www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(1): 1148-1152

www.thepharmajournal.com
Received: 22-11-2021
Accepted: 24-12-2021

RG Dudwal
Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan, India

BL Jakhar
Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan, India

ARK Pathan
Sri Karan Narendra Agriculture, University, Jobner, Rajasthan, India

AK Yadav
Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan, India

S Ramesh Babu
Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Alka Kataria
Deemed to be University, Jaipur, Rajasthan, India

Beerendra Singh
Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Corresponding Author
RG Dudwal
Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan, India

Study the persistence of spiromesifen residues in the soil of chilli field under semi-arid region of Rajasthan

RG Dudwal, BL Jakhar, ARK Pathan, AK Yadav, S Ramesh Babu, Alka Kataria and Beerendra Singh

Abstract
A field experiment was conducted at Rajasthan Agricultural Research Institute, Durgapura, Jaipur during Kharif, 2020 to study the persistence of spiromesifen residues in the soil of chilli field, when sprayed at its recommended dose (spiromesifen 96 g. ha^{-1}) and double of the recommended dose (spiromesifen 192 g. ha^{-1}). The samples were extracted and cleaned up using a modified QuEChERS method and the residues were analyzed by GC-ECD. The residues level of spiromesifen in chilli field soil collected at harvest time of chilli crop were below the detectable level (BDL) at the recommended dose and double of the recommended dose, respectively.

Keywords: dissipation, residues and spiromesifen

1. Introduction
Chilli, *Capsicum annum* L. is an important spice cum vegetable crop that belongs to the family Solanaceae. It is commonly grown throughout the year as a cash crop and used as green and red ripe dried stage for its pungency, colour and other ingredients in all culinary preparations of rich and poor alike to impart taste, flavour and colour. It is also called as sweet pepper, bell pepper or green pepper. Nutritionally, it is a rich source of capsicin, antioxidants, vitamins and minerals. It has very good export potential. Chilli is one of the most important condiments having immense commercial and therapeutic value (Reddy *et al*., 2007) [7].
Various factors are responsible for low productivity and production of chilli that include adverse climate, poor quality seeds, diseases and insect pest. The insects and mites are of prime importance which significantly affects both the quality and production of chilli. About 51 insects and 2 mite species, belonging to 27 families and 9 orders were found infesting chilli (Reddy and Puttaswami, 1988) [6]. Among these thrips, *Scirtothrips dorsalis* Hood, whitefly, *Bemisia tabaci* Genn., aphid, *Aphis gossypii* Glover, jassid, *Amrasca biguttula biguttula* (Ishida), fruit borer, *Helicoverpa armigra* (Hubner) and mites, *Polyphagotarsonemus latus* Banks are important pests contributing 60 to 75 per cent yield loss in green chilli. These cause maximum damage to the crop both during vegetative and fruit formation stages. To control these insect pests different insecticides are recommended. For managing insect pests of green chilli farmers rely mainly on the application of insecticides like acetamiprid 20% SP (thrips), chlorantraniliprole 18.5% SC (fruit borer), diafenthiuron 50% WP (mites), ethion 50% EC (mites, thrips), spinosad 45.0% SC (fruit borer,thrips), spiromesifen 22.9% SC (chilli yellow mite), buprofezin 25% SC (yellow mite) and fipronil 5% SC (thrips, aphids, fruit borers) are recommended by CIB & RC. (30.11.2021).
In modern agriculture, Enormous increase in agricultural productivity can be associated with the use of fertilizers and plant protection chemicals. The success of green and grey revolution in plants, yellow revolution in oil seed crops, white and blue revolution in animals was greatly facilitated by pesticides. The pesticides used for crop protection or public health purposes have to be safe guarded against their environmental impacts, which at times may pose serious health and environmental consequences. The fate of pesticides applied in agricultural ecosystem is governed by the transfer and degradation processes and their interaction.
During the recent years some agricultural commodities exported from India were rejected by European countries not only due to presence of physical and microbial contaminators but also due to the presence of pesticide residues. In this context monitoring of pesticide residue in agricultural produce has assumed greatest importance. Moreover, pesticide residues are equally important from the point of view of consumer's health and from the point of view of

environment as a whole. Some of these pesticides may remain on food as residues when pesticides are not applied as per the good agricultural practices (GAP), these pesticide residue can pose significant health risk to consumers. Chronic exposures to low levels of pesticides are known to cause low immunity to diseases, low learning capacity and host of other ailments. A recent report even indicts it for disturbances in sex ratio as well. Pesticide residues have created a threat to human life, biotic and abiotic factors of environment. Inspite of very low consumption of pesticides per unit area in India <0.5 kg ha^{-1} as against 6-17 kg ha^{-1} in developed countries, pesticide residues in food stuffs and feed are mainly noticed due to indiscriminate use of pesticides. The recent ICMR and ICAR (Pesticide Residue Project) reports showed that 8-10 per cent or 12 per cent of food commodities in India were with detectable levels of pesticides of which 2-3 per cent had levels exceeding MRL. Keeping in view the growing concern both at national and international scenes, concerted effort have been made by government of India through Indian council of agricultural research for safer and judicious use of pesticides.

