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Dissipation and persistence studies of certain pesticides in/on Okra

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Abstract
Persistence studies of cypermethrin and spiromesifen (50 and 125 g *a.i.*/ha) when applied on okra (var. Arka Anamika) twice at 10 days interval for controlling shoot and fruit borer and leaf hopper under field conditions during *rabi* 2015-16 at fruit initiation stage was conducted. Okra fruits from recommended dose of cypermethrin and spiromesifen detected initial residues of 0.276 mg/kg and 2.401 mg/kg which persisted upto 7 days and 10 days after final spray and dissipated to below limit of quantitation of 0.05 mg/kg by 10^{th} and 15^{th}day with half life of 1.893 and 1.954 days respectively.

Keywords: Cypermethrin, spiromesifen, dissipation behaviour, residues**,** okra fruits

1. Introduction
Okra, *Abelmoschus esculentus* L. (Moench) or ladies finger also known as Bhendi is an economically important vegetable crop with high export potential. It is grown throughout the tropical and subtropical regions. The commercial cultivation of this crop is significantly affected by the infestation of major insect pests *viz.*, shoot and fruit borer, *Earias vitella* (Fabricius) leaf hopper, *Amrasca biguttula biguttula* (Ishida) white fly *Bemesia tabaci* (Gennadius). Various scientists have reported the losses in fruit yield of okra due to insect pests in the range of 65-85 per cent (Deen *et al.* 2009) [5] from germination to harvesting. These insect pests reduce the okra fruit quality and quantity. Intensive sprays of insecticides are required to protect the crop at fruiting stage are more likely to leave toxic residues on fruits, which may be hazardous to the consumers. The consumption of pesticide treated commodity become risky due to the residual persistence of the pesticide. So, it is necessary that, pesticide should be effective against pest along with its toxicologically acceptable residue on food commodity (Singh *et al.*, 2006) [6].
There is currently an increasing concern and awareness about the hazards of pesticides to consumers. Even with the adoption of IPM, farmers believe in the control of pests using pesticide because of its quick effect. The application of pesticides pre or post-harvest could, however, leave residues on food products which pose a potential risk to the health of consumer. Hence, great significance has to be given to know the dissipation pattern of commonly used pesticides by following Good Agricultural Practices (GAP) and adopting standard QuEchERs method (Anastassiades *et al.*, 2003) [1]. Keeping this in view, an attempt was made to study the persistence of cypermethrin and spiromesifen in/on okra.

2. Materials and Methods
2.1 Field experiment
The field experiment was carried out at student farm, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India located at 17º19'19.2" latitude, 78º24'39.2" longitude and at an elevation of 534 m above MSL. The okra (var. Arka Anamika), was raised as per the package of practices of PJTSAU, 2015 was sprayed with cypermethrin 10% EC and spiromesifen 22.9% SC at recommended dose of 50 g *a.i.*/ha and 125 g *a.i.*/ha. Two sprays of each insecticide were given separately at an interval of 10 days, imposing the first spray at fruit initiation stage. The weather parameters recorded during the period of study was given in Table 1.

Table 1: Weather parameters recorded during experimental period

Month & Year	Temperature (ºC)		Relative Humidity (%)		Rainfall (mm)
	Min.	Max.	I	II	
August, 2015	22.8	31.2	87.5	64.1	126.8
September, 2015	22.4	31.0	90.0	65.0	168.2
October, 2015	19.7	30.4	90.6	45.6	11.8

2.2 Chemicals and Reagents
Certified Reference Material of cypermethrin and spiromesifen with purity of 97.8 and 98.9 percent were obtained from Sigma Aldrich and commercial formulations were purchased from local markets of Hyderabad. The solvents of HPLC grade were n-hexane, anhydrous sodium sulphate and anhydrous sodium chloride obtained from Merck India Private Limited, Mumbai (India) and PSA from Agilent Technology, Bangalore respectively.

