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MULTI-RESIDUE METHOD FOR PESTICIDE RESIDUE ANALYSIS OF OKRA CROP, ABELMOSCHUS ESCULENTUS (L.) IN MEERUT REGION

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ABSTRACT

Okra fruits samples with insecticides were extracted with acetone and partitioned into DCM solvent. It was then cleaned up separately using two adsorbent namely neutral alumina and florisil were experimented for clean-up during recovery methods. The adsorbent Florisil in method IV was found to be the best adsorbent to afford highest recovery (over 80-91%) with hexane: acetone (1:1v/v) as solvent system. In method I the recovery per cent was in the range of 65-74%. In methods II and III it was in the range of 62-82% and 72-82% respectively. The detection limit for organophosphates, synthetic pyrethroid, organochlorines and new molecules were 0.1-0.01, 0.01, 0.01 and 0.05ppm, respectively.

KEYWORDS: GLC, HPLC, Florisil, Neutral alumina, Monocrotophos, Chlorpyriphos, Dimethoate Cypermethrin, Endosulfan and Imidacloprid.

INTRODUCTION

Agriculture play an important role in economic structure in India. Okra, *Abelmoschus esculentus* (L). MOENCH, often known as bhindi, lady's finger is valued for its edible green fruit. In India, it is grown over 3.58 lakh ha area with production of 35.25 lakh tones and productivity of 9.84 tones/ha. (Anonymous, 2005). The Spotted bollworm causes 8.4-73.2% fruit infestation depending on the season. The avoidable losses in yield and fruit damage due to this pest have been estimated as 36-90% (Misra *et al.*, 2002). The jassid is causing damage throughout the growing period of the crop and reduces the plant vigour and fruit yield (Mahal *et al.*, 1994). Insecticides play vital role in management of these pests. However, repeated application of insecticides makes cultivation not only uneconomical but, leads to certain unwarranted problems *viz*., resurgence, resistance, residue and environmental pollution. On an average 13-14% of total pesticides used in the country are applied in vegetable crops. Since the produce is harvested at short intervals and consume fresh in many cases, the surveys of market samples show high level of pesticide residues in vegetables (Arora and Gopal, 2002, Agnihotri, 1999, Awasthi and Ahuja, 1997). The indiscriminate use of broad spectrum chemicals has resulted in reduction of natural enemies, increased risk of contamination of food and fodder and hazardous to environment including human being through food chain and ground water. The major cases of pesticides poisoning and death occurs in the developing world, although a greater quantities of pesticides are being used in the developed world (Arora, 2006). Examples of acute health effects include stinging eyes, rashes, blisters, blindness, nausea, dizziness, diarrhoea and even death. Examples of known chronic effects are cancer, birth defects, reproductive harm, neurological and developmental toxicity, immune toxicity, and disruption of the endocrine system. It is therefore, necessary to constantly monitor the status of pesticide residues in our food and environment so that necessary steps could be taken to ensure safe and judicious use of pesticide as well as curbing unjustified abuse of pesticide.

MATERIAL AND METHOD

Sample preparation: Okra fruits were collected from control plots of experimental trials. These were cut into small pieces and 50g samples were kept in 250ml conical flask in triplicate. The samples were spiked with 2.5ml and 0.5ml

of mixture solutions **A** & **B** to obtain deposits of 1.0µg/ml and 0.1µg/ml respectively. Similarly 1ml & 0.1ml of 50 ppm of imidacloprid solution was also added to the okra fruit samples to get deposit of 1µg /ml and 0.1µg /ml respectively. The flasks containing spiked fruits were shaken for 2 minutes. Acetone was added in the flask to submerge the piece of spiked okra fruits and left overnight (20hrs).Control samples were treated only with the solvent. Each treatment was replicated thrice including control.

Extraction
The following method for extraction was adopted.

Method- I
(A) Mechanical Extraction
The fortified samples (50g) were homogenized in a blender (Remei mixie).The extract was treated with 25ml acetone and was filtered through a Buchner funnel fitted with a Whatman No.1 filter paper. For easy and quick filtration, vaccum pump was used. The residual pieces of okra fruits on the filter paper were again transferred to the blender. This process was repeated two more times with 25ml acetone. After extraction, the blender jar was rinsed with 25ml acetone and rinsate was also filtered the same way and the final volume of the extract, around 100ml was collected.

(B) Liquid-Liquid partitioning
Solvent from the extract was evaporated off with the help of rotary vacuum evaporator to around 10ml and then transferred to a 250ml separating funnel. 100ml of sodium chloride solution (18%, w/v) followed by 50ml of distilled Dichloromethane (DCM) were added to it. Separating funnel was thoroughly shaken for 1 minute by slowly releasing pressure through stop cork and allowed to stand (for about 5 minutes) until the two layers were separated. The bottom layer was collected in a conical flask after passing through anhydrous sodium sulphate (5g) layer. The aqueous solution of the separating funnel was shaken two more times with 50ml DCM. The anhydrous sodium sulphate layer was given additional washing with 10ml DCM and the filtrate was collected in the same flask.

