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Dissipation pattern of spiromesifen in/on brinjal fruits

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Abstract
A supervised field trial was conducted to study the dissipation of spiromesifen at Naraseepuram village, Thondamuthur, Coimbatore, on the brinjal variety CO 2, during January – February, 2018. The samples were collected up to 15 days after pesticide application along with control and processed by modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method. The final estimation of residues was carried out on Ultra High Performance Liquid Chromatography (UHPLC) with diode array detector. The mean initial deposit after two spraying of spiromesifen in/on brinjal fruit @ 96 g a.i ha^{-1} and 192 g a.i ha^{-1} were 0.715 µg g^{-1} and 1.123 µg g^{-1}, respectively. More than 80 percent of spiromesifen residues got dissipated on 3 days after treatment. The residues persisted up to 5 days after treatment and further dissipated to Below Detectable Limit (BDL < 0.05 µg g^{-1}) on 7 days after treatment. Dissipation of spiromesifen followed first order reaction kinetics and the calculated half life was 1.32 and 2.18 days, respectively for 96 g a.i ha^{-1} and 192 g a.i ha^{-1} dose. The safe waiting period of 1.40 and 2.18 days is recommended for brinjal sprayed with spiromesifen @ 96 g a.i ha^{-1} and 192 g a.i ha^{-1} dose respectively.

Keywords: Brinjal, dissipation, spiromesifen, half-life, safe waiting period

Introduction
Brinjal (*Solanum melongena* L.) is popularly known as eggplant and is widely grown vegetable of Asia, parts of Europe and Africa. It is one of the most traditional vegetables in India. It is a large and diverse genus of annual and perennial plants containing nearly 2000 species of which 37 are native to Asia. In India, it is one of the most common, popular and principal vegetable crops grown throughout the country except higher altitudes. Brinjal fruit (unripe) is primarily consumed as cooked vegetable in various ways and dried shoots are used as fuel in rural areas. It is low in calories and fats, contains mostly water, some protein, fiber and carbohydrates. Such an economically important commercial crop is reported variedly to be infested by 142 species of insects, 4 species of mites and 3 species of nematodes (Sohi, 1966) [16], 26 pests (Vevai, 1970) [18], 23 species of insects and 19 diseases (Gowda and Veeresh, 1984) [2], 50 insect pests (Nair, 1995) [6], more than 36 pests (Regupathy *et al*., 1997) [12] from the time of its planting to harvest.
Chemical insecticides are used as the frontline defense sources against these insect pests and brinjal growers in India depend heavily on synthetic pesticides to combat pests and consume about 46 percent of the total insecticides used in the country against vegetables. Most of the insecticides used on agricultural crops belong to a limited number of chemically different classes. Of them, the most important organic insecticides used against these pests of brinjal belong to organophosphates, carbamates and synthetic pyrethroids (Sidhu and Dhawan, 1977) [15]. At present, the insecticide research has met with selective, neuro active and easily degradable compounds. These newer molecules always have a higher stability and superiority over the conventional pesticides to control the pest population density in classical manner at field level. In this array, spiromesifen is one such novel and superior chemical with an aim to replace the highly effective broad spectrum acaricides, which were restricted due to their high mammalian toxicity and other side effects on non-target organisms. Spiromesifen belongs to the new chemical class of spirocyclic phenyl substituted by tetronic acids with non-systemic properties and has been developed, specifically for the effective control of all important mites and whiteflies (Nauen *et al.,* 2002; Nauen *et al.,* 2003) [8, 7]. This compound has been introduced in several countries over the last few years and is becoming an important compound for controlling whiteflies and mites in resistance management programmes, along with other effective insecticides such as neonicotinoids and diafenthiuron (Kontsedalov *et. al.,* 2008) [5].

