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MONITORING OF PESTICIDE RESIDUES IN FARMGATE SAMPLES OF VEGETABLES IN KARNATAKA, INDIA

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ABSTRACT
Fifty Vegetable samples in Ramanagara district of Karnataka, India were analysed for 20 pesticide residues. Vegetable samples were extracted with acetonitrle and the pesticides are partitioned into petroleum ether and evaluated by gas liquid chromatography equipped with ECD and FTD. Recovery studies were performed at 0.1, 0.5 and 1.0 mg kg^{-1} fortification levels of each compound and the recoveries obtained ranged from 80.5% to 96.2% with relative standard deviations lower than 7%. The method showed good linearity over the range assessed 0.05-1.5mg kg^{-1} with correlation coefficient> 0.998 and the detection and quantification limits for the pesticides studied varied from 0.0001 to 0.044 mg $kg^{-1}$and 0.0005 to 0.0155 mg kg^{-1} , respectively. All the samples were found to be contaminated; the organo chlorines (83.5%) dominated followed by organophosphates (67%) and pyrethroids (55%). However, 34% of the samples were found to contain the residues of organophosphate insecticides above their respective maximum residue limits (MRL). It is therefore proposed to perform extensive monitoring studies covering all the vegetable crops from different agro-climatic regions of the state to know the exact status of pesticide contamination.

KEYWORDS: Monitoring, vegetables, Maximum residue limit (MRL), pesticides

INTRODUCTION

Vegetables exhibit a key role in the diet for maintenance of health and prevention of disease in the Indian sub continent. The total Indian meal constitutes about 150-250 g of vegetables per day (Mukherjee and Gopal, 2003) . A wide range of pesticides are used for the production of fruits and vegetables in India, due to heavy pest infestation throughout the cropping season of horticultural crops. Cauliflower (*Brassica oleracea var.botrytis*), capsicum *(Capsicum annuum* L.), okra (*Hibiscus esculenta* L.) and tomato (*Lycopersicon esculentum* L.) are the important vegetable crops of India. These are widely cultivated throughout the sub-tropical parts of south India. These crops are attacked by a number of insect pests that damage crop at various stages of growth. It is well established that repeated application of pesticides may lead to undesirable residues on consumable parts of vegetables (Agnihotri, 1999). Also, several potential hazards associated with the consumption of pesticide treated crops and the consequent impacts are known. Therefore, the sensible suggestion of a pesticide requires that it must not only provide an effective control of pests, but at the same time its residues on the commodity must also be toxicologically acceptable. These residues, when present in excessive, may prove unsafe to the health of the consumers. Chahal *et al.,* (1997 and 1999) opined surprisingly that residues of different insecticides, including those which are not recommended for use on vegetables were invariably identified. The analysis of farm gate vegetables by various workers in India have revealed contamination mostly with organo Phosphates (OPs) and synthetic pyrethroids (SPs), which denotes a clear changes in the usage pattern from organochlorines (OCs)to other groups. (Mukherjee and Gopal 1996; Madan *et al.,* 1996; Parihar *et al.,* 1997; Shah *et al.,* 2000; Kole *et al.,* 2002; Kumari *et al.,* 2003; Deka *et al.,* 2005; Battu and Joia, 2006) . An indispensable requisite to ensure quality and reliability of the results in chemical analysis is method validation. The analyst must produce information to demonstrate that a method intended for this purpose is capable of offering adequate specificity, accuracy and precision, at relevant analyte concentrations and in appropriate matrices. (Hill and Reynolds, 1999; Paschoal *et al.,* 2008). The aim of this study was to determine 20 pesticide residues related to Organophosphates, Organochlorines, and Synthetic Pyrethroids in 50 vegetable samples. Good sensitivity and selectivity of the method were obtained with the limits of quantification 0.0008 mg kg^{-1} in almost of all the cases. This method is reliable, simple, sensitive, accurate and precise and hence can be applied for routine analysis of vegetables and fruits.

