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Determination of pesticide residue levels in okra (*Abelmoschus esculentus* (L.) *monech*) plots by LC-MS/MS

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Abstract
Studies on the impact of IPM and non-IPM practices were undertaken during *rabi* season 2018-19 at College of Horticulture, Venkataramannagudem, West Godavari district, Andhra Pradesh with an objective of estimating the pesticide residue levels in fruits harvested from okra plots. IPM plot of okra includes common IPM practices and need based sequential application of botanicals and bioagents. Whereas, sequential spraying of synthetic pesticides was undertaken in non-IPM plot of okra. Levels of pesticide residues in the okra fruits obtained from IPM and non-IPM plots was estimated at Pesticide Residue and Food Quality Analysis Laboratory (PRFQAL), University of Agricultural Sciences, Raichur, Karnataka. The residues of imidachloprid (6.7 ppm), thiomethoxam (3.8 ppm), flubendiamide (7.9 ppm), chlorantraniliprole (6.5 ppm) were identified in the harvested okra fruits from non-IPM plot which are far above the maximum residue limits (MRL), however no pesticides were detected in the okra fruits from IPM plot.

Keywords: Okra, PRFQAL, IPM, Non-IPM, residues 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) 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.

The production and productivity of okra is often limited by incidence of various pests and many of the pests occurring on cotton are found to ravage okra too. Accordingly, to overcome the menace of pest complex in okra, farmers are resorting to minimum of 10 to15 rounds of pesticide sprays during a cropping season and overuse use of pesticides on okra coupled with improper waiting periods make marketed produce with toxic pesticides and may pose health hazards to consumers (Mukherjee and Gopal, 2003) [6]. To minimize the pesticide load in okra, various IPM modules have been worked out with reference to safety of the consumers and producers as well as to ensure food quality.

IPM is an effective, environmentally safe approach to pest management as it provides protection for beneficial insects as well as prevention of secondary pest outbreaks and resurgence (Preety and Bharucha, 2015) [8].

Materials and Methods
The experiment was conducted at college farm, College of Horticulture, Venkataramannagudem to estimate the insecticide residue levels in IPM and non-IPM plots of okra during *rabi* season 2018-19.

In IPM plot of okra *viz.*, Deep ploughing, maize as border crop, Reflective Plastic Mulch (Sheet gauge), marigold as trap crop, installation of yellow sticky, light traps, sex pheromone traps, erection of bird perch and need based sequential application of botanicals and bioagents such as NSKE 5 per cent @15 DAS, neem oil @ 3 ml/l at 30 DAS, sweet flag Aqueous extract 5 per cent at 45 DAS, imidachloprid 17.8 SL @ 0.3 ml/l at 60 DAS, *B. bassiana* @ 5 g/l at 75 DAS, *B. thuringiensis* @ 1 g/l at 90 DAS was carried out in IPM plot of okra.

Whereas, in non-IPM plot of okra application of chemicals was carried out on sequential basis *viz.*, imidachloprid 17.8 SL@ 0.25 ml/l at 15 DAS, lambda cyhalothrin 5 EC @ 1ml/l at 30 DAS, thiomethoxam 25WG @ 2ml/l at 45 DAS, flubendiamide 480 SC @ 1ml/l at 60 DAS,

buprofezin 25 SC @ 1ml/l at 75 DAS and chlorantraniliprole 18.5 % SC @ 0.25ml/l at 90 DAS.
Okra fruit samples (1Kg) were collected seperately from the IPM and non-IPM plots of experimental trials. They were harvested within 24 hrs of spraying and stored at $4^{o}C$ until extraction. The pesticide residues were analyzed at Pesticide Residue and Food Quality Analysis Laboratory (PRFQAL), University of Agricultural Sciences, Raichur, Karnataka. The analysis was carried out for determining the residual content of imidachloprid in fruit samples from IPM, thiomethoxam, flubendiamide, chlorantraniliprole and imidachloprid from non-IPM plots of okra by using Liquid Chromatography-Mass Spectrophotometry (LC-MS/MS).

Steps involved in the estimation of insecticide residues from IPM and non-IPM plots of okra

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 $35^{0}C$ 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.

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 ºC. 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.