It has been estimated that if the pesticides were not used in agriculture, the crop loss in the world would have been around 40 per cent. India is losing about 90,000 cores of agriculture produces annually due to insect-pests. According to an estimate, every rupee invested in chemical pest control returns 3 in crops saved (Jeyanthi and Kombairaju, 2005) [4]. Although having their unquestionably benefits for food production and storage, there is also a growing awareness of the risks to human and ecological health associated with their use (Louis and Taisen, 2012) [5], thus may also be referred as essential evil, whose injudicious and unscientific use is the main cause of pesticide residue problems. The presence of pesticide residues in food and their entry into food chain has become a topic of widespread public debate.

A number of pesticides are being frequently used, to combat insect pests. However, some of these insecticides leave residues on pods and these residues may persist up to harvest. Presence of pesticide residues in the harvested chillies was posing problem at the time of export and in recent times importing countries have rejected few consignments. To generate persistence data of spiromesifen in chilli cropped soil, the study was carried out. Insecticides applied on the crop ultimately got way into the soil (Chopra *et al.*, 2010) [2]. Sushil *et al.*, 2018 [13] studied the persistence of spiromesifen residues in soil of hot pepper (*Capsicum annuum*) field. So persistence of these recommended insecticides in soil also carried out during the study. Thus, keeping in view, this paper reports dissipation and residues of formulation (spiromesifen) in chilli field soil at recommended dose and double of the recommended dose.

2. Materials and Methods

2.1. Reagents and Instruments

Certified Reference Material (CRM) as procured from accu standard and all the solvents used were HPLC grade. The chemicals (Na_2SO_4, primary secondary amine (PSA) and $mgSO_4$ were used analytical regent grade and activated by heating at 30 ^oC for 12 hrs and kept in desiccators.GC-Shimadzu-2010, Analytical balance, Mixer, Centrifuge and Turbovap.

2.2. Pesticides and application rate

Commercial formulations, of spiromesifen (22.9 % SC) received from UPL Ltd, Mumbai recommended dose @ 96 g. ha^{-1} and double of recommended dose @ 192 g. ha^{-1}

2.3. Field experimental design

The field experiment was conducted at Rajasthan Agricultural Research Institute, Durgapura, Jaipur during Kharif, 2020. The soil of the experimental field was sandy loam with pH 8.1, EC 0.18 dSm^{-1} , organic carbon 2.1 g kg^{-1}, available N 178 kg $^{ha-1}$, P_2O_5 21.8 kg $^{ha-1}$ and K_2O 193.4 kg $^{ha-1}$. The experiment consisted of three treatments viz. control, recommended dose of spiromesifen 22.9 % SC (96 g. ha^{-1}) and double of the recommended dose spiromesifen 22.9 % SC (192 g. ha^{-1}) with four replications. All the agronomic practices were followed. No rainfall received during the experimental period. The first spray of insecticide was done at fruit formation stage and second spray at 10 days interval at recommended dose (96 g. ha^{-1}) and double of recommended dose (192 g. ha^{-1}), where as one plot was left untreated and used for the sampling of fruits as control in each treatment. About 1 kg of soil sample was collected randomly and separately from the control and treated plots of each treatments at harvest of chilli crop.

2.4. Sampling

Soil

Soil samples (1 kg) from the sprayed field of chilli were drawn from each replication at harvest time for analysis. During sampling, soil samples were collected from the depth of 0-15 cm. The samples were placed into plastic containers and allowed to shade dry at room temperature in the laboratory. The air dried samples were desegregated manually using a pestle and a marble mortar, passed through a No. 20 mm brass soil sieve and mixed thoroughly to achieve homogeneity.

