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Multi residue analysis of insecticides in okra (*Abelmoschus esculentus* (L.) *monech*) fruits of tadepalligudem market, west Godavari district, Andhra Pradesh, India

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Abstract
Levels of pesticide residues in the okra fruits obtained from local tadepalligudem market was estimated at Pesticide Residue and Food Quality Analysis Laboratory (PRFQAL), University of Agricultural Sciences, Raichur, Karnataka. As sample of okra fruits were collected from tadepalligudem market and analyzed for multiresidues. Surprisingly, out of 74 chemicals tested, profenophos with 0.048 ppm was detected which was above the Maximum Residual Limit (MRL) of 0.01 ppm.

Keywords: okra, PRFQAL, multiresidues and MRL

Introduction
Pesticides, such as insecticides, herbicides, fungicides and acaricides, have been widely applied during the cultivation and the post-harvest storage of crops. These pesticides were used to prevent the destruction of edible crops by controlling agricultural pests or unwanted plants and thereby increases and improve food production.

Okra (*Abelmoschus esculentus* (L.) Moench) is originated in Africa, is one of the important vegetable crops and placed in under Malvaceae family. It is rich source of dietary fiber, antioxidants, ascorbic acid and folate. Mucilage from okra has been reported to be effective as blood volume expander and has the potential to alleviate renal disease, reduce proteinuria and improve renal function (Siemonsma and Kouame, 2004) [4]. The production and productivity of okra is limited by incidence of various pests.

Indiscriminate use of pesticides to manage the pest complex in bhendi and negligence to follow proper waiting periods make marketed produce with toxic pesticides and may pose health hazards to consumers (Lakshminarayana and Menon, 1975 and Mukherjee and Gopal, 2003) [2, 3]. The presence of insecticide residues *viz*., flubendiamide (0.142 mg/kg), profenophos (0.042 mg/kg), acephate (0.193 mg/ kg), fipronil (0.028 mg/kg), chlorpyrifos (0.275 mg/kg), lambda-cyhalothrin (0.241 mg/kg), acetamiprid (0.167 mg/kg), monocrotophos (0.011 mg/kg), and imidacloprid (0.032 mg/kg) were found in the okra samples collected from Rythu bazaar vegetable market of karimnagar during 2014-15 and these pesticide residues find their way into the human body through food, water, and environment. Among the samples analyzed for different insecticide residues, two of the insecticides *i.e.*, chlorpyriphos and acetamiprid have shown above MRL value established by Codex alimentarius, while the remaining insecticide residues detected in the samples were below the MRL (Anil *et al.*, 2017) [1].

Materials and methods
A sample of 1 Kg okra fruits were collected from tadepalligudem market and analyzed for residues by using Liquid Chromatography-Mass Spectrophotometry (LC-MS/MS) and Gas Chromatography-Mass Spectrophotometry (GC-MS/MS). The analysis was undertaken at Pesticide Residue and Food Quality Analysis Laboratory (PRFQAL), University of Agricultural Sciences, Raichur, Karnataka. The following methods were adopted for estimation of insecticide residues in okra fruits of tadepalligudem market.

Steps involved in the estimation of insecticide residues sample of okra from Tadepalligudem market, West Godavari District, Andhra Pradesh.

A. Extraction

Okra fruits were chopped into small pieces and blended in the grinder. The fortified sample (10 g) was taken in a 50 ml centrifuge tube and added with 5ml distilled water. After 30 min, the blended mixture was added with 10 ml ethyl acetate and 10 g anhydrous sodium sulphate (activated at 500℃ for 4 hours).

B. Homogenization

After extraction, the sample mixture was homogenized at 10000-13000 rpm for 3 minutes.

C. High volume centrifugation

The content was subjected to high volume centrifugation at 5000 rpm for 5 minutes at 10℃.

D. Clean up

After centrifugation, 7ml of extract was transferred to 15ml centrifuge tube containing 25 mg of primary secondary amine (PSA) and 150 mg of magnesium sulphate ($MgSO_4$). The mixture was homogenized in vortex for 1min and centrifuged again at 12000 rpm for 5 minutes followed by the addition of 25 mg of activated charcoal for the removal of coloured impurities.

