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Residue Analysis in Farmgate Samples Of Brinjal Using Gas Chromatograph with ECD and FTD

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Abstract: *The application of pesticide for agricultural production is even more severe in developing countries to eradicate insect-borne, endemic diseasesand to protect the forests, plantations and fibrecrops. India being the second largest producer of vegetables after China, accounts for 13.4% of world production.Long persistence of some agrochemicals in the environment leads to series of undesirable effects through contamination of food and feed. Survey carried out by various institutions spread throughout the country indicated that 50-70% of vegetables are contaminated with insecticide residues and 30 million non-target bioforms are shocked with threat of extinction.In India, DDT and BHC are partially banned, butstill very much used in agriculture and public health programs use because of their wide spectrum of activity and low cost. Residue analysis infarmgate samples of brinjal collected from five districts of Karnataka, India namely Bengaluru urban, Bengaluru rural, kolar, Chickballapura and Ramanagara.Variation in acephate, chlorpyriphos, dichlorvos, monocrotophos, phorate, cyfluthrin-β, cyhalothrin-λ, cypermethrin, deltamethrin and fenvalerate residues in brinjal samples were analysed.Brinjalsamples contaminated with pesticide residues accounted for 37.5% acephate, 20% chlorpyriphos, 27.5% phorate,5% cyfluthrin-β, 20% deltamethrin and 17.5% fenvalerate.*

Key words:*Brinjal, Gas chromatography, Residues, Farmgate samples, Karnataka*

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I. Introduction

Pesticide residues in food and crops are a direct result of application of pesticides to crops growing in the field, and to a lesser extent from pesticide residues remaining in the soil (Businelli *et al.,* 1992).Gips (1987)considered a pest as "an organism which may be a plant or animal, grown for food, fibre or pleasure or health, well-being or peace of mind". About 10,000 species of insects out of 75,000 and 50,000 species of fungi causing more than 1,500 plant diseases have been reported worldwide. In India, crops are considerably damaged by more than 200 pests and 100 plant diseases. Approximately, 30% of Indian crop yield potential is being lost due to insects, diseases and weeds, which in terms of quantity accounts to 30MT of food. Available reports also indicate that loss of food grains is estimated to be 23% and 25% respectively due to insects and diseases (Mauskar, 2007).Almost, 85-90% of pesticides applied to agricultural crops never reaches the target organisms, but gets dispersed through air, soil and water **(**Repetto and Baliga, 1996).Developing country like India consumes 600gof pesticides / hectare, whereas that of developed countries is nearly 3000g/hectare. The low consumption can be attributed to fragmented land holdings, lower level of irrigation, dependence on monsoons and lack of awareness in using the pesticides on grains(Mauskar, 2007).

Pesticides are one among the chemicals that were added to an agro-ecosystem and hence they are referred to as agrochemicals. Hammerton and Reid (1985)considered pesticides, fertilizers, hormones and growth regulators as agrochemicals. They are defined as "any substance or mixture of substances of natural or synthetics origin, used alone or in mixtures, to estimate or regulate the growth of, or to control the pests of, agricultural,horticultural, plantation crops, and of domesticated livestock/farm animals."Agri-Horticultural activities are the main sources of pesticides. Large quantities of pesticides are being used to combat the various pests, which normally infest the economically important plants(Ramesh andYogananda Murthy,2013). It also includes indiscriminate emission from the manufacturing industry, defence units, where chemical welfare is manufactured and commercial products used to kill household pests.

The objectives of the present study are,

- Identify and assess the pesticide levels in the selected vegetables from the point view of safety to consumers.
- Comparing the observed residue levels with PFA and Codex Standards.

- Enumerate the most prevalent pesticides among three different chemical groups i.e., organochlorines (OC), organophosphorus(OP), and synthetic pyrethroids (SP) for guidance to farmers and policy makers for future use.
- Making appropriate recommendations to facilitate extension workers to adopt safe pesticide usage in crop cultivation

II. Materials And Methods

This chapter deals with the description of the materials used, study area, sampling period, the sampling procedure adopted, the questionnaire survey and analytical tools and techniques employed. The present study was conducted during the year 2017-2019 in five districts of Karnataka. Field study was conducted to collect the information about various aspects of pesticide use and their safety. This information was used as the baseline data to investigate the residue level of the analyzed pesticides in vegetables.