Pesticides are applied to the crops, they may interact with the plant surfaces, be exposed to the environmental factors such as wind and sun and may be washed off during rainfall. The pesticide may be absorbed by the plant surface (waxy cuticle and root surfaces) and enter the plant transport system (systemic) or stay on the surface of the plant (contact). While still on the surface of the crop, the pesticide can undergo volatilization, photolysis chemical and microbial degradation. All these processes can reduce the original pesticides concentration but can also introduce some metabolites in the crops. The products formed may be less or more toxic than the parent chemical. Hence it is important to know the dissipation pattern, half life and waiting period of insecticides applied on the brinjal field to ensure the food safety. With the above background, this study was undertaken to study the dissipation pattern of spiromesifen in brinjal agro ecosystem.

2. Materials and Methods

A supervised field trial was conducted to study the dissipation of spiromesifen at Naraseepuram village, Thondamuthur, Coimbatore, on the brinjal variety CO 2, during January – February, 2018. The experiment was laid out in randomized block design in a plot size of 50 m^2 and replicated thrice, including untreated control. The brinjal plots were sprayed with spiromesifen 22.9 SC - X dose (96 g a.i. ha^{-1}) and 2X dose (192 g a.i. ha^{-1}) twice i.e., first spraying at fruit initiation stage and subsequent spraying at 10 days interval using hand operated knapsack sprayer. It was ensured that the insecticide under investigation had not been used earlier in the experimental plot.

The reference standard of spiromesifen (99.9% purity) was purchased from M/S Sigma Aldrich, Bangalore, India. Acetonitrile of HPLC grade, sodium chloride and anhydrous magnesium sulphate of analytical grade were purchased from Merck (Mumbai, India). Primary Secondary Amine (PSA) (Bondesil 40 µm) and Graphitized Carbon Black (GCB) were purchased from M/S Agilent technologies, USA. Primary stock solutions of spiromesifen (400 µg ml^{-1}) standard was prepared with HPLC grade acetonitrile in a volumetric flask. An intermediate stock solution of 100 µg ml^{-1} and 10 100 µg ml^{-1} was prepared from primary stock solution and working standards was prepared from intermediate stock. All the stock and working standard solutions were stored in the refrigerator at -20 °C.

The linearity study was conducted by injecting five different concentrations of standard solution following three replications of spiromesifen. The limit of detection (LOD) was calculated by considering signal-to-noise ratio of three with reference to the background noise obtained from blank sample and the limit of quantification (LOQ) by considering a signal to noise ratio of ten. Recovery studies were conducted to assess the validity of the present method. The homogenized untreated brinjal fruit samples (10g) were spiked at three different concentrations viz., 0.05, 0.25 and 0.5 µg g^{-1} of separately using analytical standard solution of spiromesifen. Each treatment was replicated three times with untreated control. The spiked samples were equilibrated for one hour and residues were extracted and estimated as per the method mentioned above. The control brinjal fruit samples were analysed and the result indicated that blank sample did not contribute any interference with the target compounds. The percentage recovery was calculated by comparing the peak area of the spiked standards with those of the pure standards by using the below formula.

$$\text{Percent recovery} = \frac{\text{Residue quantified in fortified level}}{\text{Fortified level}} \text{ x } 100$$