E. Evaporation

After clean up, 3 ml of extract was transferred into two test tubes containing 300µl of 10 per cent diethylene glycol in methanol and evaporated to dryness using nitrogen concentrator at 35^0C temperature. The residue was reconstituted for Liquid Chromatography-Mass Spectrophotometry (LC-MS) analysis with 1.5ml LC compatible solvent (methanol). The mixture was homogenized in vortex for 30 seconds and sonicated for one minute to dissolve the residues.

F. Filtration

The extract of 1.5 ml was then filtered to LC autosampler vials through 0.22µ Poly tetra fluoro ethylene (PTFE) membrane filter. These steps involved are presented in plate 1.

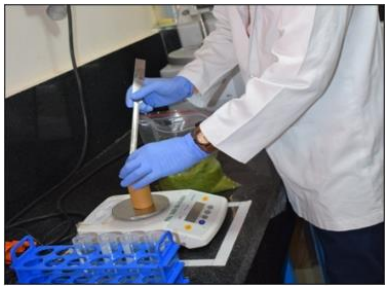
G. Sample Injection

Sample of 2µl filtrate was injected into LC-MS/MS with below conditions.

Chopping of okra fruits

Blending in a grinder

Blended mixture



10gm of blended mixture was taken

Addition of 10 ml ethyl acetate

Weighing of 10 g anhydrous Na_2SO_4

Plate 1: Steps involved in pesticide residue analysis……contd

Addition of 10 g anhydrous Na_2SO_4

Addition of 10 g anhydrous Na_2SO_4

High Volume Centrifugation

Transfer of 7 ml extract to 15ml centrifuge tube containing 25mg PSA and 150mg $MgSO_4$

Homogenization, high volume centrifugation, evaporating to dryness using nitrogen concentrator

H. Analysis of pesticide residue

The sample was analyzed using Liquid Chromatography-Mass Spectrophotometry (LC-MS/MS) to determine the residual content. LC was equipped with mega bore column Shimpack XR with dimensions 2 mm id x 150 mm. The working conditions were as follows: ECI probe source, total run time 25 min, Nitrogen gas flow rate 0.4 ml per minute, Heart block temperature 400°C and dissolution temperature 200°C. Nebulizing and Drying gas flow (Nitrogen) rate of 2.9 l/min and 15 l/min. The mobile phase was 0.0314g ammonium formate (5mM) + 2ml methanol + 10µl formic acid (0.01%) made up the volume with HPLC water to 100 ml (or) 0.0314g ammonium formate (5mM) + 10µl formic acid (0.01%) made up the volume with 100% methanol to 100ml.

GC was equipped with column HP-5 MS with dimensions of 0.25µ film thickness, 30 m length. The working conditions were as follows: Total run time 41.07 min, total flow rate 4.5 ml per minute, column flow rate 1.5 ml per minute, mass range 50 to 550, injector temperature 280^{0}c, transfer line temperature 280^{0}c, source temperature 300^{0}c.

I. Method validation

Blank samples of okra were analyzed to verify the absence of interfering species. The matrix-dependent limit of detection (LOD) and limits of quantification (LOQ) was calculated for analytical methodology using the blank and calibration standards of okra. The LOD value of insecticide is the concentration that produces a signal to noise (peak to peak) ratio of 3. The LOQ is defined based on the signal-to-noise ratio of 10 and estimated from the chromatogram to the lowest point used in the matrix-matched calibration. The retention time was noted.

Calculation: The recovery (%) and residues from the fortified sample were calculated by using the following formula.

$$\text{Recovery (\%)} = \frac{\text{Concentration of fortified sample (mg/kg)}}{\text{Concentration of analytical standard of pesticide}} \times 100$$

$$\text{Residue (mg/kg)} = \frac{\text{Peak area of sample} \times \text{Conc. of Std.} \times \mu\text{l std. injected}}{\text{Final volume of sample (1.5ml) x Peak area (standard)} \times \text{weight of the sample (g)} \times \mu\text{l sample injected}}$$

$$\text{Weight of sample (g)} = \frac{\text{Sample weight x aliquot taken (ml)}}{\text{Volume of extractant (ml)}}$$

Results and discussion

The farm gate sample of okra fruits were collected from tadepalligudem market and analyzed for residues to detect 74 chemicals using Liquid Chromatography - Mass Spectrophotometry/Mass Spectrophotometry (LC-MS/MS) and Gas Chromatography-Mass Spectrophotometry/Mass Spectrophotometry (GC-MS/MS).