III. Study Area

Karnataka State is situated in the west central part of peninsular India, geographically located between 11^0 30' N to 18^0 30' N latitudes and 74^0 E to 78^0 30' E longitude. The state covers 191,976 square kilometers (74,122 sq mi) or 5.83% of the total geographical area of India.Five districts of Karnataka (Table-1) namely, Bangalore rural, Bangalore urban, Chikkaballapura, Kolar and Ramanagara are presenting the hub of agricultural activities were selected as study areas forthedetermination of pesticide residues inbrinjal. A sporadic information and data was collected from farmers with respect to use ofdifferentpesticides and harvesting period of vegetables and fruits in the study area. The sampling period covered the pre- and post-rainy seasons, coinciding with maximum harvest period of vegetable samples coming from different sources of agriculture in the region were procured randomly between 2009and 2013.

Table 1: Profile of various districts selected for the present study

District	Bangalore rural	Bangalore urban	Chikkaballapura	Kolar	Ramanagara
Area	5,814sq. km	2,260 sq. km	4254 sq.km	3969 sq.km	3566 sq.km
Elevation	3,020 ft above MSL	2490 ft above MSL	2727 ft above MSL	2727 ft above MSL	2488 ft above MSL
Latitude	12° 15' N and 13° 35' N, Av. = 12° 58' N	12° 58' N and 12° 97' N, Av. = 12° 77' N	13° 19' N and 13° 39' N, Av. = 13° 29' N	12° 46'N and 13° 58'N, Av. = 12° 77' N	12° 54'N and 13° 53'N, Av. = 12° 78'N
Longitude	77° 05' E and 78° E, Av = 77° 38' E	77° 34' E and 77° 57' E Av. = 77° 45' E	77° 35' E and 77° 52' E, Av =77° 43' E	77° 21' E and 78° 35' E, Av=77° 77'E	75° 04'E and 76° 21' E, Av=75° 37' E
Population (2011 census)	0.98 Million	0.98 Million	1.25 Million	1.54 Million	1.08 Million
Population density	441 inhabitants per square kilometer	4378 inhabitants per square kilometer	298 inhabitants per square kilometer	384 inhabitants per square	303 inhabitants per square kilometer
Population growth rate (over the decade 2001-2011):	16.02%	46.68%	9.17%	11.04%	5.06%
Rainfall	790 mm	790 mm	750.4 mm	760 mm	854mm
Temperature range	Min: 15℃ Max: 34℃	Min: 15℃ Max: 34℃	Min: 14℃ Max: 34℃	Min : 14℃ Max: 40℃	Min: 14℃ Max: 31℃

Sampling procedure transportation and storage

Randomly selected vegetable sample brinjal collected from the growing areas and headquarters of five districts (Including major markets of Bangalore urban). Samples of marketable size (1kg each) were collected and transported to the laboratory in freshplastic bags and kept in refrigerator (5℃) until analysis in order to avoid any degradation of residues between sampling and analysis. Only the edible parts of vegetables were processed for residue analysis.

Questionnaire survey

Field study was carried out through questionnaire so as to collect the information on awareness level of farmers regarding pesticide use, type and amount of pesticide they were using, their knowledge regarding the safety measures to be undertaken during and after pesticide.Twenty five farmers were interviewed. On the basis of these survey results, different pesticides that they were used in their farm arerecognized and were selected for the laboratory analysis of the collected samples to study their residue level. Some banned pesticides were also considered for analysis.

Standard solutions

Pesticide standard stock solutionswereprocured from Indian Agricultural Research Institute (IARI), New Delhi.Working standard solutions containing a mixture of the analyte were prepared from the stock by appropriate solvent dilutions in n-hexane.

For standards preparation, the required amount of pesticide standards were mixed with required volume of n-hexane (HPLC grade).The stock solutions, 100ppm of each pesticide were prepared, labeled and stored in airtight clean bottles. From this, a single mix standard of 100ppm was prepared, which was diluted firstly to 10ppm and then to 1ppm. The 1ppm mix standard was used to make the calibration standards of 0.01, 0.5 and 1.0ppm. In this way, a series of calibration standards ranging from 1.0 to 0.01ppm was prepared. The single and mixed stock solutions were stored at -5^0C while, the calibration standards were made on the day of analysis. The calibration standards so prepared (1µl) were injected to GC and analyzed.