MATERIALS AND METHODS

Chemicals

All of the glassware was rinsed with acetone and dried in an oven at around 350 ℃ prior to use. 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. Sodium chloride (NaCl) and anhydrous sodium sulfate (Na_2SO_4), AR grade was procured from Himedia Pvt. Ltd. India. Before use, anhydrous sodium sulfate (Na_2SO_4) was purified with acetone and heated for 4 h at 400℃ in muffle furnace to remove possible phthalate impurities. Florisil, 60-100 mesh, purchased from Merck India limited was

activated at 450 ℃ and reheated at 130 ℃ for 5 h before use. The Pesticide Standards were procured from All India Network Project on pesticide residues, Division Of Agricultural Chemicals, Indian Agricultural Research Institute (IARI) Delhi, India.

Preparation of Standard Solutions

An accurately weighed 10 mg amount of an individual analytical grade pesticide was dissolved in a 10 ml volumetric flask using n-hexane to prepare the standard stock solution to 1000 mg kg^{-1}. The standard stock solution of each pesticide was serially diluted to obtain intermediate lower concentration of 100 mg kg^{-1}. A mixture of standard stock pesticide solution was prepared by taking 0.1m1(100 mg kg^{-1}) solution of each compatible (acephate, aldrin, chlorpyriphos, cyfluthrin-β, cyhalothrin, cypermethrin, delta methrin, dichlorvos (DDVP), dieldrin, endosulfan-α,endosulfan-β, endosulfan-sulphate, fenvalerate, HCH-α, HCH-β, HCH -γ, heptachlor, monocrotophos, phorate and profenofos) pesticide in a 10 ml volumetric flask and making the volume up to the mark with n- hexane. This standard mixture contained 10 mg kg^{-1} of each pesticide. This was further diluted serially with n-hexane to obtain working standard 1.0, 0.1, and 0.01 mg kg^{-1} of solution to determine the limit of detection (LOD) of the instrument. They were stored in a refrigerator at 5℃.

Sampling

Fifty samples of marketable size vegetables (1 kg each) like cauliflower, capsicum, okra, and tomato were collected from the farms of vast vegetable growing areas of Ramanagara district of Karnataka state, India in 2011. Twelve samples of cauliflower, capsicum and okra each and fourteen tomato samples were analyzed. The samples were kept in a refrigerator (5℃) till analysis. Only the edible parts of each vegetable was processed for residue analysis. All the samples were extracted fresh. The information regarding pesticide applied to vegetable crops was collected from the farmers at the time of sampling. Composite sample consisted of 1 kg was cut into small pieces and macerated in a grinder.

Sample extraction and clean up

A representative 50 g sample of macerated vegetable was extracted with 100 ml acetonitrile in a warring blender (Super mixer grinder, Model, No.Mx-216E, National, India) for 2-3 min. The supernatant liquid was filtered through a Buchner funnel with Whatman filter paper No.1. The vegetable residue was re-extracted with 50ml acetonitrile, two more times. The extracts were pooled and transferred to a separating funnel (1000 ml). 600 ml of 5% brine solution was added and the extract was partitioned three times with petroleum ether (100+70+50ml). The aqueous phase was discarded. The combined organic layers containing the insecticide residues were drained into 500ml beaker through 5cm layer of anhydrous sodium sulphate (10g) supported on a pre washed glass wool in a funnel. The sodium sulphate was washed with an additional 25 ml of petroleum ether. The combined extracts obtained was concentrated to 5ml in a rotary evaporator at 35-40℃. The extracts so obtained were cleaned up by column chromatography using florisil adsorbent. (AOAC . 2000). Before use, the florisil was activated at 110℃ for 2 h. A glass column (60 cm x 2.0 cm i.d.) was packed with a mixture of florisil (10g), anhydrous sodium sulphate (10 g) and activated charcoal (0.2g) supported on a cotton plug was used for cleanup and the sample was wetted with 50 ml petroleum ether. A sample slurry was prepared using petroleum ether and 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 45 min. Subsequently the petroleum ether present in the column was eluted drop wise (5ml/ min). When about 5 ml petroleum ether remained on the surface of the adsorbent, the extract was eluted with 200 ml of freshly prepared 6% solvent mixture (diethyl ether in petroleum ether) , 200 ml 15% solvent mixture (diethyl ether in petroleum ether) and finally 200 ml 50% solvent mixture successively. The eluents were concentrated to dryness in a rotary evaporator under vacuum and reconstituted in 10 ml n-hexane for GC analysis.