One kilogram of fruit sample was collected randomly at 0 (one hour after spraying), 1, 3, 5, 7 and 10 days after spraying from spiromesifen treated field along with untreated control separately. The collected samples were transported to the laboratory and processed immediately. The fruits were chopped into small pieces, from which a sub sample of 500 g was taken and homogenized with a mixer grinder. The residues were extracted by following the modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method (Anastassiades *et al.,* 2003) [1]. A representative sample of 10 g was transferred into a 50 ml centrifuge tube and mixed using a vortexer for one minute after adding 20 ml of acetonitrile. Four gram of anhydrous magnesium sulphate ($MgSO_4$) and 1 g of sodium chloride (NaCl) were subsequently added, shaken well by vortexer and then centrifuged at 6000 rpm for 10 minutes. Nine milli liter of supernatant was transferred to test tube containing anhydrous sodium sulphate (Na_2SO_4) and 6 ml of supernatant aliquot was transferred into a 15 ml centrifuge tube containing 100 mg Primary Secondary Amine (PSA), 600 mg anhydrous magnesium sulphate ($MgSO_4$) and 10 mg Graphitised Carbon Black (GCB). The mixture was vortexed for 1 minute and then centrifuged for 10 minutes at 3000 rpm. The upper extract (4 ml) was transferred into a turbovap tube and concentrated to dryness under a gentle stream of nitrogen in a turbovap LV at 40° C. HPLC grade acetonitrile (1ml) was added to test tube, shaken well reconstituted 1 ml was transferred into a 1.5 ml glass auto sampler vial for analysis. The residues of spiromesifen were estimated by Ultra High Performance Liquid Chromatography (UHPLC) (Shimadzu, series 2020) equipped with diode array detector (SPD-M20A), Chromatographic separation was achieved with reverse phase - C18 (Agilent) column, 250 mm length x 4.6 mm id x 5 µ particle size in a column oven, at 40 °C. The low pressure gradient condition employed with a mobile phase of acetonitrile and water (80:20) with a flow rate of 1.0 ml $minute^{-1}$ and the injection volume was 20 µl.

The final quantification of pesticide residues was calculated using the following formula

$$Residues\ (ppm) = \frac{As}{Astd} x \frac{Wstd}{Ws} x \frac{Vs}{Asj}$$

Where, As: Peak area of the sample; Astd: Peak area of the standard; Wstd: Weight of the standard in ng; Ws: Weight of the sample in g; Vs: Volume of the sample (final extract in ml); Asj: Aliquot of the sample injected in ml. The insecticide degradation pattern was analyzed by applying seven transformation functions as suggested by Hoskins (1961) [4] and Timme *et al.* (1986) [17]. The half-life was calculated using Pesticide Residue Half Life Calculator software developed by Department of Soil Science, Tamil Nadu Agricultural University, Coimbatore based on Regupathy and Dhamu (2001) [11] and best fit degradation model was determined. The safe waiting period was worked out as per the formula given by Handa *et al.* (1999) [3] using Codex Maximum Residual Limit (MRL)

$$\text{Safe waiting period } (T_{tol}) = \frac{\log K_2 - \log(\text{MRL/tolerance})}{\log K_1}$$

Where, K_1 is the slope of the regression line (b), always negative sign used as a positive number and K_2 is the apparent initial deposit obtained by extrapolating the line back to zero time.

3. Results and Discussion

Supervised field trial was conducted in farmers field to study the degradation behavior of spiromesifen residues in/on brinjal. Further, fruit samples were collected from insecticides treated plots and subjected to residue analysis in order to study the degradation behavior of the spiromesifen. The linearity of the calibration curves was established in the range of 0.05 to 0.8 μg g^{-1} and the correlation coefficient (R^2) obtained was 0.992 (Figure 3). The LOQ and LOD values for spiromesifen were 0.015 and 0.05 μg g^{-1} in UHPLC. The results of the recovery study of spiromesifen carried out in brinjal fruits (Table 1) revealed that the mean percent recoveries of spiromesifen were 89.22, 92.26 and 93.13 in brinjal fruits with Relative Standard Deviation (RSD) percentage of 2.43, 4.66 and 3.88 when samples were spiked at 0.05, 0.25 and 0.5 μg g^{-1}, respectively. The mean percent recoveries of spiromesifen residues in brinjal ranged from 80 to 120 percent and RSD were below 20 percent. According to SANTE (2017) (Document No.SANTE/11813/2017), any recovery range of 60 to 140 percent is acceptable for method validation. Based on the recovery study, the suitability of modified QuChERS method for residue analysis of spiromesifen in brinjal thus confirmed.