Among the 74 chemicals tested for residues, okra fruits were observed to be contaminated with profenophos (0.048 ppm), which was above maximum residue limits (MRL) of 0.01 ppm (table 1). Similar reports were made by Anil *et al.* (2017) [1] who detected residues of profenophos from Rythu bazaar market (0.042 mg/kg), Tower circle (0.065 mg/kg) and Ramnagar vegetable market (0.052 mg/kg) of karimnagar. While spinosad, emmamectin benzoate, tetraconazole, propanil, chlorpyriphos methyl and cypermethrin were below quantification level (BQL), while the remaining chemicals were not detected in the sample. The LC-MS/MS chromatogram is presented in plate 2.

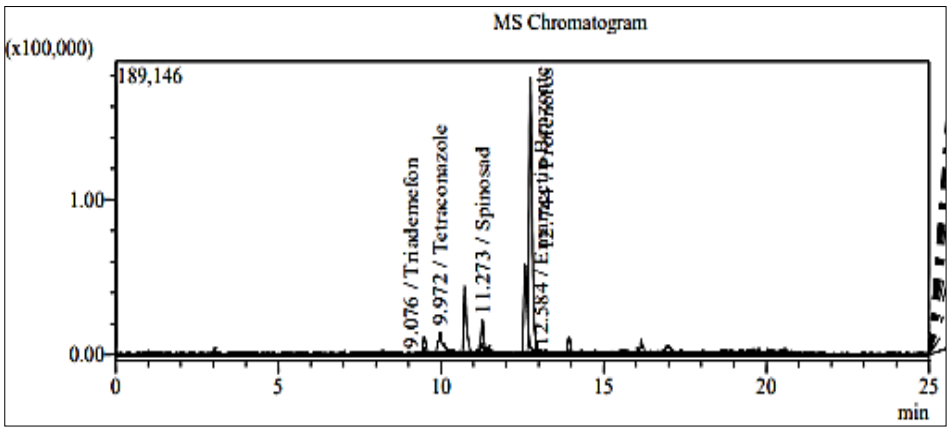


Plate 2: LC-MS Chromatogram of okra market sample for 39 pesticides

Conclusion

The present pattern of insecticides detected in the okra sample collected from tadepalligudem market does not seem to contribute toward excessive residues. However, the insecticides should be applied in a need based manner and recommended insecticides should be applied as and when required. Further to safeguard the consumer's interest, proper waiting period must be practiced by the producer before marketing vegetables.

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Table 1: Insecticide residues detected in okra fruits grown in Tadepalligudem (West Godavari district, Andhra Pradesh) market