GC Analysis

The quantification of 20 residues , 9 OCs (aldrin, dieldrin, endosulfan -α, endosulfan-β, endosulfan sulphate, HCH-α, HCH- β ,HCH- γ , heptachlor), 6 Ops (acephate, chlorpyriphos, dichlorvos (DDVP), monocrotophos, phorate, profenofos), 5 SPs (cyfluthrin - β, cyhalothrin - λ, cypermethrin, delta methrin, fenvalerate) were carried out with a gas liquid chromatography (GLC) (Shimadzu Model GC-2010) equipped with an electron-capture detector (ECD) and flame thermionic detector (FTD). Organochlorines (OCs) and pyrethroids (SPs) insecticides were analysed using ECD (63Ni) and a capillary column BP-5 (60 m x 0.25 mm i.d. x 0.25 μm film thickness) with split ratio 1:10. The GLC working conditions were as follows: nitrogen flow rate, 30 ml min-1; injection port, 250℃; and detector, 300℃. The column temperature was initially maintained at 80 ℃ for 5 min, then increased to 260℃ at the rate of 10℃ min-1, for 5min and finally increased to 290℃ for 5min.The injection volume was 1μl. Organophosphates (OPs) insecticides were analysed with FTD and a splitless capillary column DB-5 (60 m x 0.25 mm i.d. x 0.25 μm film thickness). The GLC working conditions involved : nitrogen flow rate, 60 ml min-1; hydrogen flow rate, 3 ml min^{-1}; air flow rate, 150 ml min^{-1}; injection port temperature: 280℃; and detector temperature: 300℃; The column temperature was initially maintained at 180℃ for 5 min, then increased gradually to 260℃ for 5min. The injection volume was 1μl with split ratio of 1:10. Residues were estimated by comparison of peak heights/ peak areas of the standards with that of the unknown or spiked samples run under similar conditions.

Method Validatation

Efficiency of the method was validated with recovery, repeatability, specificity, linearity, LOD and LOQ.

1. Recovery: The accuracy of an analytical method is the closeness of experimental results obtained by that method to true value. The accuracy of the method was estimated through recovery experiment.

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TABLE 1: Average recoveries and R.S.Ds,% of different insecticides from three samples of cauliflower, capsicum, okra and tomato, fortified at 1.0,0.5 & 0.1 mg kg^{-1} Levels