Chopping of okra fruits

Blending in a grinder

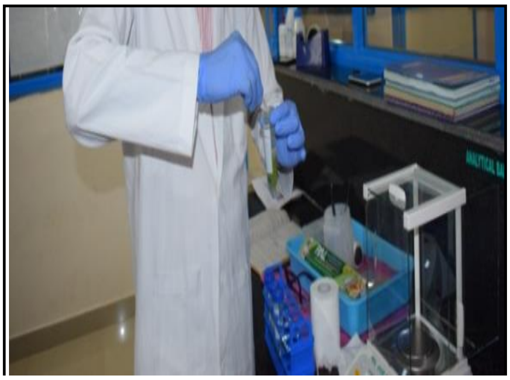
Blended mixtures

10gm of blended mixture was taken

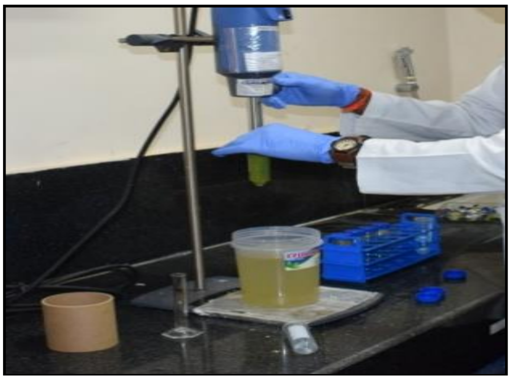
Addition of 10 ml ethyl acetate

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



Homogenization

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

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 results in the table 1 and figure 1 revealed that insecticide residues were detected in okra fruits collected from non-IPM plot of okra. All the samples were fortified at equal level of 10 g kg^{-1}. The Limit of Detection (L.O.D) and Limit of Quantification (L.O.Q) values are 2 and 10 parts per billion (PPB) for all the samples collected from non-IPM plots and per cent recoveries were ranged from 70-120 per cent.

The residues of imidacloprid (6.7 ppm), thiomethoxam (3.8 ppm), flubendiamide (7.9 ppm) and chlorantraniliprole (6.5 ppm) were determined which were exceeding their maximum residue limits (MRL) with retention times of 1.964, 3.389, 12.360, 11.590 min respectively. The LC-MS/MS chromatograms are presented in plates 2, 3, 4 and 5. In the present investigations it was found that residues were not detected in okra fruits grown in IPM plot which is in accordance with Kole *et al*. (2002) [3] who reported that IPM trials were safe for consumption as the residues of insecticides were either below MRL or not detectable.

While, in okra fruits grown in non-IPM plot persistence of imidachloprid, thiomethoxam, flubendiamide and chlorantraniliprole was detected. The results were in accordance with the findings of Pandit *et al.* (2016) [7] and Joshi *et al.* (2019) [2] who reported that imidachloprid residue persisted upto 5 days after treatment. Aly (2016) [1] concluded that okra fruits could be consumed safely after 15 days of treatment with thiamethoxam. The result goes in line with Vemuri *et al.* (2014) [9] and Meenambigai *et al.* (2017) [5] observed that the persistence of flubendiamide upto 7 days after spraying. Mandal *et al.* (2014) [4] reported that half-life of chlorantraniliprole was 0.93-1.33 days in berseem, whereas it was 1.31 days in cauliflower as per the studies of Vijayasree *et al*. (2013) [10].