Reagents

All solvents like n-hexane, acetonitrile, petroleum ether and diethyl ether (HPLC grade) were procured from Sigma Aldrich Co., and were glass distilled before use. AR grade sodium chloride (NaCl) and anhydrous sodium sulphate (Na_2SO_4)was procured from HIMEDIA Pvt. Ltd., India. Before use, anhydrous sodium sulphate (Na_2SO_4) was purified with acetone and heated for 4hr at 400^0Cin a muffle furnace to remove possible phthalate impurities. Florosil(60-100 mesh) purchased from Merck India limited was activated at 450^0C and reheated at 130^0C for 5hr before use.

Pesticide analysis

During this investigation, residues of insecticides in vegetablebrinjalmonitored in vegetable samples from five districts of Karnataka using Gas Chromatograph with ECD and FTD (Shimadzu make, Model GC-2010). Preparation of the samples and determination of insecticide residues was based on the method described by AOAC (2000).

Pesticide residue analysis involves three steps: Extraction, Cleanup and Analysis. The samples of vegetables were extracted by following acetonitrile extraction / petroleum ether partitioning and cleaned up by column chromatography using florisil adsorbent. Efficiency of the method was validated with recovery.

Extraction

The most suitable approach in the determination of the pesticide residue contents in food samples is sample preparation method (Extraction and Cleanup) with various chromatographic methods. Sample preparation is often a neglected area, which over the years has received much less attention and research than the chromatographic separation or detection stages. The most efficient approach to pesticide analysis involves the use of multiclass, multi-residue methods (MRMs). The first notable MRM was the Mills method developed in the 1960s for the determination of non-polar organochlorine pesticides in non-fatty food. The Mills method was based on acetonitrile extraction, the extract was then diluted with water, and the pesticides were partitioned into a non-polar solvent. The follow-up research was oriented towards extending the analytical polarity range to cover wider range of polarity of pesticides analyzed in a single procedure. New solvents for initial extraction and addition of NaCl for the partitioning step were tested to reach higher recoveries of the more polar analytes. In 80s environmental and health concerns led to the avoidance of dangerous solvents, later Solid-Phase Extraction (SPE) was established to avoid Liquid-Liquid Partitioning (LLP) and as a cleanup step. Increased urgency to further reduce solvent usage and manual labor led to the introduction of several alternative extraction approaches Accelerated Solvent Extraction/Pressurized Liquid Extraction (ASE/PLE),Gel Permeation Chromatography (GPC), Matrix Solid-Phase Dispersion (MSPD), Microwave-Assisted Extraction (MAE), Solid-Phase Extraction (SPE) / Dispersive Solid-Phase Extraction (DSPE), Solid-Phase Micro Extraction (SPME),Stir-Bar Sorptive Extraction (SBSE), Supercritical Fluid Extraction (SFE). Anastassiades*et al.*,(2003) developed quick, easy, cheap, effective, rugged and safe method (QuEChERS) which aimed to overcome critical deficiencies and practical limitations of existing methods.

In the present study, only the edible parts of vegetable samples (1.0kg) werechopped and 50gof samples were extracted in a warring blender with 100 ml acetonitrile for 2-3min. The solvent was filtered through a Buchner funnel. The fruit residue was again subjected to extraction with 50ml acetonitrile two more times. The extracts were evaporated under vacuum to about 5ml and then transferred to a separator funnel of capacity 1000 ml. 600 ml of 5% sodium chloride was added and the extract was exchanged into petroleum ether layer by liquid-liquid partitioning thrice (100ml, 2 × 50ml). The extract was then passed through a layer of sodium sulfate (5g) and evaporated to dryness in a rotary evaporator at a temperature below 40℃.

Clean up

Glass column (60cm length × 2.0cm I.D) was packed with a mixture of florisil(10g), anhydrous sodiumsulphate(10g) and activated charcoal (0.2g) supported on a cotton plug was used for cleanup and the sample was wetted with 50ml petroleum ether. Sample slurry prepared using petroleum etherwas transferred to the column. The glass beaker containing extract was rinsed with acetone and was transferred to the column, which was allowed to stand for 45min. Subsequently, the petroleum ether present in the column was eluted drop-wise (5ml/min). When about 5ml petroleum ether remained on the surface of the adsorbent, the extract was eluted with 200ml each of freshly prepared6% solvent mixture (diethyl ether in petroleum ether), 15% solvent mixture (diethyl ether in petroleum ether) and 50% solvent mixture successively. The eluents were concentrated to dryness in a rotary evaporator under vacuum and diluted to 10ml with n-hexane for further analysis. From the dissolved residues, 1µl was injected to gas chromatograph and peak areas were compared with those obtained from similar injections of standards.