2.3 Residue Analysis
2.3.1 Standard preparation: Standard stock solution of (500 ppm) of cypermethrin and spiromesifen was prepared in n-hexane and serially diluted (0.5 ppm, 0.25 ppm, 0.1 ppm, 0.05 ppm, 0.025 ppm, 0.01 ppm) with n hexane from the intermediate stock standard solution (20 ppm) for instrument analysis. They were stored in refrigerator at -40 ºC. From intermediate standards, working standards were prepared by suitably diluting the stock solution in n-hexane and used as standard check in analysis, linearity and recovery studies.

2.3.2 Method validation: Prior to analysis of samples, linearity of cypermethrin and spiromesifen was established on GC-ECD and GC-MS/MS. Accuracy and precision of the method was determined by per cent mean recovery and per cent relative standard deviation. Linearity was studied by injecting standard solution of insecticides (0.05, 0.1, 0.25, 0.5, 0.75 and 1.0 mg/kg for cypermethrin; 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/kg for spiromesifen). The linearity curve was established with concentration of the standard and corresponding peak area. Recovery study was conducted in order to establish the reliability of the method of analysis. The okra samples from control plots were used for recovery studies. 15 g of the homogenized fruit sample was taken in 50 ml polypropylene tube. The samples were spiked with three different concentrations *viz*., 0.05 mg/kg (LOQ), 0.25 mg/kg (5xLOQ) and 0.5 mg/kg (10xLOQ) in triplicate. The extraction and clean up were performed as per modified QuEChERS method. Percent recovery was calculated by using following formula

$$\text{Per cent recovery} = \frac{\text{Quantity of pesticide recovered}}{\text{Quantity of pesticide added}}$$

2.3.3 Sampling: Treated okra fruit samples (2 kg) were collected randomly from each plot in polythene bags treatment wise and replication wise separately along with control samples at regular time interval of 0 (2 hrs after spraying), 1, 3, 5, 7, 10 and 15 days after the second spray. The collected samples were brought to the laboratory in polythene bags and processed immediately.

2.3.4 Extraction and Clean up: The okra fruit samples were homogenized with Robot coupe blixer. Homogenized 15±0.1g sample was taken in 50 ml centrifuge tube and 30±0.1 ml acetonitrile was added. The samples were homogenized at 14000-15000 rpm for 2-3 min using Heidolph silent crusher. The sample was then added with 3±0.1 g sodium chloride, mixed by shaking gently followed by centrifugation for 3 min at 2500-3000 rpm to separate the organic layer. The top organic layer of about 16 ml was taken into the 50 ml centrifuge tube and added with 9±0.1g anhydrous sodium sulphate to remove the moisture content. 8 ml of extract was taken in to 15 ml tube containing 0.4±0.01gr PSA sorbent (for dispersive solid phase (d-SPE) cleanup) and 1.2±0.01 g anhydrous magnesium sulphate. The sample tube was vortexed for 30 sec then followed by centrifugation for 5 min at 2500 – 3000 rpm. The extract of about 2 ml was transferred into test tubes and evaporated to dryness using Turbovap with nitrogen gas and reconstituted with 1 ml n-Hexane for GC analysis with ECD for cypermethrin analysis and GC MS/MS analysis for spiromesifen analysis under standard operational conditions (Table 2 and 3).

2.3.5 Kinetic Study
To calculate the rate of degradation, waiting period and half-life of deltamethrin on green pods of chickpea, Hoskin's (1961) [3] linear regression equation was followed. The period to be allowed to expect the residues to reach below the tolerance limit after treatment was calculated by using the formula (Gunther and Blinn, 1955) [2].

Y = a + b X where,
Y - Log of tolerance limit
a - Log of initial deposit
b - Slope of the regression line

Table 2: Instrument Parameters for cypermethrin

Gas chromatograph	AGILENT GC- 7890B.
Detector	Electron Capture Detector (ECD)
Column	GC capillary column, 35-MS 30mts, 0.25 mm ID, 0.25 µm Film Thickness
Injector temperature	260ºC
Injector status	Split 5
Carrier gas	Nitrogen
Carrier gas flow	1.8 ml/min
Column oven	180 ºC-2 min @10 ºC -260 ºC 5-min hold@2 min-280ºC-10min hold TOTAL 35 min
ECD temperature	300ºC
Makeup flow	35 ml/min
Retention time (RT)	19.9 min