2.5. Extraction (QuEChERS)

10 g representative soil sample were taken in a 50 ml centrifuge tube and 20 ml acetonitrile was taken (Asensio-Ramos *et al.* 2010) [1]. Shaken vigorously for one minute, 4 g of magnesium sulphate and 1 g of sodium chloride were added. To improve the recovery values, citrate buffered medium (1g trisodium citrate dehydrate and 0.5 g of disodium hydrogen citrate sesquihydrate was added). Centrifuge at 3,300 rpm for 5 minutes. 10 ml of the supernatant were taken into 15 ml centrifuge tube containing 1.5 g of magnesium sulphate and 250 mg of PSA. The content was shaken for few seconds and then sonicated for 1 minute; the tube was centrifuged for 10 minutes at 4,400 rpm. From the above centrifuge tube, 4 ml aliquot were taken of the supernatant and evaporated to dryness using rotavapour at 40 ^0C. The dry residue was redissolved in 1 ml hexane. In case, aqueous phase is noticed, little amount of anhydrous sodium sulphate were added and filtered through PTFE filters.

2.6. Standards

The reference standard of spiromesifen obtained from Pesticide Residues Laboratory, Division of Entomology, RARI, Durgapura, Jaipur, Rajasthan, was used for quantification.

Spiromesifen

a. Standard stock solution: The analytical grade spiromesifen with 98.2% purity was dissolved in 100 ml volumetric flask with hexane to get 1000 mg kg^{-1} standard stock solution.

b. Intermediates stock solution: The standard stock solution was brought at room temperature and 1 ml from the standard stock solution was transfer to 100 ml volumetric flask, made up the volume and shaken well to obtain a homogenous intermediates stock solution of 10 mg kg $^{-1}$. This was utilized for fortification of samples.

c. Working standard: From the intermediate stock solution, after brining to room temperature, working standard of 0.01 to 1 mg kg^{-1} were prepared by serial dilution techniques and labeled graduated test tubs. The working standards were used to find out retention time of these compounds and for quantitative determination of residues in samples.

2.7. Recovery studies: The soil samples were fortified at 0.05, 0.25 and 0.50 mg kg^{-1} for spiromesifen by adding required quantity of 10 mg kg^{-1} intermediates stock solution to work out the recovery per cent of analytical methodology.

2.8. Instruments parameters: Spiromesifen residues were estimated by Shimadzu-2010 gas chromatograph fitted with capillary column DB 5, 30 m x 0.25 mm ID 0.25 µm film thicknesses.

The following were the operating parameters

Column temperature ^{0}C	**Rate (^{0}C/min.)**	**Temp. (^{0}C)**	**Hold time (min)**
	------	160.0	1.00
	7.0	280.0	5.00
Injector temperature ^{0}C	280		
Detector temperature ^{0}C	300		
Gas flow rate (ml min^{-1})			
Total flow (Detector)	12.0		
Column flow	1.50		
Aliquot injected	1µl		

2.9. Analysis of spiromesifen residues

The detection and quantification of spiromesifen residue in soil the GC, Shimadzu-2010 instrumental parameters were used. Prior to injection of the sample extract, standard solutions of different concentrations of pesticides were prepared and injected in the instrument. Insecticide compound were qualitatively identified by comparing the retention time of peaks and quantitatively estimated on the basis of area of chromatograms obtained in each test sample with that of the analytical standard. Sample results were expressed in mgkg^{-1}. From this value of actual amount of insecticide residue presented in the sample was determined by using the following formula,

Residue in Sample (mgkg^{-1})

Soil:

$$\text{Residues (ig/g)} = \frac{\text{Peak area (Sample) X Conc.std (ppm) X µL std injected X Final volume of the sample (1 mL)}}{\text{Peak area (Std) x weight of the sample (2 g) xµL of sample injected}}$$

$$\text{Wt. of sample analyzed (g)} = \frac{\text{Sample wt. (10 g) X Aliquot taken (4mL)}}{\text{Volume of extract (20 ml)}} = 2\text{g}$$

Recovery

$$\text{Percent Recovery} = \frac{\text{Sample peak area}}{\text{Standard peak area}} \times 100$$

2.10. Statistical analysis

Statistical analysis was performed on Microsoft Excel-2016 (Microsoft Corporation, USA). All analysis was performed in triplicate and the results were expressed as mean ± SD.

3. Results and Discussion

3.1 Recovery

To ensure the reliability of the results the recovery study was also conducted for spiromesifen in soil. The soil samples were spiked with spiromesifen at 0.05, 0.25 and 0.50 mg kg^{-1} fortification levels and analysed as per the methodology described earlier.