Pesticide residue analysis

The pesticide residue analysis was performed on Gas Chromatograph GC-2010 (Shimadzu make)equipped with ECD (Electron Capture Detector) and FTD (Flame Thermionic Detector). A fused silica capillary column (BP5- 5% Phenyl, 95%Dimethylpolysiloxane) was used for the analysis.

Insecticides like organochlorines (OCs) and pyrethroids (SPs) were analyzed using ECD (63Ni) and a capillary column BP-5 (60m × 0.25mm I.D. × 0.25µm film thickness) with split ratio 1:10. Nitrogen flow rate of 30ml/min, injection port temperature of 250^0C and temperature of detector of 300^0C and an injection volume of 1µl were the Gas - Liquid Chromatography(GLC) working conditions maintained during the analyses. The column temperature was initially maintained at 80^0C for 5min and then slowly increased to 260^0Cat the rate of 10^0Cper minfor 5min and finally increased to 290^0C for 5min.In contrast, organophosphates (OPs) residues were analyzed with FTD and a split less capillary column DB-1 (10m × 0.53mm I.D. × 2.65µm film thickness). The GLC working conditions maintained during the analyses were nitrogen flow rate of 60ml/min, hydrogen flow rate of 3ml/ min, air flow rate of 150ml/min, injection port temperature of 280^0C, detector temperature of 300^0C and an injection volume of 1µl with split ratio of 1:10.The column temperature was initially maintained at 180^0C for 5min and then gradually increased to 260^0C for 5min.

Estimation/ Quantification of residues

The carrier gas obtained from a steel gas cylinder passes through a flow regulator for the adjusted flow rate and enters into the sample injector. A little amount of the sample is introduced into the sample injector with the help of a hypodermic syringe. The sample injector is maintained at a temperature higher than the boiling point of the highest boiling component of sample in order to ensure rapid vaporization of the liquid samples. The carrier gas entering the sample injector sweeps off the vaporized sample and passes down the thermostat or temperature programmed column. The components of the sample are distributed between the stationary and the mobile phases and pass down the column at different rates. This results in the separation of the components of the sample. The carrier gas with the separated components enters the detector, which measure the change in composition of the carrier gas as it passes through it. This change is amplified before it is fed into a recorder which drives the recording pen on a moving strip of paper and a chromatogram is obtained. Currently rapid instrumental methods are available for data processing and obtaining chromatograms in computer compatible formats.

The pesticide residue concentration was calculated using the equation,

$$\text{Residues value } (\mu g/g) = \frac{A_s \times V_{std} \times C_{std} \times D_f}{A_{std} \times V_s \times W_s}$$

Where

A_s = peak area of sample injected (mv)
A_{std} =peak area of standard injected (mv)
V_s= volume of sample injected (µl/ml)
V_{std}= volume of standard injected (µl/ml)
C_{std}= concentration of standard (µg/ml)
W_s= weight of sample taken (g)
D_f= dilution factor (ml)

Among the quantification of the targeted 20 residuesnine areorganochlorines(viz., aldrin, dieldrin, endosulfan-α, endosulfan-β, endosulfansulphate, HCH-α, HCH-β, HCH-γ, heptachlor),six are organophosphorus (i.e., acephate, chlorpyriphos, dichlorvos (DDVP), monocrotophos, phorate, profenophos) and five aresynthetic pyrethroids (cyfluthrin-β, cyhalothrin-λ, cypermethrin, deltamethrin, fenvalerate). By injecting 1µl of the standard solution or the cleaned up extract into the GC, retention times (RT) and peak areas of analytes were

recorded. Residueswere estimated by comparison of peak heights/peak areas of the standards with that of the unknown or spiked samples run under similar conditions. Efficiency of the method was validated with recovery.

Fortification/ Recovery studies

The recoverystudies forthree replicates for each pesticide at three different fortification levels (1.0, 0.5 and 0.01mg/kg) were carried out. For this purpose, vegetable samples were spiked with 1ml of desired concentration of pesticide. Resulting samples were mixed and allowed to stand for 30 min before extraction and then processed separately as per the methodology described above. The amount of pesticide residues in vegetable samples were calculated by measuring peak areas from extracted current profiles and comparing with those obtained from matrix-matched standards of a concentration similar to that of samples. Spiked samples were calculated in the same way as regular samples (Harry *et al*., 1993; Michel *et al*., 2003; Anna *et al*., 2004).