		Cauli flower (n=3)			capsicum(n=3)			okra (n=3)			Tomato (n=3)		
	Insecticide(s)	Mean recovery ± R.S.D			Mean recovery ± R.S.D			Mean recovery ± R.S.D			Mean recovery ± R.S.D		
		Level of fortification(mg kg^{-1})			Level of fortification(mg kg^{-1})			Level of fortification(mg kg^{-1})			Level of fortification(mg kg^{-1})		
		1	0.5	0.1	1	0.5	0.1	1	0.5	0.1	1	0.5	0.1
1.	Aldrin	89.1(6.1)	86.5(3.6)	87.4(3.7)	90.8(3.7)	86.4(3.6)	90.8(3.7)	90.8(3.7)	86.4(3.6)	92.5(3.5)	90.8(3.7)	86.4(3.6)	89.1(6.2)
2.	Dieldrin	85.6(5.9)	95.5(2.3)	82.5(5)	85.6(5.9)	95.5(2.3)	87.1(5.2)	82.5(5)	95.5(2.3)	82.5(5)	82.5(5)	95.5(2.3)	85.6(5.9)
3.	Endosulfan-α	73(2.2)	93.5(0.9)	75.9(5.8)	73(4.5)	93.5(0.9)	75.9(5.8)	71.4(0.9)	93.5(0.9)	75.9(5.8)	73(4.5)	93.5(0.9)	75.9(5.8)
4.	Endosulfan -β	78.7(2.2)	91.8(5.7)	80.6(3.6)	78.7(2.2)	91.8(5.7)	80.6(3.6)	77.9(0.4)	91.8(5.7)	80.6(3.6)	78.7(2.2)	91.8(5.7)	80.6(3.6)
5.	Endosulfan-Sulphate	85.2(3)	96.2(1.4)	88.5(5.6)	85.2(3)	96.2(1.5)	88.5(5.6)	85.2(3)	96.2(1.4)	88.5(5.6)	85.2(3)	96.2(1.4)	88.5(5.6)
6.	HCH - α	79.9(4.5)	88.7(5.6)	80.4(3.5)	81.5(2.1)	88.7(5.6)	81.5(2.1)	79.3(5.6)	88.7(5.6)	80.4(3.5)	79.9(2.5)	88.7(5.6)	81.5(2.1)
7.	HCH - β	83.7(3.5)	81.8(1.3)	85.1(4.6)	83.7(3.5)	81.8(1.3)	85.1(4.6)	83.7(3.5)	81.8(1.3)	85.1(4.6)	83.7(3.5)	81.8(1.3)	85.1(4.6)
8.	HCH - γ	79.7(5.3)	93(2.5)	75.9(1.6)	78.4(4.9)	93(2.5)	75.9(1.6)	79.1(6.2)	93(2.5)	75.9(1.6)	79.8(5.3)	93(2.5)	75.9(1.6)
9.	Heptachlor	83.6(3.7)	95.2(0.7)	83.6(3.7)	83.6(3.7)	95.2(0.7)	83.6(3.7)	83.6(3.7)	95.2(0.7)	83.6(3.7)	85(6)	95.2(0.7)	83.6(3.7)
10.	**Acephate**	79.1(4.9)	87.9(3.9)	82.4(2.1)	79.1(4.9)	90.5(4.6)	82.4(2.1)	79.1(4.9)	90.5(4.6)	82.4(2.1)	77.7(6.7)	90.5(4.7)	82.4(2.2)
11.	Chlorpyriphos	84.5(5.3)	94.7(3.4)	84.5(5.4)	84.5(5.4)	91.2(7.4)	84.5(5.4)	84.5(5.3)	94.7(3.4)	84.5(5.3)	84.5(5.4)	92.9(4.7)	84.5(5.4)
12.	Dichlorvos (DDVP)	96.1(2.6)	92.2(2.3)	92.4(4.8)	96.1(2.6)	92.4(2.4)	92.4(4.8)	95.2(4)	92.2(2.3)	92.4(4.8)	96.1(2.6)	92.2(2.3)	92.4(4.8)
13.	Monocrotophos	88.4(1.5)	87(5)	88.4(1.5)	88.4(1.6)	87(5)	88.4(1.5)	88.4(1.5)	87(4.9)	88.4(1.5)	88.4(1.5)	86.9(5)	88.4(1.5)
14.	Phorate	84.3(6.1)	92.3(0.2)	86.3(2.2)	84.3(6.1)	92.2(0.3)	84.3(6.1)	84.3(6.1)	92.2(0.3)	84.3(6)	84.2(6.1)	92.1(0.3)	84.2(6.1)
15.	Profenofos	80.3(3.1)	88.5(2.7)	80.3(3.1)	80.3(3.1)	88.5(2.7)	80.3(3.1)	80.3(3.1)	88.5(2.7)	80.3(3.1)	80.3(3.1)	88.5(2.7)	80.3(3.1)
16.	**Cyfluthrin - β**	81.1(2.4)	86.9(5)	83(3.7)	81.1(2.4)	86.9(5)	83(3.7)	81.1(2.4)	86.9(5)	83(3.7)	81.1(2.4)	86.9(5)	83(3.7)
17.	Cyhalothrin - λ	91(5.1)	82.7(2.2)	82.7(7.8)	84.2(4.5)	82.7(2.2)	85.7(1.4)	87.2(3.9)	82.7(2.2)	85.7(1.4)	87.2(3.9)	82.7(2.2)	85.7(1.4)
18.	Cypermethrin	85.7(2)	83.4(3.4)	91.5(5.6)	85.7(2)	83.4(3.4)	89.6(4.3)	85.7(2)	83.4(3.4)	86.7(3.4)	85.7(2)	83.4(3.4)	86.2(2.6)
19.	Delta methrin	94.7(3.8)	91.6(1.7)	94.8(3.6)	94.9(3.6)	91.9(1.7)	95.1(3.4)	95.2(3.7)	91.8(1.9)	94.6(3.7)	94.5(3.7)	91.5(1.6)	91.2(7.1)
20.	Fenvalerate	89.1(6.1)	88.8(2.4)	77(2.6)	74.3(1.6)	88.8(2.4)	77(2.6)	74.3(1.6)	88.8(2.4)	77(2.6)	74.3(1.6)	88.8(2.4)	77(2.6)