The results of persistence and dissipation of spiromesifen in brinjal fruits sprayed at 96 g a.i ha^{-1} and double the recommended dose 192 g a.i ha^{-1} are presented in Table 2. The mean initial deposit (1 hour after spraying) of spiromesifen on brinjal fruits was found to be 0.715 and 1.123 μg g^{-1}, at single and double the doses. At recommended dose, spiromesifen residue dissipated to 0.37,0.123 and 0.052 μg g^{-1} on 1, 3 and 5 days of treatment with dissipation percentage of 48.15, 82.79 and 92.69 percent and reached Below Detectable Limit (BDL) of less than 0.05 μg g^{-1} on 7 days after treatment. At double the recommended dose, spiromesifen mean residues were 0.548, 0.157 and 0.090 μg g^{-1} with percent loss of 51.16, 85.98, 83.3 and 92.02 after 1, 3 and 5 days after spraying, respectively and reached BDL on 7 days after treatment.

The dissipation pattern of spiromesifen in brinjal fruit was computed following seven transformations and the best fit observed was first order kinetics for both the doses (96 g a.i ha^{-1} and 192 g a.i ha^{-1}) (Table 3). The statistical parameters like intercept (a), slope of regression lines (b) and half life were presented in Table 3. The half life values of spiromesifen on brinjal fruit were found to be 1.32 and 1.35 days at recommended and double the recommended dose. Since, Maximum Residual Limit (MRL) for spiromesifen is 0.5 μg g^{-1} for brinjal by Food Safety Standards Authority of India (FSSAI), the calculated safe waiting periods were 1.40 and 2.18 days for recommended dose and double the recommended dose.

Present study is in accordance with the results of Sharma *et al.* (2005) [14], who reported the persistence of spiromesifen in apple in four locations and the initial deposits of spiromesifen were 0.91, 0.99, 0.99 and 0.88 μg.kg^{-1} at recommended dose, respectively. Raj *et al.* (2012) [10] reported the dissipation of spiromesifen on okra and the initial deposits 0.96 and 1.81μ g g-1 at standard (48 g.a.i.ha^{-1}) and double (96 g.a.i.ha^{-1}) dose, gradually declined and persisted up to 3rd and 5th day at lower and higher dose. Initial deposits of 1.61 mg kg^{-1} of spiromesifen detected at 2 hours after last spray, dissipated to BDL at 10th day after spray. The half-life and safe waiting period for harvest was 2.09 and 10.00 days, respectively (Pathipati *et al.*2017) [9]. To conclude, present study indicated that the residues of spiromesifen 22.9 SC @ 96 g a.i ha^{-1} and @ 192 g a.i ha^{-1} in brinjal dissipated BDL (< 0.05 μg g^{-1}) on 7 days after treatment with calculated half-life of 1.32 and 2.18 days, respectively. The present study provided adequate information to the farmers for safe harvesting period (1.40 and 2.18 days for X and 2X dose) for brinjal sprayed with spiromesifen.