Calculation of Percentage Recovery

The percentage recovery was calculated using the formula

$$\% \text{ Recovery } = \frac{\text{Amount recovered}}{\text{Amount spiked}} \times 100$$

Maximum Residue Levels (MRLs)

Maximum residue levels may be defined as the maximum levels of pesticide residue present in or on a produce when pesticide used under supervision following good agricultural practices (GAP) (Table-2). According to Environmental Protection Agency (EPA), it is the concentration of a pesticide residue that can remain in food and feed products, or commodities. Itis also known as 'pesticide residue limits' or tolerances, which are set to protect human from harmful levels of pesticides in food. Food and Drug Administration (FDA) and the United States Department of Agriculture monitor the food in interstate commerce to ensure that these limits are not exceeded.MRL is primarily intended to be used in international trade. MRL is the maximum concentration of a pesticide residue (expressed as mg/kg), recommended by the Codex Alimentarious Commission (CAC) to be legally permitted in food commodities and animal feeds. MRL are based on GAP data and foods derived from commodities that comply with the respective MRL are intended to be toxicologically acceptable.

Table 2: MRLs /Tolerance limits (mg/kg)

Sl. no.	Pesticides	MRL (mg/kg) for Vs and Fs	Reference	MRL (mg/kg) for Vs and Fs	Reference
1.	Acephate	2.0	Agnihotri NP(1999)	-----	Codex Alimentarius (2008, 2009)
2.	Cyfluthrin-β	NA	-----	3.0	
3.	Cyhalothrin-λ	0.2	Agnihotri.NP(1999)	1.0	
4.	Cypermethrin	0.2	Agnihotri.NP(1999)	1.0	
5.	Cypermethrin	0.5	FAO/WHO-1996	---	
6.	Deltamethrin	0.2	FAO/WHO-1996	1.0	
7.	Fenvalerate	2	Agnihotri NP(1999)	1.0	
8.	Fenvalerate	0.2-1.0	FAO/WHO-1996	----	
Note: VsandFs= vegetablesand fruits					

Pesticide residues in brinjal

Variation in acephate, chlorpyriphos, dichlorvos, monocrotophos, phorate, cyfluthrin-β, cyhalothrin-λ, cypermethrin, deltamethrin and fenvalerate residues in brinjal samples are deliberated below:

Acephateresidues

In brinjal samples from Bangalore rural, the concentration of acephate residue varied from 0231 to 0.481mg/kg (mean = 0.138mg/kg), 0.116 to 0.512 mg/kg (mean = 0.12mg/kg), 0.321 to 0.512mg/kg (mean = 0.165mg/kg), 0.011 to 0.513mg/Kg (mean = 0.121mg/kg) and 0.371 to 0.416mg/Kg (mean = 0.148mg/kg) respectively for Bangalore rural, Bangalore urban, Chikkaballapura, Kolar and Ramanagara districts. Among the samples collected from five districts, 37.5% of samples showed contamination with acephate and none of the samples crossed the MRL value of 2.0mg/kg. The trend of mean concentration of acephate residue in different districts is Chikkaballapura>Ramanagara> Bangalore rural >Kolar >Bangalore urban (Table-3).