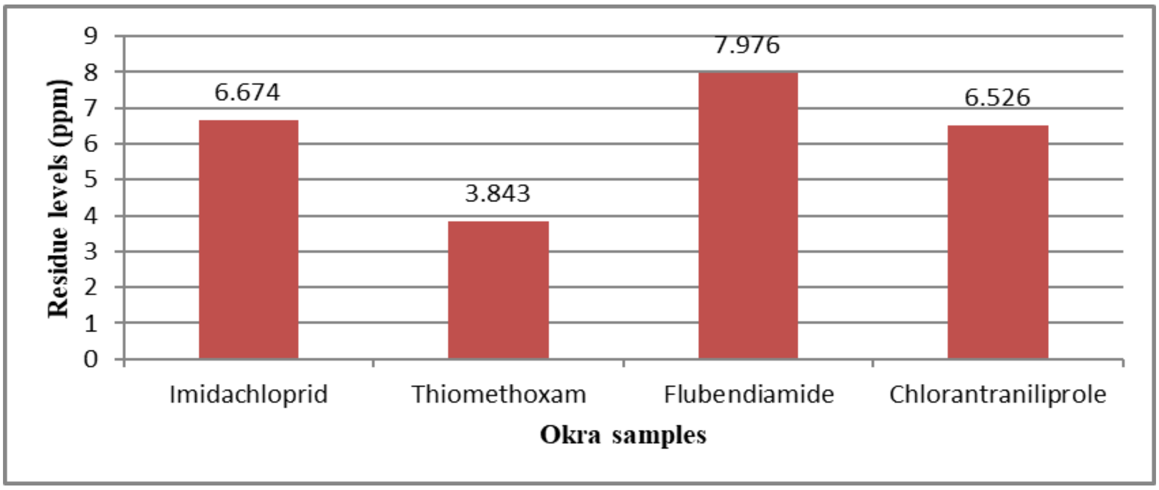


Fig 1: Residue levels of various insecticides in non-IPM plots of okra

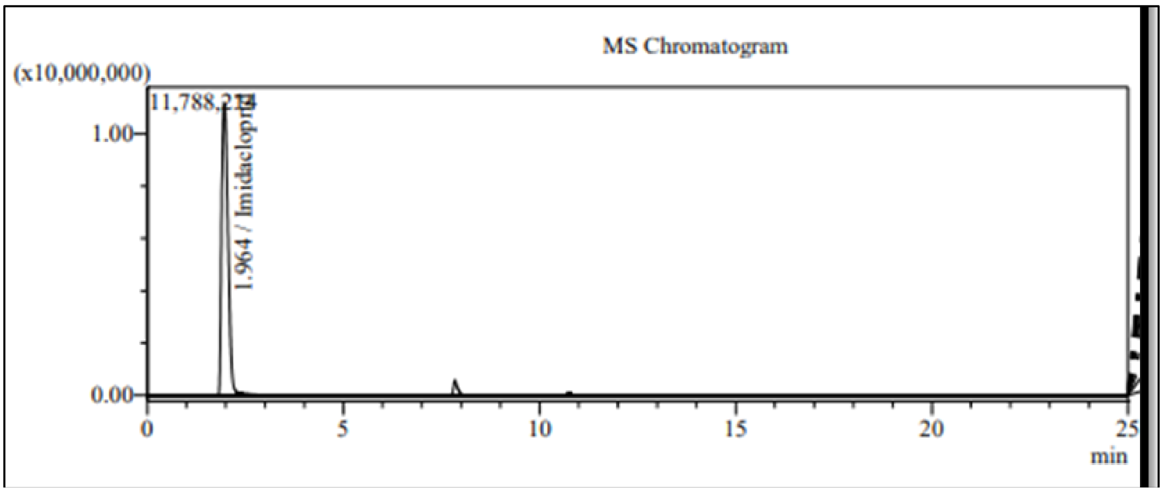


Plate 1: LC-MS Chromatogram of imidachloprid in okra sample of non-IPM plot

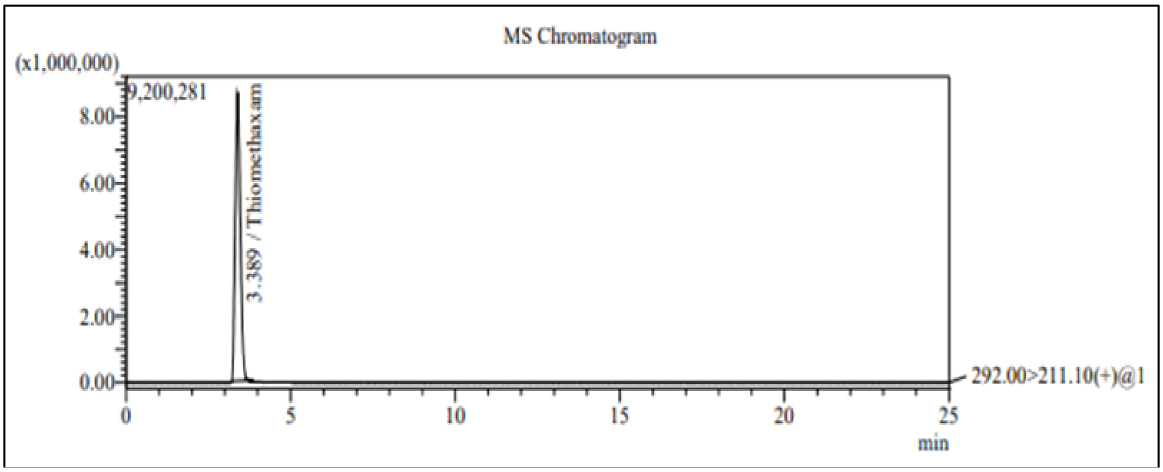


Plate 2: LC-MS Chromatogram of thiomethoxam in okra sample of non-IPM plot

Table 1: Residue levels of various insecticides in okra fruits of IPM and non-IPM plots

S. No	Plot	Matrix	Spiked level (gm Kg^{-1})	Sample analysed	L.O.D (PPB)	L.O.Q (PPB)	Recovery (%)	Retention time (min.)	Residues detected (ppm)	MRL values (ppm)
1.	Non-IPM	Okra	10	Imidachloprid	2	10	70-120	1.964	6.7	2.00
2.	Non-IPM	Okra	10	Thiomethoxam	2	10	70-120	3.389	3.8	0.50
3.	Non-IPM	Okra	10	Flubendiamide	2	10	70-120	12.360	7.9	0.20
4.	Non-IPM	Okra	10	Chlorantraniliprole	2	10	70-120	11.520	6.5	0.30
5.	IPM	Okra	10	Imidachloprid	-	-	-	-	ND	2.00