Table 3: Instrument parameters for spiromesifen

Gas Chromatograph	Bruker Scion 436 GC
Detector	MS/MS TQD
Column	Zebron 5 MS (Phenomenex) 30 m length, 0.25 mm I.D, 0.25 µm FT
Injector temp	260° C
Column oven temp	150°C 3.00 min hold, 260°C- 20°C/min, 11.50 min hold TOTAL: 20.00 min
Carrier gas	Helium
Carrier gas flow	1 ml/min
Source Temp	220° C
Transfer line Temp	250° C
RT	10.6
Precursor ion	272
Product ions	209, 226, 231, 254
Quantifier ion	209

3. Results and Discussion

3.1 Linearity studies: The detector response to the neat standards of the insecticides was studied by injecting five linear concentrations of cypermethrin and spiromesifen separately. The graph was plotted with detector response against respective concentrations and linearity plot was drawn. The response of the instrument was linear over the range tested and R^2 value was 0.99 for cypermethrin and spiromesifen (Fig 1 and 2). The results indicated that the method is valid for residue determination of the tested insecticides in okra fruits. Accuracy of the analytical method was determined by recovery studies. The percent recovery was within the acceptable range of 70-120 per cent (SANTE, 2015) [7] prescribed in Table 4.

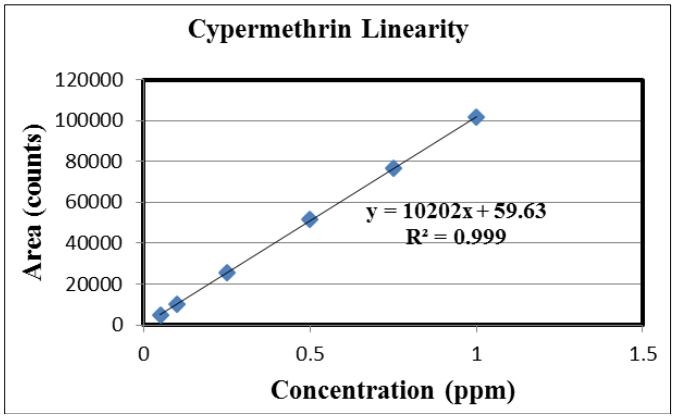

Fig 1: Linearity of cypermethrin

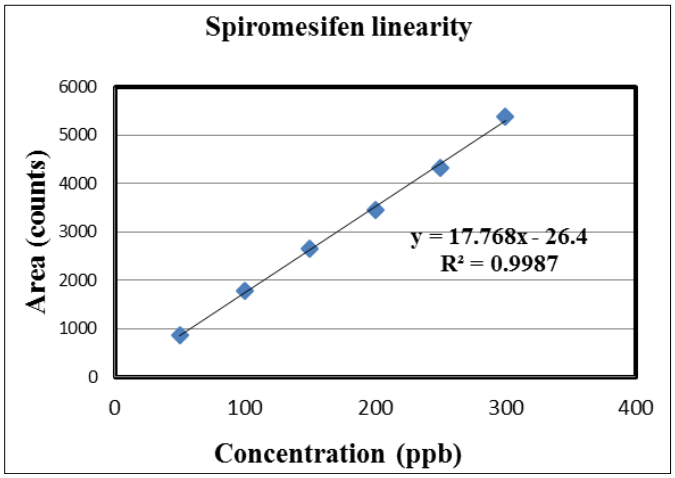

Fig 2: Linearity of spiromesifen

Table 4: Recovery studies for Cypermethrin and spiromesifen in okra

Fortification level	Recovery (%)± SD	
	Cypermethrin 10% EC	Spiromesifen 22.9% SC
0.05 mg/kg	100.00 (±2.00)	96.00 (±7.94)
0.25 mg/kg	88.66 (±2.00)	91.61 (±3.11)
0.5 mg/kg	87.73 (±1.48)	88.80 (±4/18)

The recovery of cypermethrin is 100.0% from the okra fruit samples fortified at 0.05 mg/kg and 88.66% at 0.25 mg/kg fortified level while the samples fortified with 0.50 mg/kg have shown the recovery of 87.73%. The recovery of spiromesifen is 96.00% from the okra samples fortified at 0.05 mg/kg and 91.61% at 0.25 mg/kg fortified level while the samples fortified with 0.50 mg/kg have shown the recovery of 88.80%.