Table 3: Pesticide residues (mg/kg) in brinjal samples

Sl. No.	Pesticides	Residue range (mg/kg)																			
		Bangalore rural (n=8)				Bangalore urban (n=8)				Chikkaballapura (n=8)				Kolar (n=8)				Ramanagara (n =8)			
		a(b)	min	max	mean	a(b)	min	max	mean	a(b)	min	max	mean	a(b)	min	Max	mean	a(b)	min	max	mean
1	Acephate	3(37.5)	0.231	0.481	0.138	3(37.5)	0.116	0.512	0.12	3(37.5)	0.321.	0.512	0.165	3(37.5)	0.011	0.513	0.121	3(37.5)	0.371	0.416	0.148
2	Chlorpyriphos	2(25)	0.072	0.083	0.019	2(25)	0.041	0.088	0.016	2(25)	0.071	0.085	0.019	2(25)	0.012	0.086	0.012	BDL	BDL	BDL	BDL
3	Dichlorvos (DDVP)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
4	Monocrotophos	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
5	Phorate	1(12.5)	BDL	0.042	0.005	3(37.5)	0.022	0.058	0.016	3(37.5)	0.021	0.034	0.01	2(25)	0.032	0.045	0.038	2(25)	0.026	0.075	0.013
6	Cyfluthrin-β	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2(25)	0.102	0.106	0.015	BDL	BDL	BDL	BDL
7	Cyhalothrin-ʎ	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
8	Cypermethrin	2(25)	0.011	0.036	0.006	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2(25)	0.017	0.047	0.008	BDL	BDL	BDL	BDL
9	Delta methrin	2(25)	0.361	0.387	0.094	2(25)	0.226	0.368	0.074	BDL	BDL	BDL	BDL	2(25)	0.293	0.321	0.077	2(25)	0.312	0.491	0.1
10	Fenvalerate	2(25)	0.011	0.022	0.004	BDL	BDL	BDL	BDL	1(12.5)	BDL	0.023	0.003	2(25)	0.011	0.024	0.004	1(12.5)	BDL	0.024	0.003

Note: BDL= Belowdetectionlimit, n = No. ofsamplesanalyzed, a = Contaminated, b = % of contamination

Chlorpyriphosresidues

The concentration of chlorpyriphos residue in the brinjal samples for Bangalore rural, Bangalore urban, Chikkaballapura and Kolar districts respectively ranged from 0.072 to 0.083mg/kg (mean = 0.019mg/kg), 0.041 to 0.088mg/kg (mean 0.016mg/kg), 0.071 to 0.085mg/kg (mean = 0.019mg/kg) and 0.012 to 0.086mg/kg (mean = 0.012mg/kg) with 25% of samples each from all the four districts contaminated with chlorpyriphos. In contrast, the concentration of chlorpyriphos residue in brinjal collected from Ramanagara district is below detectable level. None of samples had chlorpyriphos concentration crossing the MRL value of 0.2mg/kg. The trend of mean concentration of chlorpyriphos residue in brinjal in different districts is Bangalore rural=Chikkaballapura>Bangalore urban >Kolar>Ramanagara.

Phorate residues

In Bangalore rural, the concentration of phorate residue in brinjalsamples varied fromBDL to 0.042mg/kg (mean = 0.005mg/kg) with 12.5% of samples being contaminated. In Chikkaballapura district, it ranged from 0.021 to 0.034mg/kg (mean = 0.01mg/kg) having 37.5% of samples contamination while in Kolar, it ranged from 0.032 to 0.045 mg/kg (mean = 0.038mg/kg) and 25% of samples found to be contaminated with phorate. None of samples in all these three districts showed phorate residue concentration crossing the MRL of 0.05mg/kg. In Bangalore urban and Ramanagara samples, it respectively varied from 0.022 to 0.058mg/kg (mean = 0.016mg/kg) and 0.026 to 0.075mg/kg (mean = 0.013mg/kg) and their respective sample contamination accounted for 37.5% and 25%. It is also found that 12.5% of samples each fromBangalore urban and Ramanagara districtswere having phorate residue value above the MRL of 0.05mg/kg. The trend of mean concentration of phorate residue in brinjal in different districts is Kolar > Bangalore urban >Ramanagara>Chikkaballapura> Bangalore rural.

Cyfluthrin-β residues

The cyfluthrin-β residue is not detected in the brinjalsamples collected from Bangalore rural, Bangalore urban, Chikkaballapura and Ramanagara districts. In contrast, it ranged from 0.102 to 0.106mg/kg (mean = 0.015mg/kg) in the samples collected from Kolar. It is found that 25% of samples are contaminated with Cyfluthrin-β and none had residue values above the MRL of 3.0mg/kg.

Cypermethrinresidues

Cypermethrinresidues in brinjal samples are found to be range from 0.011 to 0.036mg/kg (mean = 0.006mg/kg) and 0.017 to 0.047mg/kg (mean = 0.008mg/kg) respectively in the samples collected from Bangalore rural and Kolar districts with nearly 25% of samples being contaminated. None of samples contained residue above the MRL value of 0.2mg/kg. Also, cypermethrin residues are not detected in Bangalore urban, Chikkaballapura and Ramanagara districts. The mean concentration of cypermethrin residue in brinjalof Kolar is more than that of Bangalore rural.