The results of the recovery study are presented in Table 1. The recovery study of spiromesifen was carried out at the fortification level of 0.05, 0.25 and 0.50 mgkg $^{-1}$ in soil. The mean recovery of spiromesifen at 0.05, 0.25 and 0.50 mg kg^{-1} fortification level was 89.0, 87.5 and 90.6 per cent in soil, respectively.

These present recovery experiment are in agreement with those of Raj *et al.* 2012 conducted a recovery experiment at fortification level of 0.01 µg g^{-1} of spiromesifen in soil and recovered mean recovery of spiromesifen from soil was 80 per cent, respectively.

According to the SANTE (2015) [12] guidelines, any analytical method which records mean recovery in the range of 70-120 per cent is accurate and precise. Hence, the method employed in the present study for the extraction of fipronil, its metabolites and spiromesifen from chilli fruits and soil was accurate and precise.

3.2 Residues

It appears that relatively low doses (96 g. and 192 g. a.i. ha^{-1}, respectively) may play a role in the faster dissipation/degradation of spiromesifen under the cover of chilli crop and favorable climatic conditions such as high temperature, some other factors, e.g. Evaporation, leaching and crop uptake may be assumed to play some role, leading to rapid dissipation of these pesticides.

Table 1: Percent recovery of spiromesifen in soil at different fortification levels.

Level of Fortification (mg kg^{-1})	Replications	Soil	
		µg recovered	Recovery (%)
0.05	R_1	0.043	86.0
	R_2	0.045	90.0
	R_3	0.043	86.0
	R_4	0.047	94.0
Mean±SD			89.0±3.317
0.25	R_1	0.222	88.8
	R_2	0.218	87.2
	R_3	0.214	85.6
	R_4	0.221	88.4
Mean±SD			87.5±1.245
0.50	R_1	0.449	89.8
	R_2	0.456	91.2
	R_3	0.445	89.0
	R_4	0.462	92.4
Mean±SD			90.6±1.304

Spiromesifen

Persistence and dissipation of spiromesifen residues in soil under cover of chilli crop have been studied (obtained from two treatments *i.e.* recommended dose (96 g. a.i. ha^{-1}) and double of the recommended dose (192 g. a.i. ha^{-1}) are given in Table 2). The soil samples were collected at harvest time of chilli crop. In case of soil samples the residues at harvest time of chilli crop was not detected in the recommended dose (96 g. a.i. ha^{-1}) and double of the recommended dose (192 g. a.i. ha^{-1}). The control samples of soil did not show the residues also.

Present studies are in agreement with Sharma *et al*. 2005a [9] who did not found the residues of spiromesifen in soil samples collected from apple orchard at 40 days after spray. Similarly, Sharma *et al*. 2006 [10] studied the soil samples, collected from egg plant field 15 days, and did not show presence of spiromesifen residue. The results are also similar with Sharma *et al*. 2007a [11] who studied in cotton and chilli field soil and no residues were detectable at harvest time. This is strongly support to present findings. Raj *et al*. 2012 analysed the soil samples collected on the 20th day after the last spray and found the spiromesifen residues at below quantitation limit of 0.01 µg g^{-1}.

Further, In 2018 Sushil *et al*. studied the persistence of spiromesifen residues in soil of hot pepper (*Capsicum annuum*) field. No residue was found in soil at the time of harvesting. This is also strongly supported to the present experiment. The observations in the present studies are in accordance with the findings of all the above researchers.

Table 2: Residues (mg kg^{-1}) of spiromesifen in soil under chilli crop at recommended dose (96 g. a.i. ha^{-1}) and double of the recommended dose (192 g. a.i. ha^{-1}).

Days	Replications	Recommended dose (96 g. a.i. ha^{-1})		Double of the recommended dose (192 g. a.i. ha^{-1})	
		Average* Residues ± SD	%Dissipation	Average* Residues ± SD	%Dissipation
Soil Control	R_1	ND	-	ND	-
	R_2	ND	-	ND	-
	R_3	ND	-	ND	-
	R_4	ND	-	ND	-
Soil at Harvest time	R_1	ND	-	ND	-
	R_2	ND	-	ND	-
	R_3	ND	-	ND	-
	R_4	ND	-	ND	-

3.3 Limit of quantiation and Limit of detection

Limit of quantiation (LOQ) and Limit of detection (LOD) of fipronil and its metabolites in soil was 0.001 and 0.0003 mg kg $^{-1}$. The residues level of both pesticides in field soil collected at harvest time of chilli crops were below detectable level (BDL) at recommended dose and double of the recommended dose.