(C) Column Chromatography
Glass column (50cm × 1.5cm) was plugged with cotton at the bottom, then dry packed with 5g of anhydrous sodium sulphate followed by 5g of florisil and finally again with 5g of anhydrous sodium sulphate. It was prewashed with 20ml hexane. The extract from DCM was taken 10ml hexane and was added to the adsorbent column. The column was sequentially eluted with 100ml hexane: acetone (7:3, v/v) and the eluate were collected in a conical flask for final estimation of pesticides using GLC & HPLC.

Method- II
A) Mechanical Extraction
The fortified samples (50g) were homogenized in a blender (Ramie mixie).The extract was treated with 50ml solvent mixture (1:1 hexane: acetonitrile) and was filtered through a Buchner funnel fitted with a Whatman No.1 filter paper. For easy and quick filtration, vacuum pump was used. The residual pieces of okra fruits on the filter paper were again transferred to the blender. This process was repeated two more times with 25ml acetone. After extraction, the blender jar was rinsed with 25ml acetone and rinsate was also filtered the same way and the final volume of the extract, around 100ml was collected.

(B) Liquid-Liquid partitioning
Solvent from the extract was evaporated off with the help of rotary vacuum evaporator to around 10ml and then transferred to a 250ml separating funnel.100ml of sodium chloride solution (18%, w/v) followed by 50ml of distilled Dichloromethane were added to it. Separating funnel was thoroughly shaken for 1 minute by slowly releasing pressure through stop cork and allowed to stand (for about 5 minutes) until the two layers were separated. The bottom layer was collected in a conical flask after passing through anhydrous sodium sulphate (5 g) layer. The aqueous solution of the separating funnel was shaken two more times with 50ml DCM. The anhydrous sodium sulphate layer was given additional washing with 10ml DCM and the filtrate was collected in the same flask.

(C) Column Chromatography

Glass column (50cm × 1.5cm) was plugged with cotton at the bottom, then dry packed with 5g of anhydrous sodium sulphate followed by 5g of activated neutral alumina and finally again with 5g of anhydrous sodium sulphate. It was prewashed with 20ml hexane. The extract from DCM was taken 10ml hexane and was added to the adsorbent column. The column was sequentially eluted with 100 ml hexane: acetone (7:3, v/v) and the eluate was collected in a conical flask for final estimation of pesticides using GLC & HPLC.

Method- III

(A) Mechanical Extraction

The fortified samples (50g) were homogenized in a blender (Remei mixie).The extract was treated with 25ml acetonitrile and was filtered through a Buchner funnel fitted with a Whatman No. 1 filter paper. For easy and quick filtration, vacuum pump was used. The residual pieces of okra fruits on the filter paper were again transferred to the blender. This process was repeated two more times with 25ml acetonitrile. After extraction, the blender jar was rinsed with 25ml acetonitrile and rinsate was also filtered the same way and the final volume of the extract, around 100ml was collected.

(B) Liquid-Liquid partitioning

Solvent from the extract was evaporated off with the help of rotary vacuum evaporator to around 10ml and then transferred to a 250ml separating funnel.100ml of sodium chloride solution (18%, w/v) followed by 50ml of distilled Dichloromethane were added to it. Separating funnel was thoroughly shaken for 1 minute by slowly releasing pressure through stop cork and allowed to stand (for about 5 minutes) until the two layers were separated. The bottom layer was collected in a conical flask after passing through anhydrous sodium sulphate (5 g) layer. The aqueous solution of the separating funnel was shaken two more times with 50ml DCM. The anhydrous sodium sulphate layer was given additional washing with 10ml DCM and the filtrate was collected in the same flask.

(C) Column Chromatography

Glass column (50cm × 1.5cm) was plugged with cotton at the bottom, then dry packed with 5g of anhydrous sodium sulphate followed by 5 g of neutral alumina and finally again with 5g of anhydrous sodium sulphate. It was prewashed with 20ml hexane. The extract from DCM was taken 10 ml hexane and was added to the adsorbent column. The column was sequentially eluted with 100ml hexane: acetone (7:3 v/v) and the eluate were collected in a conical flask for final estimation of pesticides using GLC & HPLC.

Method- IV

(A) Mechanical Extraction

The fortified samples (50g) were homogenized in a blender (Remei mixie).The extract was treated with 100ml solvent mixture (1:1 hexane : acetone) and was filtered through a Buchner funnel fitted with a Whatman No.1 filter paper. For easy and quick filtration, vacuum pump was used. The residual pieces of okra fruits on the filter paper were again transferred to the blender. This process was repeated two more times with 25ml acetone. After extraction, the blender jar was rinsed with 25ml acetone and rinsate was also filtered the same way and the final volume of the extract, around 100ml was collected.