3.2 Dissipation

The results revealed that there was reduction in residue levels of two test insecticides in/on okra with time (Table 5). Residues were not detected from okra fruits collected from control plots.

3.2.1 Dissipation of cypermethrin in okra fruits

At recommended dose of 50 g *a.i.*/ha, mean initial residues of cypermethrin were 0.276 mg/kg at 0 day (two hours after final spray) which dissipated to 0.158 mg/kg (42.75%) by 1st day, 0.110 mg/kg (60.14%) by 3rd day, 0.081 mg/kg (70.65%) by 5th day, 0.06l mg/kg (77.89%) by 7th day and to less than limit of quantitation of 0.05 mg/kg by 10th day with half life of 1.893 days. As there is no MRL available for cypermethrin in okra, 0.05 mg kg^{-1} may be taken as a default MRL. On the basis of this, Pre-Harvest Interval (PHI) of five days can be suggested for cypermethrin. The present research findings are in line with findings of Prasad *et al.* (1993) [9] wherein the dissipation of cypermethrin residues degraded from 55.7to 73.5 percent within seven days and reached BDL on 10th day. Khan *et al.* (1999) [10] reported half-life of 2.25 days for cypermethrin in okra fruits. However, in contrast to this, the half-life values of cypermethrin on chickpea green pods were 8.36 and 9.40 days following application of cypermethrin @ 60 and 90 g *a.i.* ha^{-1} (Kumar *et al.*, 1998) [11].

3.2.2 Dissipation of spiromesifen in okra fruits

In case of spiromesifen, mean initial residues of 2.401 mg/kg dissipated to 1.724, 1.053, 0.767, 0.238 and 0.062 mg/kg with a reduction of 28.19, 56.14, 68.05, 90.08 and 97.41 per cent at

1, 3, 5, 7 and 10 days respectively. The residues of spiromesifen reached below LOQ on 15th day of final treatment with half life of 1.954 days respectively. The present results were supported by the work of Hymavathi and Kiranmayi, 2020 who reported initial deposits of 2.401 mg/kg which dissipated to below quantitation limit of 0.05 mg/kg on the 15th day with half life of 1.943 days respectively whereas the results were contradicted by the work of Roy *et al.*, 2012 wherein the initial deposits documented was 0.964 mg/kg which dissipated to below quantitation limit of 0.01 µg g^{-1}on the 5th day with half life of 1.68 days.

Table 5: Residues of cypermethrin and spiromesifen in okra

Days after final Treatment	Control	Cypermethrin 10 5 EC @ 50 g *a.i.*/ha	Per cent dissipation	Spiromesifen 22.9% SC @ 125 g *a.i.*/ha	Per cent dissipation
0 day	ND	0.276 (±0.004)	-	2.401 (±0.199)	-
1 Day	ND	0.158 (±0.021)	42.75	1.724 (±0.078)	28.19
3 Days	ND	0.110 (±0.001)	60.14	1.053 (±0.139)	56.14
5 Days	ND	0.081 (±0.006)	70.65	0.767 (±0.030)	68.05
7 Days	ND	0.061(±0.001)	77.89	0.238 (±0.053)	90.08
10 Days	ND	LOQ	-	0.062 (±0.006)	97.41
15 Days	ND	LOQ	-	LOQ	-
Regression Equation	-	Y=0.159x+2.555	-	Y=0.1549+3.4534	-
R^2	-	0.978	-	0.963	-
Half life (days)	-	1.893	-	1.954	-

*ND – Not detected, LOQ - Limit of Quantitation (0.05 mg/kg)

4. Conclusion

The study clearly showed that cypermethrin and spiromesifen when used as foliar spray, did not record residues (<LOQ) at 10 and 15 days after treatment. Hence, cypermethrin and spiromesifen may be recommended as foliar spray to protect okra fruits from shoot and fruit borer and sucking pests, without the problem of presence of residues in fruits at harvest.

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