Deltamethrin residues

In the brinjalsamples collected from Bangalore rural, Bangalore urban, Kolar and Ramanagara districts, the deltamethrin residue values respectively ranged from 0.361 to 0.387mg/kg (mean = 0.094mg/kg), 0.226 to 0.368mg/kg (mean = 0.074mg/kg), 0.293 to 0.321mg/kg (mean = 0.077mg/kg) and 0.312 to 0.491mg/kg (mean = 0.1mg/kg). 25% of total samples collected are contaminated with deltamethrin, but none crossed the MRL value of 0.2mg/kg. While in the samples from Chikkaballapura district, the concentration of the residue in brinjalis below detectable level. The trend of mean concentration of deltamethrin residue in brinjal in different districts is Bangalore urban>Ramanagara>Bangalore rural>Kolar >Chikkaballapura.

Fenvalerateresidues

In Bangalore rural, Chikkaballapura and Kolar samples, the concentration of fenvalerate in brinjalsamplesvaried from 0.011 to 0.022mg/kg (mean = 0.004mg/kg), from BDL to 0.0.023mg/kg (mean = 0.003mg/kg) and 0.011 to 0.024mg/kg (mean = 0.004mg/kg) respectively, with 25% of samples each having fenvalerate contamination. In contrast, it is BDL to 0.024mg/kg (mean = 0.003mg/kg) with 12.5% of samples contamination in Ramanagara district. None of samples from the three districts had fenvalerate residue crossing the MRL value of 2.0mg/kg. The fenvalerate residue is not detected in brinjal from Bangalore urban and Chikkaballapura districts.

Dichlorvos, monocrotophos and cyhalothrin-λ residues

The dichlorvos, monocrotophos and cyhalothrin-λ residuesare below the detectable level in brinjalsamplesfrom all the five districts.

IV. Discussion

Recovery studies

In order to check the authenticity of the experimental procedure followed for extraction of different pesticides from vegetable samples, recovery studies were performed. All the targeted pesticides (aldrin, dieldrin, endosulfan-α endosulfan-β, endosulfansulphate, HCH-α, HCH-β, HCH-γ, heptachlor, acephate, chlorpyriphos, dichlorvos(DDVP), monocrotophos, phorate, profenofos, cyfluthrin-β, cyhalothrin-λ, cypermethrin, delta methrin and fenvalerate) were spiked at 0.01, 0.5 and 1.0 mg/kg onBrinjal (Table-4).The average recovery rates ranged from 77.3 to 94.7% in brinjal.The variation in the recovery is not always possible to predict accurately but may be due to complete evaporation of the solvents on the rotary evaporator,oxidation due to solvent evaporation through direct air anddegradation of pesticides(Wheeler *et al.* 1983).Our findings are corroborated by the earlier works listed. BeenaKumari*et al.,* (2002)reported the percent recoveries for OC, OP, SP and carbamate respectively varied from 80–111, 83–125, 73–95 and 82–104% at spiking levels of OC (0.01–0.1ppm), SP (0.25ppm), OP (0.25–0.5ppm) and carbamates (0.5 –1ppm). Darinka and Lucija (2003)reported for 90 pesticides in fruits from Slovenia in the concentration range of 0.01 to 0.50mg/kg, with the pesticide recoveries greater than 80%. Patel *et al.,* (2004) obtained average recoveries of 70 and 116% for pesticides spiked at 0.01mg/kg and 0.1mg/kg respectively and their associated relative standard deviation (RSD) values was less than 20%.Martinez *et al.,* (2005) determined residues of twenty pesticides in eight commodities (cucumber, tomato, pepper, green bean, eggplant, zucchini, melon and watermelon) of Spain at a recovery rate of 65–115% and RSD value of 4-16%.PadalaVenkateswarlu*et al*., (2007) obtained mean recoveries of 78-104% at fortification levels of 0.010 - 0.100mg/kg and their relative standard deviations (RSDs) as ≤15%. Abhilash *et al.,* (2009)reported the recoveries of pesticides in vegetable samples ranged from 93% to 103% and RSD between 5% and 10%.Seyed and Somashekar (2010)recorded an average recoveryof 73.5 to 83.5% for residues like α-cypermethrin and fenvalerate in grapes. Doyeli*et al.,* (2011)reported mean recoveries of pesticides in spinach and eggplant in the range of 70-120% and RSD less than 10%.Gouri*et al.,* (2011) reported recoveries of pesticides in fruits and vegetables which ranged between 72 and 114% with a RSD < 20%.