(B) Liquid-Liquid partitioning

Solvent from the extract was evaporated off with the help of rotary vacuum evaporator to around 10ml and then transferred to a 250ml separating funnel. 100ml of sodium chloride solution (18%, w/v) followed by 50ml of distilled Dichloromethane were added to it. Separating funnel was thoroughly shaken for 1 minute by slowly releasing pressure through stop cork and allowed to stand (for about 5 minutes) until the two layers were separated. The bottom layer was collected in a conical flask after passing through anhydrous sodium sulphate (5 g) layer. The aqueous solution of the separating funnel was shaken two more times with 50ml DCM. The anhydrous sodium sulphate layer was given additional washing with 10ml DCM and the filtrate was collected in the same flask.

(C) Column Chromatography

Glass column (50cm × 1.5cm) was plugged with cotton at the bottom, then dry packed with 5g of anhydrous sodium sulphate followed by 5g of florisil and finally again with 5g of anhydrous sodium sulphate. It was prewashed with

20ml hexane. The extract from DCM was taken 10ml hexane and was added to the adsorbent column. The column was sequentially eluted with 100ml hexane: acetone (7:3 v/v) and the eluate were collected in a conical flask for final estimation of pesticides using GLC & HPLC.

ANALYSIS OF PESTICIDES EXTRACT

The samples were analyzed using GLC for determination of organochlorines, synthetic pyrethroides and organophosphates and HPLC for imidacloprid insecticide. GLC (HP 5890 series II) equipped with Ni^{63}, mega bore column (10m, 0.53mm id, 2.65 mm film thickness) and ECD detector. The GLC working conditions were as flows: Nitrogen gas flow rate 12 ml/min Detector, 260°C: and Injector 250°C. The column temperature 150°C maintained for 10 minutes raised @ 5°C/min to 220°C and held for 5 minutes. The retention time (Rt) of monocrotophos 3.0 min, Endosulfan 13.1 min, chlorpyriphos 15.4 min, Dimethoate 18.8 min, Cypermethrin 31.6 min. The HPLC working conditions as were as flows: Column -BEH C-18 (1.7 µm), Column dimensions-100 mm x 2.1 mm id. Column temperature -50 ^{o}C, Mobile phase -Acetonitrile –water (30:70, v/v),Flow rate -0.5 ml/min, Sample size -5 µl ,Detector -PDA ,λ_{max} -278nm, Retention time -1 min ,BDL-up to the ng.

Recovery percentage formula

$$\textbf{Recovery \%} = \frac{\text{Concentration of pesticides in fortified samples x100}}{\text{Concentration of analytical standard of pesticide}}$$

RESULT

An important step in any recovery experiment is to standardize the methodology for the extraction and clean-up of the pesticide from the treated samples; so that the accuracy of extraction becomes maximum and the amount of co-extractives are minimum. Therefore various methods extraction was adopted. The samples with insecticides were extracted with acetone from okra fruits and partitioned into DCM solvent. It was then cleaned up separately using two adsorbent namely neutral alumina and Florisil were experimented for clean-up during recovery methods. The adsorbent Florisil in method IV was found to be the best adsorbent to afford highest recovery (over 80-91%) with hexane: acetone (1:1v/v) as solvent system. Recovery data of insecticides from okra fruits are presented in Table-1. The recovery of insecticides in method IV was found to be the best to afford highest recovery (over 80-91%) with Florisil as adsorbent. In method I the recovery per cent was in the range of 65-74%. In methods II and III it was in the range of 62-82% and 72-82% respectively**.**

Table - 1: The recovery analysis of insecticide on okra fruits

S. N.	Methods	Adsorbent	Insecticides	Added amonut (µg)	Residue (ppm)	Recovery (%)	Mean of recovery (%)
1	I	Florisil	Monocrotophos	1.0	0.69	69	70.0
				0.1	0.069	71	
			Chlorpyriphos	1.0	0.72	72	73.0
				0.1	0.072	74	
			Dimethoate	1.0	0.70	70	69.0
				0.1	0.070	68	
			Cypermethrin	1.0	0.73	73	71.5
				0.1	0.073	70	
			Endosulfan	1.0	0.74	74	72.5
				0.1	0.074	71	

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			Imidacloprid	1.0	0.65	65	67.5
				0.1	0.065	70	
2	II	Neutral alumina	Monocrotophos	1.0	0.68	68	69.5
				0.1	0.068	71	
			Chlorpyriphos	1.0	0.78	78	79.0
				0.1	0.078	80	
			Dimethoate	1.0	0.70	70	72.5
				0.1	0.070	75	
			Cypermethrin	1.0	0.79	79	80.5
				0.1	0.079	82	
			Endosulfan	1.0	0.78	78	78.5
				0.1	0.078	79	
			Imidacloprid	1.0	0.62	62	68.5
				0.1	0.062	67	