TABLE 2 : Pesticides residues (mg/kg) detected in farm gate samples of cauliflower, capsicum, okra and tomato in 2011

			Cauliflower(n=12)							Capsicum(n=12)						
			No of samples		Residue Range (mg/kg)			samples		No of samples		Residue Range (mg/kg)			samples	
	Pesticides	RT (min)	a	b	min	max	Mean	MRLs	>M	a	b	min	max	Mean	MRLs	>M
1	Aldrin	20.7	5	42	0.0043	0.0541	0.0156	0.1	Nil	5	42	0.0025	0.0355	0.0201	0.1	Nil
2	Dieldrin	22.6	6	50	0.0190	0.0660	0.0428	0.1	Nil	3	25	0.0077	0.0116	0.0092	0.1	Nil
3	Endosulfan-α	22.2	4	33	0.0230	0.0470	0.034	2	Nil	3	25	0.0009	0.0067	0.0043	2	Nil
4	Endosulfan-β	23.2	2	17	0.0150	0.0460	0.0305	2	Nil	3	25	0.0035	0.0048	0.0043	2	Nil
5	Endosulfansulfate	24.4	4	33	0.0150	0.0425	0.0281	2	Nil	5	42	0.0032	0.0099	0.0053	2	Nil
6	HCH-α	17.6	6	50	0.0021	0.0092	0.0052	1	Nil	4	33	0.0035	0.0840	0.0266	1	Nil
7	HCH-β	18.4	3	25	0.0233	0.0375	0.0325	1	Nil	4	33	0.0020	0.0028	0.0024	1	Nil
8	HCH-γ	19.0	2	17	0.0172	0.0353	0.0263	1	Nil	5	42	0.0025	0.0057	0.0039	1	Nil
9	Heptachlor	19.8	3	25	0.0011	0.0098	0.006	0.05	Nil	5	42	0.0027	0.0088	0.0045	0.05	Nil
1	Acephate	14.5	3	25	0.1308	0.2113	0.165	NA	Nil	6	50	0.0160	0.3240	0.2133	NA	Nil
2	Chlorpyriphos	20.6	ND	ND	ND	ND	ND	0.01	Nil	4	42	0.1202	0.1473	0.0959	0.2	Nil
3	Dichlorvos (DDVP)	11.6	5	42	0.0133	0.0236	0.0183	0.15	Nil	5	42	0.0026	0.0144	0.0075	0.15	Nil
4	Monocrotophos	17.5	ND	ND	ND	ND	ND	0.2	Nil	ND	ND	ND	ND	ND	0.2	Nil
5	Phorate	17.6	3	25	0.1138	0.2533	0.1931	0.05	3	5	42	0.2863	0.5766	0.4253	0.05	5
6	Profenofos	22.4	3	25	0.0754	0.0868	0.0797	NA	Nil	ND	ND	ND	ND	ND	NA	Nil
1	Cyfluthrin-β	30.1	2	17	0.1130	0.2312	0.1721	NA	Nil	3	25	0.0213	0.0360	0.0268	NA	Nil
2	Cyhalothrin-λ	26.8	4	33	0.1122	0.2214	0.1828	NA	Nil	3	25	0.0031	0.0040	0.0036	NA	Nil
3	Cypermethrin	30.7	ND	ND	ND	ND	ND	NA	Nil	ND	ND	ND	ND	ND	NA	Nil
4	Delta methrin	34.5	ND	ND	ND	ND	ND	NA	Nil	3	25	0.2456	0.3237	0.2949	NA	Nil
5	Fenvalerate	33.2	ND	ND	ND	ND	ND	2	Nil	4	33	0.0072	0.1011	0.0384	NA	Nil