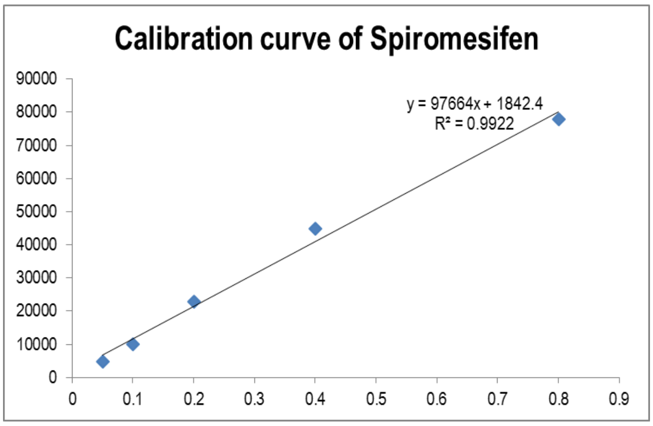

Fig 1: Linearity calibration curve of spiromesifen – UHPLC

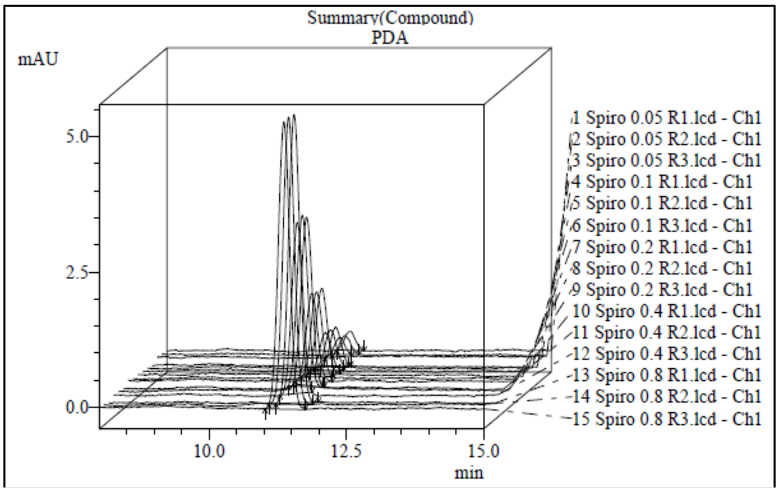

Fig 2: Standard curve of spiromesifen – UHPLC

Table 1: Recovery percentage of spiromesifen 22.9 SC in brinjal fruit

Spiked level (µg g^{-1})	Percent recovery (%)			Mean ± SD	RSD
	R1	R2	R3		
0.05	91.19	86.88	89.58	89.22 ± 2.18	2.43
0.25	87.32	95.63	93.82	92.26 ± 4.37	4.66
0.5	96.51	89.29	93.61	93.13 ± 3.63	3.88

Table 2: Persistence and dissipation of spiromesifen 22.9 SC residues in/on brinjal fruit

DAA	Spiromesifen residues (µg g^{-1})									
	Spiromesifen 22.9 SC @ 96 g a.i. ha^{-1}					Spiromesifen 22.9 SC @ 192 g a.i. ha^{-1}				
	R1	R2	R3	Mean	Dissipation (%)	R1	R2	R3	Mean	Dissipation (%)
0 (1 hr)	0.697	0.734	0.712	0.715	0.00	1.115	1.076	1.177	1.123	0.00
1	0.362	0.379	0.372	0.371	48.15	0.561	0.516	0.568	0.548	51.16
3	0.133	0.113	0.123	0.123	82.79	0.158	0.153	0.161	0.157	85.98
5	0.057	0.049	0.051	0.052	92.69	0.076	0.091	0.101	0.090	92.02
7	0.000	0.000	0.000	0.000	100.00	BDL	BDL	BDL	BDL	100.00

Table 3: Correlation coefficient and half life for spiromesifen in/on brinjal by different methods of transformations of residues data

Function	Spiromesifen 22.9 SC @ 96 g a.i.ha^{-1}					Spiromesifen 22.9 SC @ 192 g a.i.ha^{-1}				
	a	b	r	R^2	T Half	a	b	r	R^2	T Half
First order	-0.416	-0.523	-0.997**	0.993	1.32	-0.036	-0.511	0.997**	0.994	1.35
1.5th order	1.065	0.644	0.996**	0.992	0.68	0.929	0.492	0.993**	0.988	0.78
2nd order	-0.163	3.563	0.971*	0.944	-0.05	0.299	2.108	-0.982*	0.965	0.14
RF First order	-0.037	-1.229	-0.982*	0.965	0.31	0.365	-1.224	-0.988*	0.977	0.32
RF 1.5th order	0.676	1.451	0.942^{NS}	0.887	0.08	0.598	1.136	0.966*	0.933	0.09
RF 2nd order	-1.957	7.751	0.887^{NS}	0.786	0.06	-0.924	4.714	0.932^{NS}	0.869	0.04
Inverse power law	-0.204	0.354	0.964^{NS}	0.746	7.07	-0.187	0.361	0.888^{NS}	0.789	6.83

a - Intercept; b – Slope; r - regression coefficient; RF- root function; * significant at 5% level; ** significant at 1% level,

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