S. No.	Name of the sample	Result	Unit	Method of Analysis/technique
1.	Thiachloprid	ND	mg/kg	LC-MS/MS
2.	Buprofezin	ND	mg/kg	LC-MS/MS
3.	Methalachlor	ND	mg/kg	LC-MS/MS
4.	Imidachloprid	ND	mg/kg	LC-MS/MS
5.	Dimethoate	ND	mg/kg	LC-MS/MS
6.	Coumatetryl	ND	mg/kg	LC-MS/MS
7.	Triademeneol	ND	mg/kg	LC-MS/MS
8.	Triademefon	ND	mg/kg	LC-MS/MS
9.	Thiodicarb	ND	mg/kg	LC-MS/MS
10.	Spinosad	BQL	mg/kg	LC-MS/MS
11.	Phosalone	ND	mg/kg	LC-MS/MS
12.	Methoxyfenozide	ND	mg/kg	LC-MS/MS
13.	Hexythiazox	ND	mg/kg	LC-MS/MS
14.	Fenpyroximate	ND	mg/kg	LC-MS/MS
15.	Carbendazim	ND	mg/kg	LC-MS/MS
16.	Carbaryl	ND	mg/kg	LC-MS/MS
17.	Triazophos	ND	mg/kg	LC-MS/MS
18.	Carbofuran	ND	mg/kg	LC-MS/MS
19.	Bitertenol	ND	mg/kg	LC-MS/MS
20.	Bendiocarb	ND	mg/kg	LC-MS/MS
21.	Benalaxyl	ND	mg/kg	LC-MS/MS
22.	Acephate	ND	mg/kg	LC-MS/MS
23.	Pymetrozine	ND	mg/kg	LC-MS/MS
24.	Omethoate	ND	mg/kg	LC-MS/MS
25.	Metribuzin	ND	mg/kg	LC-MS/MS
26.	Metalaxyl	ND	mg/kg	LC-MS/MS
27.	Isoproturon	ND	mg/kg	LC-MS/MS
28.	Emmamectin benzoate	BQL	mg/kg	LC-MS/MS
29.	Tetraconazole	BQL	mg/kg	LC-MS/MS
30.	Quinalphos	ND	mg/kg	LC-MS/MS
31.	Profenofos	0.048	mg/kg	LC-MS/MS
32.	Phosphomidon	ND	mg/kg	LC-MS/MS
33.	Pendimethaliun	ND	mg/kg	LC-MS/MS
34.	Difenconazole	ND	mg/kg	LC-MS/MS
35.	Pretilachlor	ND	mg/kg	LC-MS/MS
36.	Penconazole	ND	mg/kg	LC-MS/MS
37.	Paclobutrazole	ND	mg/kg	LC-MS/MS
38.	Hexaconazole	ND	mg/kg	LC-MS/MS
39.	Chlorantraniliprole	ND	mg/kg	LC-MS/MS
40.	Phenol, 4- bromo 2- chloro	ND	mg/kg	GC-MS/MS
41.	Trifluralin	ND	mg/kg	GC-MS/MS
42.	Alpha-BHC	ND	mg/kg	GC-MS/MS
43.	Beta-BHC	ND	mg/kg	GC-MS/MS
44.	Diazinone	ND	mg/kg	GC-MS/MS
45.	Fluchloralin	ND	mg/kg	GC-MS/MS
46.	Tri-allate	ND	mg/kg	GC-MS/MS
47.	Iprobenfos	ND	mg/kg	GC-MS/MS
48.	Propanil	BQL	mg/kg	GC-MS/MS
49.	Clorpyriphos methyl	BQL	mg/kg	GC-MS/MS
50.	Parathion methyl	ND	mg/kg	GC-MS/MS
51.	Alachlor	ND	mg/kg	GC-MS/MS
52.	Heptachlor	ND	mg/kg	GC-MS/MS
53.	Fenitrothion	ND	mg/kg	GC-MS/MS
54.	Chlorpyriphos	ND	mg/kg	GC-MS/MS
55.	Parathion	ND	mg/kg	GC-MS/MS
56.	Chlorfenvinphos	ND	mg/kg	GC-MS/MS

57.	Parathion	ND	mg/kg	GC-MS/MS
58.	Butachlor	ND	mg/kg	GC-MS/MS
59.	p, p' –DDE	ND	mg/kg	GC-MS/MS
60.	Endrin	ND	mg/kg	GC-MS/MS
61.	Beta-Endosulfon	ND	mg/kg	GC-MS/MS
62.	p,p' –DDT	ND	mg/kg	GC-MS/MS
63.	o,p' –DDT	ND	mg/kg	GC-MS/MS
64.	Endosulfon sulphate	ND	mg/kg	GC-MS/MS
65.	Bifenthrin	ND	mg/kg	GC-MS/MS
66.	Fenpropathrin	ND	mg/kg	GC-MS/MS
67.	Lambda-cyhalothrin	ND	mg/kg	GC-MS/MS
68.	Permethrin	ND	mg/kg	GC-MS/MS
69.	Cyfluthrin	ND	mg/kg	GC-MS/MS
70.	Cypermethrin	BQL	mg/kg	GC-MS/MS
71.	Ethofenprox	ND	mg/kg	GC-MS/MS
72.	Fenvelerate	ND	mg/kg	GC-MS/MS
73.	Deltamethrin	ND	mg/kg	GC-MS/MS
74.	Aldrin	ND	mg/kg	GC-MS/MS

N.D – Not Detected,
B.Q.L – Below Quantification Level (<0.01 mg/kg),
LC-MS/MS – Liquid Chromatography – Mass Spectrophotometry,
GC-MS/MS – Gas Chromatography – Mass Spectrophotometry.

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