3.4. Standard Curve

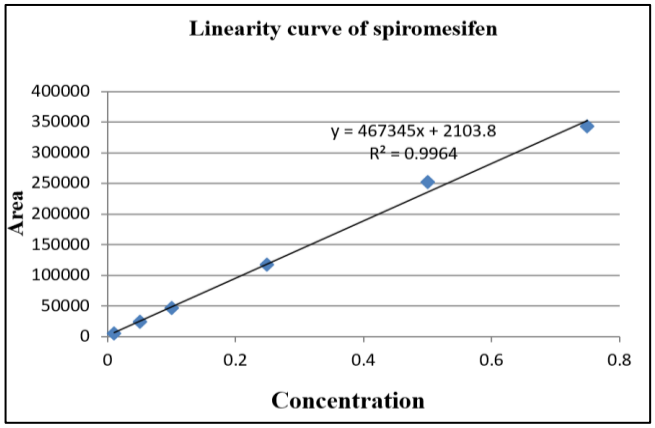

Fig 1: A standard curve of spirsomesifen

4. Conclusion

In this study, the field trial was conducted to investigate dissipation and residues of spiromesifen insecticide in soil. Residue of spiromesifen was successfully extracted from soil samples using the QuEChERS method. The developed method demonstrated acceptable accuracy and precision and was successfully applied to the dissipation kinetics of spiromesifen in soil. The present result suggested that the soil was free with pesticides residues of spiromesifen at harvest of chilli crop. This finding would help the government to provide guidance on the proper and safe use, and establishing local Maximum Residue Levels (MRL) for spiromesifen.

5. References

1. Asensio-Ramos, Hernandez-Borges M, Ravelo-Perez J, Riguez-Delgado MA. Anal Bioanal Chem. 2010;396:2307-2319.
2. Chopra I, Chauhan R, Kumari B, Dahiya KK. Fate of fipronil in cotton and soils under Tropical climatic conditions. Bulletin of Environmental Contamination and Toxicology. 2010;86:242-245.
3. Central Insecticide Board and Registration Committee. Major uses of pesticides. 31 October, 2019.
4. Jeyanthi H, Kombairaju S. Pesticide use in vegetable crops: frequency, intensity and determinant factors. Agricultural Economics Research Review. 2005;18:209-221.
5. Louis JG, Taisen I. Life in a contaminated world. Science. 2012;337:1614-1615.
6. Reddy DNR, Puttaswami. Pests infesting chilli (*Capsicum annum* L.) in the nursery. Mysore Journal of Agricultural Sciences. 1988;18(2):122-125.
7. Reddy KD, Reddy KN, Mahalingappa PB. Dissipation of fipronil and profenofos residues in chillies (*Capsicum annuum* L.). Pesticide Research Journal. 2007;19:106-107.
8. Raj MF, Solanki PP, Singh S, Vaghela KM, Shah PG, Patel AR, *et al*. Dissipation of spiromesifen in/on okra under middle Gujarat condition. Pesticide Research Journal. 2012;24(1):25-27.
9. Sharma KK, Dubey JK, Kumar A, Gupta P, Kalpana Singh B, Sharma ID, *et al*. Persistence and safety evaluation of spiromesifen on apple (*Maius domestica* L) in India, a multi-location study. Pesticide Research Journal. 2005a;17(2):77-81.
10. Sharma KK, Dubey JK, Mukherjee I, Parihar NS, Battu RS, Singh B, *et al*. Residual behavior and risk assessment of spiromesifen (oberon 240 sc) on eggplant (*Solanum melonongena* L) in India, A multi-locational study. Bulletin of Environmental Contamination and Toxicology. 2006;76:760-765.
11. Sharma KK, Rao CS, Dubey JK, Patyal SK, Parihar NS, Battu RS, *et al*. Persistence and dissipation kinetics of spiromesifen in chilli and cotton. Environmental Monitoring and Assessment. 2007a;132:25-31.
12. SANTE. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Document No. /11945, 2015, 20p.
13. Sushil, Reena Chauhan, Beena Kumari, RS Jaglan. Studies on persistence and dissipation behavior of selected pesticides in hot pepper (*Capsicum annuum*). International Journal of Chemical Studies. 2018;6(1):1791-1794.
14. Wu X, Yu Y, Xu J, Dong F, Liu X, Du P, *et al*. Residue analysis and persistence evaluation of fipronil and its metabolites in cotton using high-performance liquid chromatography- tandem mass spectrometry. PLoS ONE. 2017;12(3):e0173690. https://doi.org/10.1371/journal.pone.0173690.