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3	III	Neutral alumina	Monocrotophos	1.0	0.78	78	80.5
				0.1	0.078	83	
			Chlorpyriphos	1.0	0.80	80	82.0
				0.1	0.080	84	
			Dimethoate	1.0	0.76	76	74.5
				0.1	0.076	73	
			Cypermethrin	1.0	0.80	80	81.5
				0.1	0.080	83	
			Endosulfan	1.0	0.82	82	83.5
				0.1	0.082	85	
			Imidacloprid	1.0	0.72	72	71.0
				0.1	0.072	70	
4	IV	Florisil	Monocrotophos	1.0	0.82	82	81.0
				0.1	0.082	80	
			Chlorpyriphos	1.0	0.88	88	89.5
				0.1	0.088	91	
			Dimethoate	1.0	0.86	86	87.5
				0.1	0.086	89	
			Cypermethrin	1.0	0.87	87	88.5
				0.1	0.087	90	
			Endosulfan	1.0	0.84	84	85.5
				0.1	0.084	87	
			Imidacloprid	1.0	0.80	80	81.0

For method I, extraction was carried out using distilled acetone solvent, DCM solvent was used for liquid-liquid partitioning and for clean-up florisil adsorbent was used. For method II, extraction was carried out using distilled acetone solvent, DCM solvent was used for liquid-liquid partitioning and for clean-up neutral alumina adsorbent was used. For method III, extraction was carried out using distilled acetonitrile solvent, DCM solvent was used for liquid-liquid partitioning and for clean-up neutral alumina adsorbent was used and for method IV, extraction was carried out using distilled hexane : acetone (1:1) solvent, DCM solvent was used for liquid-liquid partitioning and for clean-up florisil adsorbent was used.

Table – 2 Retention time & limit of detection for different pesticides

S.N.	Insecticides	Retention(min)	Limit Of Detection (LOD)
1.	Monocrotophos	5.6	0.1
2.	Chlorpyriphos	13.8	0.01
3.	Dimethoate	2.4	0.1
4.	Cypermethrin	3.5	0.01
5.	Endosulfan	4.1	0.01
6.	Imidacloprid	1.0	0.05

DISCUSSION

For recovery experiment, the control plot of okra fruit samples were fortified at the level of 1.0 and 0.1ppm and the recovery of monocrotophos (81%), chlorpyriphos (89.5%), dimethoate (87.5%), cypermethrin (88.5%), endosulfan (85.5%), and imidacloprid (81%) with used as florisil adsorbent. The detection limit for organophosphates, synthetic pyrethroid, organochlorines and new molecules were 0.1-0.01, 0.01, 0.01 and 0.05ppm, respectively. According to Chandrasekaran *et al.* (1997) okra fruit samples fortified at 0.5, 1.0 and 2.0 mg/kg level was found to be from 80-92 per cent recovery, with used as florisil adsorbent and instrument sensitivity for organochlorinated hydrocarbon and synthetic pyrethroides was 0.001µg and 0.01µg for organophosphorus and carbamate compounds.

Awasthi and Anand (1993) reported known quantities of synthetic pyrethroid (cypermethrin) for residues analysis, the efficiencies of analytical procedure with neutral alumina adsorbent and found average recovery of 91.5%. Similar used as florisil adsorbent but recovery differed at fortified level of 0.5 and 1.0 mg/kg were 94.3 and 86.2% for cypermethrin and 90.2 and 88.1% for endosulfan (Deen *et al*., 2009). The recovery of dimethoate reported were similar with florisil adsorbent but different recovery of monocrotophos (80%), chlorpyriphos (86%), dimethoate (87%) and cypermethrin (80%) at fortified level of 0.5 and 1µg/g (Deka *et al*., 2005). With respect to cypermethrin (88.5%) level at 1.0 and 0.1ppm, average recovery reported from fortified sample at 0.5 and 1.0 mg/kg level was 87.3% with neutral alumina (Singh *et al*., 2004). Fortified okra fruit samples at 6.5 and 5.5µg/g level with neutral alumina was found to 83 and 96% recovery for cypermethrin and chlorpyriphos, respectively (Arora, 2004).

Other than okra fruit samples, with known quantities of imidacloprid in whole tomato plant, fortified at 0.1 and 1.0ppm level as similar but recovery varied from 75 to 80% with sodium hydrochloride solution, chloroform, anhydrous sodium sulfate and acetonitrile-water gradient system and detection limit for method was 0.01ppm (Gajbhiye *et al.*, 2000).

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