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Pesticides residues (mg/kg) detected in farm gate samples of cauli flower, capsicum, okra and tomato in 2011

			Okra(n=12)							Tomato(n=14)						
			No of samples		Residue Range (mg/kg)			Samples		No of samples		Residue Range (mg/kg)			Samples	
	Pesticides	RT (min)	a	b	min	max	Mean	MRLs	>M	a	b	min	max	Mean	MRLs	>M
1	Aldrin	20.7	5	42	0.0025	0.0861	0.0417	0.1	Nil	5	35.7	0.0023	0.0155	0.0076	0.1	Nil
2	Dieldrin	22.6	ND	ND	ND	ND	ND	0.1	Nil	4	28.6	0.0007	0.0025	0.0015	0.1	Nil
3	Endosulfan-α	22.2	ND	ND	ND	ND	ND	2	Nil	ND	ND	ND	ND	ND	2	Nil
4	Endosulfan-β	23.2	3	25	0.0025	0.0056	0.0042	2	Nil	ND	ND	ND	ND	ND	2	Nil
5	Endosulfansulfate	24.4	3	25	0.0035	0.0213	0.0141	2	Nil	ND	ND	ND	ND	ND	2	Nil
6	HCH-α	17.6	4	33	0.0141	0.04	0.0276	1	Nil	5	35.7	0.0031	0.0325	0.0139	1	Nil
7	HCH-β	18.4	ND	ND	ND	ND	ND	1	Nil	4	28.6	0.0005	0.0171	0.0091	1	Nil
8	HCH-γ	19.0	3	25	0.0026	0.0045	0.0033	1	Nil	6	42.8	0.0006	0.1183	0.0752	1	Nil
9	Heptachlor	19.8	3	25	0.005	0.0107	0.0077	0.05	Nil	6	42.8	0.0015	0.0069	0.0048	0.05	Nil
1	Acephate	14.5	7	58	0.053	0.7982	0.4019	NA	Nil	8	57.1	0.1531	0.2564	0.1911	NA	Nil
2	Chlorpyriphos	20.6	4	33	0.0523	0.077	0.0636	0.2	Nil	ND	ND	ND	ND	ND	0.2	Nil
3	Dichlorvos (DDVP)	11.6	4	33	0.0008	0.018	0.0095	0.15	Nil	4	28.6	0.0025	0.1688	0.0472	0.15	Nil
4	Monocrotophos	17.5	ND	ND	ND	ND	ND	0.2	Nil	2	14.2	0.0148	0.0264	0.0206	0.2	Nil
5	Phorate	17.6	4	33	0.0536	0.2144	0.1303	0.05	4	5	35.7	0.2742	0.3432	0.3087	0.05	5
6	Profenofos	22.4	ND	ND	ND	ND	ND	NA	Nil	ND	ND	ND	ND	ND	NA	Nil
1	Cyfluthrin-β	30.1	3	25	0.2367	0.5512	0.3777	NA	Nil	2	14.3	0.0447	0.0729	0.0588	NA	Nil
2	Cyhalothrin-λ	26.8	ND	ND	ND	ND	ND	NA	Nil	3	21.4	0.0007	0.2841	0.0956	NA	Nil
3	Cypermethrin	30.7	3	25	0.0509	0.358	0.2402	0.2	Nil	ND	ND	ND	ND	ND	NA	Nil
4	Delta methrin	34.5	ND	ND	ND	ND	ND	NA	Nil	2	14.3	0.2235	0.2418	0.2327	NA	Nil
5	Fenvalerate	33.2	2	17	0.0267	0.2002	0.1135	2	Nil	ND	ND	ND	ND	ND	NA	Nil