IPM - Integrated Pest Management
L.O.D – Limit of Detection
L.O.Q – Limit of Quantification
PPB - Parts Per Billion
MRL – Maximum Residue Limit

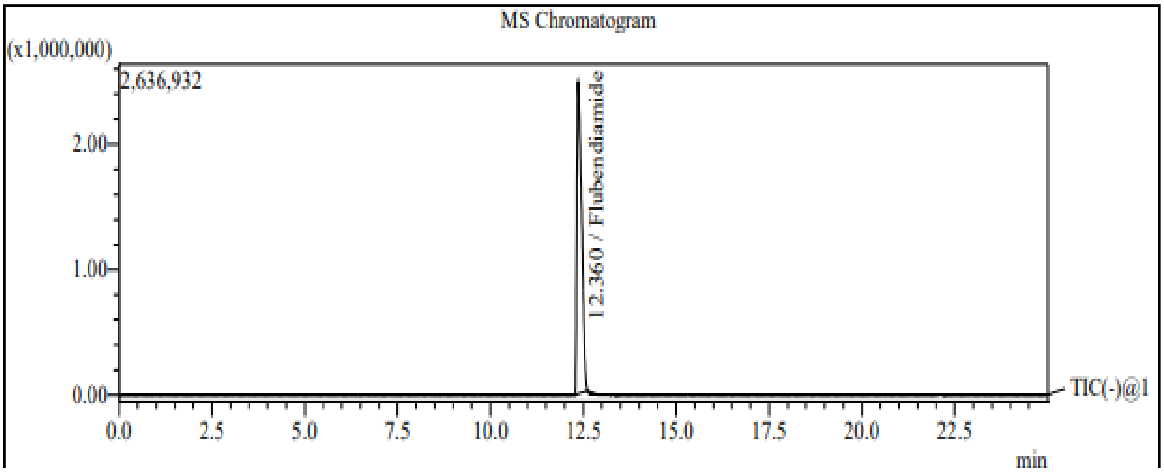


Plate 3: LC-MS Chromatogram of flubendiamide in okra sample of non-IPM plot

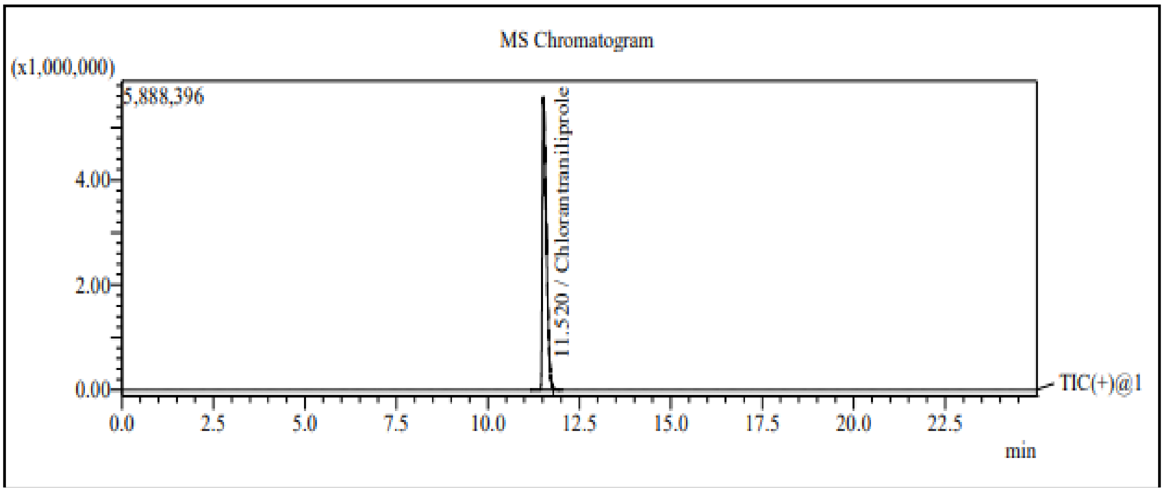


Plate 4: LC-MS Chromatogram of chlorantraniliprole in okra sample of non-IPM plot

Conclusion

Pesticide Residue Analysis was carried out in okra fruits to determine the amount of residues present in IPM and non-IPM plots and the results revealed that no pesticide residues were found in or on okra fruits grown and harvested in IPM plots. However, the pesticides used in the non-IPM plots were detected above MRL's through high end LC-MS machines.

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