Pesticide residue in brinjal:

In the presentinvestigation,the concentration of deltamethrin residuein the range of0.024-0.668mg/kg with maximum mean concentration(viz., 0.112mg/kg) was present in brinjal samples collected from Bangalore urban district and lowest mean concentration (0.007mg/kg) in the capsicum samples from Ramanagara district. Delta-methrin residuewasbelow detectable level in 67.5% of the total vegetable samplesanalysed and none of the samples showed mean concentration of deltamethrinexceeding the MRL value of 0.2mg/kgfrom five districts. According to a study by BeenaKumari*et al.,* (2003)deltamethrin residue concentration ranging from 0.002 – 0.051mg/kg in cabbage, cauliflower, pea grains, brinjal, tomato, potato and green chilly samples collected from whole sale markets of Hisar, Haryana, but well below the MRL values in all the vegetables was recorded. The results of the present investigation are higher than the findings of a similar survey conducted by BeenaKumari and Kathpal (2009)whoreported 68% contamination in vegetarian diet samples with deltamethrin residue (0.008 -0.102mg/kg), represented by samples from homes, hostels and hotels from Hisar, Haryana. The

levels of deltamethrin varied between 0.007- 0.010mg/kg (mean: 0.008mg/kg) in pear and 0.026-0.062mg/kg (mean: 0.044mg/kg) in pineapple samples collected from markets in Kumasi(Crentsil Kofi Bempah*et al.,* 2011).

Table 4: Average recoveries and RSDs % of different insecticides from three samples of be brinjal at fortification levels of 1.0, 0.5, 0.01 mg/kg

Sl No	Pesticide groups	Name of the pesticide	Mean recovery (R.S.D) Brinjal (n=3) Level of fortification (mg/kg)		
			1	**0.5**	**0.01**
1	OCPs	**Aldrin**	85.3(4.4)	86.4(3.6)	91.1(3.3)
2		Dieldrin	83.9(3)	97.5(1.8)	84.3(3.5)
3		Endosulfan-α	81(3.3)	93.7(1.7)	76.9(3.6)
4		Endosulfan-β	83.7(4.9)	92.9(6)	81.1(2.7)
5		Endosulfan-Sulphate	83.7(5.3)	96.2(1.5)	88.5(5.6)
6		HCH-α	84.1(3.8)	90.1(3.2)	95.8(1.3)
7		HCH-β	83.7(1.2)	81.6(1.3)	85.1(4.6)
8		HCH-γ	82.8(2.2)	94.9(4.1)	76.1(1.4)
9		Heptachlor	83.9(4.4)	96(0.8)	83.6(3.7)
10	OPPs	**Acephate**	80.4(1.9)	89.4(6.6)	82.9(2.9)
11		Chlorpyriphos	84.6(3.1)	91.6(7.5)	84.5(5.4)
12		Dichlorvos	88.7(7.3)	92.2(2.3)	92.4(4.8)
13		Monocrotophos	89.1(2.8)	88.3(4.9)	90.3(3)
14		Phorate	83.4(4.7)	92.7(1.4)	84.5(5.9)
15		Profenofos	80(2.8)	88.5(2.7)	80.3(3.1)
16	SPs	**Cyfluthrin-β**	86.1(3.5)	89.9(9.6)	83(3.7)
17		Cyhalothrin	87.2(3.9)	82.6(2.2)	85.8(1.5)
18		Cypermethrin	86.2(3.6)	83.4(3.4)	86.2(2.6)
19		Deltamethrin	90.9(3.3)	92.1(1.4)	94.5(3.7)
20		Fenvalerate	74.3(1.6)	90.3(1.4)	77.2(2.6)

V. Recommendation:

Following are the recommendations made keeping in mind sample contamination rates and type of pesticides being used in the study area. It is necessary that these recommendations are addressed from time to time for improvement(AnandaGowda*et al*., 2017).

- Regular monitoring of the vicinity should be encouraged to avoid possible consumption of contaminated foodstuff.
- For accurate and rapid analysis of pesticide residues, standard methods must be developed.
- Farmers should be educated and encouraged not to use higher dosage of pesticides. In order to avoid residue problems, rotation of pesticides to combat the pests and diseases are recommended.
- A regular training/workshop on the use and safety measures to be followed should be imparted to farmers, retailers, distributors, consumers policy makers and other stake holders.
- Multimedia awareness activities in local language should be massively conducted on the dangers posed by pesticides contamination in the food.

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