 73, 787-793.

[2] Chevallier, A. 1996. The encyclopedia of medicinal plants.Darling Kindersley, London, UK.

[3] Chowdhury, B.K., Jha, S., Bhattacharyya, P. and Mukherjee, J. 2001. Two new carbazole alkaloids from
Murraya koenigii. Indian Journal of Chemistry 40: 490-494.

[4] Khanum, Farhath, Sudarshana Krishna, K.R.,Viswanathan, K.R., and Santhanam, K.2000. Anticarcinogenic
effects of curry leaves in dimethylhydrazine treated rats.Plant Food and Human Nutrition 55:347-355.

[5] Fytianos, K., Vasilikiotis, G., Weil, L, Kavlendis, E. and Laskaridis, N. (1985) Preliminary study of organochlorine compounds in milk products, human milk and vegetable. Bull Environ Contam Toxicol.34:504-508.

[6] Gupta, A., Singh, B., Parihar ,N.S. and Bhatnagar, A. (1998) Pesticide residues in the farm gate samples of bottle gourd, cauliflower, cabbage and fenugreek at Jaipur. Pesticide Res. J., 10 (1):86-90.

[7] Khan, A.D., M.M. Bhatti, F.A. Khan, S.T. Naqvi and A. Karam, Adverse effects of Pesticides Residues on Biochemical Markers in Pakistani Tobacco Farmers, Int. J. Clin. Exp. Med., 1, 2008, 274-282.

[8] Khanum, F., Anilakumar, K. R., Sudarshana Krishna, K. R., Viswanathan, K. R., &Santhanam, K. (2000). Anticarcinogenic effects of curry leaves in dimethylhydrazine-treated rats. Plant Foods for Human Nutrition, 55, 347–355.

[9] Lee, J.C., Kim, H.R., Kim, J. and Jang, Y. S. 2002. Antioxidant activity of ethanol extract of the stem Of Opuntia ficus-indica var. saboten. Journal of Agriculture and Food Chemistry 50: 6490–6496.

[10] Ningappa, M.B., Dinesha, R., and Srinivas, L. 2008. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf (Murraya koenigii L.) extracts. Food Chemistry 106: 720-728.

[11] Ramsewak, R. S., Nair, M. G., Strasburg, G. M., De Witt, D. L., &Nitiss, J. L. (1999). Biologically active carbazole alkaloids from Murraya koenigii. Journal of Agricultural and Food Chemistry,47,444 – 447.

[12] Salikutty Joseph and K. V. Peter, Curry leaf (Murrayakoenigii), perennial, nutritious, leafy vegetable , Economic Botany 2008 , 39 (1): 68-73.

[13] Tachibana, Y., Kikuzaki, H., Lajis, N.H. and Nakatani, N. 2003. Comparison of antioxidative properties of carbazole alkaloids from Murrayakoenigii leaves. Journal of Agriculture and Food Chemistry 51: 6461-6467.

[14] Wu, T.S., Chan, Y.Y., Liou, M.J., Lin, F.W., Shi, L.S. and Chen, K. T. 1998. Platelet aggregation inhibitor from Murraya keonigii. Phytotherapy Research, 12: 380- 382