RT=Retetion time, ND=Not detected, NA=Not assigned, n= No. of samples analysed a= Contaminated, b= % of contamination, MRLs= MRLs Values(mg/kg) by PFA, >M= No. of samples >MRLs

The recovery studies for 5 replicates for each pesticide at three different fortification levels was carried out. For this purpose, blank samples (cauliflower, capsicum, okra, and tomato) were spiked with a mixture of 20 insecticides at three levels 1.0, 0.5 and 0.1 mg kg^{-1} and processed separately as per the methodology described above. Matrix- matched calibration solutions were used for all the analysis. The percentage average recoveries of 20 pesticides are in the range of 80.5 - 96.2 **(**Table 1) and revealed that the method was found accurate for all above purposes.

2. Repeatability: Precision is the degree of reproducibility or repeatability of the analytical method under normal operating conditions. It was determined in terms of relative standard deviation (RSD, in %) from recovery experiments at each fortification level for 5 replicates of each pesticide, the RSD of 20 pesticides are in the range of 0.2 -6.1%. (Table1).

3. Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix. The specificity of the method was determined by analyzing blank vegetable samples. The absence of background peaks, above a signal-to-noise ratio of 3, at the retention times of the target pesticides, showed that no interferences occurred.

4. Linearity: The linearity of an analytical method is its ability to obtain test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity of the calibration plots was studied using calibration solutions prepared in the blank /pesticide free matrix extract. Under the chromatographic conditions described above calibration plots were constructed by plotting peak area against concentration. The ECD response for all pesticides was good linear in the range of concentration studied (0.05-1.5 mg kg^{-1}).The correlation coefficients derived from linear regression were always >0.998, demonstrating its suitability for analysis.

5. Limit Of Detection (LOD) and Limit Of Quantitation(LOQ)

The limit of detection (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 3 with reference to the background noise obtained from blank sample. The limit of detection (LOD) of each pesticide, individually and in a mixture was determined by injecting standard solutions of different concentrations in duplicate to GLC. The lowest concentration of the pesticide that gave peak area five times greater than background level was considered as LOD, and the values are in the range of 0.0001 – 0.044 mg kg^{-1}.

Limit of Quantitation (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10 can be taken as LOQ of the method. The LOQ values are in the range of 0.0005-

0.015 mg kg^{-1}. The results obtained with these methods are comparable with those reported in vegetables by others (Nakamura *et al.,* 1994; Nguyen *et al.,* 2008; Tao *et al.,* 2009).

RESULTS AND DISCUSSION

A multi residue procedure was applied to monitor 20 pesticide residues by GC-ECD and FPD with sufficient sensitivity among four vegetable samples collected in 2011.The targeted twenty pesticides were detected and quantified based on calibration standard at 0.1mgkg-1 and the results of analysis (Table 2) are compared with MRL values of each pesticide fixed by Food Safety and Standards Authority of India (FSSAI) Regulations -2010. Of the twelve cauliflower samples analysed, all of them contained OCs, 67% Ops and 40% SPs. Specially 6 (50%) samples were highly contaminated with dieldrin and HCH-α while 2(17%) samples were least contaminated with Cyfluthrin-β and endosulfan-β. Residues of cypermethrin, deltamethrin , chlorpyriphos , fenvalerate and monocrotophos were not detected. Residue levels of phorate was high (0.2533mgkg^{-1}) followed by Cyfluthrin-β (0.2312mg/kg) while heptachlor was least (0.0011mgkg^{-1}). Three (25%) out of 12 samples, contained residues of phorate (MRL value = 0.05, 0.1138 – 0.2533mg kg^{-1}) above MRL values.(Table 2). All the twelve Capsicum samples were contaminated with OCs, 67% with OPs and 60% with SPs. Six (50%) samples were highly contaminated with acephate while 3 (25%) samples were least contaminated with endosulfan-α, endosulfan-β, cyhalothrin-λ, cyfluthrin-β, delta methrin and dieldrin,. Residues of monocrotophos, profenofos and cypermethrin were not detected. Residue levels of phorate was high (0.2863 – 0.5766 mgkg^{-1}) while endosulfan-α was least (0.0009mgkg^{-1}). Of the 12 samples of Capsicum, five (41.6%) samples contained phorate (MRL value =0.05, 0.2863 – 0.5766 mgkg^{-1}) residues exceeded MRL values.

Among 12samples of Okra analysed, OCs, OPs and SPs were present in 67%, 67% and 60% samples respectively. While 7(58%) samples were highly contaminated with acephate and 2 (17%) samples were least contaminated with Fenvalerate. Residues of dieldrin, endosulfan-α, HCH-β, monocrotophos, profenofos, cyhalothrin-λ, and delta methrin were not detected. Residue levels of acephate was high (0.7982 mgkg^{-1}) while Dichlorvos (DDVP), was least (0.0008 mgkg^{-1}). Out of 12 samples of Okra, four contained residues of phorate (MRL value =0.05, 0.0536-0.2144) values are above tolerance levels. In 14 samples of Tomato analysed , 67% contained OCs, 67% OPs and 60% SPs. Eight (57%) samples were highly contaminated with acephate while 2 (14%) samples were least contaminated with monocrotophos, cyfluthrin-β, Endosulfan-α and delta methrin. Residues of chlorpyriphos, cypermethrin , endosulfan-Sulphate,endosulfan-β and profenofos were not detected. Residue of phorate was high (0.3432mgkg^{-1}) while HCH-β was least (0.0005 mgkg^{-1}). Out of 14 samples of Tomato, five contained residues of phorate , (MRL value =0.05, 0.2742-3432) above tolerance levels. In general, among 50 samples, 100% contamination was found with one or other residues, 83.3% were found contaminated with OC, 67% with OP and 55% with SP. Residue levels of OP were highest followed by OC and SP. However, about 34% samples showed residues of OP (phorate) above their

respective MRL values, OCs and SPs not exceeded the MRL value in any sample. This exhibits the shift from OC to OP and SP insecticides and the restricted use of OC insecticides. Slightly low level of pesticidal contamination was noticed (Kumari et al. 2001)), in case of summer vegetables viz. okra, brinjal, bitter gourd etc., where 23% samples were found to contain OP insecticide residues above MRLvalues. Acephate (58%) is highly contaminated followed by aldrin (42%) and monocrotophos (4%) is least. The results obtained in the present investigations are in agreement with earlier reports. (Fytianos et al.1985, Gupta et al.1998;).

CONCLUSION

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 four vegetables 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. It is, therefore, suggested that the vegetable collected from in and around of Rama nagara district of Karnataka, India are comparatively unsafe from pesticide residues. A periodical monitoring studies of pesticide residues may be extended to other vegetables in other vegetables /food commodities grown in 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.

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