ENTOMON 41(2): 149-156 (2016)
Article No. ent. 41210

Persistence and effect of processing on reduction of spiromesifen residues in chilli pepper (*Capsicum annum* L.) and soil

George Xavier[1*] and M. Chandran[2]

[1]Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamil Nadu. India 627012
[2] Department of Chemistry, Vivekananda College, Agasteeswaram, Kanyakumari, Tamil Nadu, India. Email: george.xavier@rediffmail.com

ABSTRACT: Residue dynamics of spiromesifen (Oberon 240 SC) in chilli fruits was studied using Ultra high performance liquid chromatography coupled with tandem mass spectrometer (UPLC-MS/MS). The initial deposits of spiromesifen in chilli fruits after application at 96 and 192 g ai ha^{-1}, were found to be 0.62 and 1.20 ìg g^{-1}, which reached below quantitation level after 21 and 27 days, respectively. Half-life of spiromesifen at single and double recommended dose were 3.65 and 3.19 days, respectively and the corresponding waiting period calculated was 0.74 and 4.49 days. The removal of spiromesifen residues using different simple decontamination 2 h after spraying and 3 days of spraying were 77.74 -88.18 % and 55.46 -71.00 % respectively. Processing factor due to sun drying in dry chilli fruits ranged from 2.12 to 1.33 during 0 to 15th day after application.

KEY WORDS: Chilli, Spiromesifen, Dissipation,Decontamination

INTRODUCTION

Chilli is the most important condiment, which adds pungency, taste, flavor and color to various cuisines around the world. Currently, India is the world leader in chilli production followed by China and Pakistan. Among the different constraints contributing to low chilli productivity, the pest complex that attack chilli crop at different crop stages is most important. Chilli farmers often resort to the use of more frequent and higher doses of pesticides, to overcome the losses due to these pests in commercial cultivation, thereby leading to unusual levels of their residue in the final produce. Also, the sprays of the popular conventional insecticide chemistries like organochlorines, organophosphates, carbamates and synthetic pyrethroids become a threat to chilli ecosystem causing resurgence of pest and threat to beneficial fauna. Since, insects and other animals have similar reproductive, hormonal and nervous systems; these compounds have potential for non target effects, including humans. Spiromesifen is a non-systemic insecticide-cum accaricide belonging to the chemical class of cyclic ketoenoles effective against the sucking pest complex in chilli. Spiromesifen is an acetyl CoA carboxylase inhibitor and the biological activity of cyclic keto enoles correlates with inhibition of lipogenesis, resulting in decreased lipid contents, especially of triglycerides and free fatty acids, in treated insects (The United Kingdom, 2004). Owing to its unique chemical structure it is a useful tool in resistance management in many cropping systems including cotton, tea, vegetables, fruits and ornamentals (Nauen et al., 2002).

* Author for correspondence

Farmers often resort to the application of pesticides at recurrent intervals, resulting in substantial residues in the chilli fruits. Pesticide residue will be strictly monitored in future owing to the high concern about the toxic residues. There are reports of chilli consignment rejection from India in European countries due to the presence of pesticide residues of ethion, triazophos, chlorpyriphos, phosphamidon, cypermethrin, fenvalerate and dicofol (Rao, 2005). Chilli being consumed more as dried products rather than green chillies, the residue accumulation will be more on dry weight basis. Degradation studies in soil are required for evaluating the persistence of pesticides in the environment. Chillies can be subjected to some simple household practices e.g., washing, cooking, removal of non edible parts, etc. before actual consumption. The effects of these processing techniques on residue levels are extremely important in evaluating the risk associated with residues.

A literature survey reveals that data pertaining to the dissipation of spiromesifen in/on fresh, dry chilli fruit and soil in Indian condition and the effect of various household preparations in reduction of residues in chilli is scanty. Therefore, the present investigation was carried out with the objective to examine the persistence of spiromesifen in chilli fruits both fresh and dry and in the soil and the effect of culinary practices on the reduction of residues for ensuring consumer safety.

MATERIALS AND METHODS

Chemicals and reagents

Analytical standards of spiromesifen purity 98 per cent, sodium citrate tribasic dihydrate and sodium hydrogen citrate sesquihydrate were procured from M/S Sigma-Aldrich, India and the formulation Oberon 240 SC was from Bayer Crop Science India Ltd. Solvents like acetonitrile, water (HPLC grade), sodium chloride, anhydrous sodium sulfate, and anhydrous magnesium sulfate (ACS reagent grade) were obtained from Merck Germany. Primary secondary amine (PSA) was obtained from Agilent Technologies, USA. All the glass wares were thoroughly washed as per the standard operating procedure to avoid the interferences from any contaminants during analysis. The suitability of the solvents and other chemicals were ensured by running reagent blanks before actual analysis.

Preparation of standard solution

Single laboratory method validation was done initially to test the efficiency of extraction and clean up procedures and to standardize the procedure for residue estimation of fresh, dry chilli fruits and soil. Standard stock solution of spiromesifen (1000 µg ml^{-1}) was prepared in HPLC grade methanol and serially diluted with acetonitrile to obtain the solutions required for forming a calibration curve (1.0, 0.50, 0.25, 0.10, 0.075, 0.05, 0.025, and 0.0l µg ml^{-1}. The standard solutions were stored at 4°C before use. The fortification study of the untreated fresh, dry chilli fruits and soil was carried out by spiking at 0.01, 0.05, 0.1, and 0.5 µg g^{-1} levels.

Instrument Parameters

The chromatographic separation was achieved using Waters Acquity UPLC system equipped with a reversed phase Atlantis C-18 (2.1 x 100 mm, 5 micron particle size) column. The operation of the LC gradient involved the following two eluent components: A: 10 % methanol in water + 0.1 % formic acid + 50 mM ammonium acetate; B: 10 % water in methanol + 0.1 % formic acid +50 mM ammonium acetate. The gradient elution was as follows: 0 minutes isocratic 5 % B, 0.0–3.0 minutes linear from 5 % to 80 % B, 3.0–4.0 minutes linear from 80 % to 100 % B, 4–6.5.0 minutes linear from 100 % to 50 % B, with 6.5–8.0 minutes for initial conditions of 5 % B. The flow rate remained constant at 0.8 mL min^{-1} and injection volume was 10µL. The column temperature was kept at 40°C. The effluent from the LC system was introduced into Triple quadrapole API 3200 MS/MS system equipped with an electrospray ionization interface (ESI), operating in the negative ion mode. The source parameters were temperature 550^0C; ion gas (GS1) 50 psi, ion gas (GS2) 50 psi, ion spray voltage 5500 V, curtain gas 13 psi and compound dependent parameters are Declustering potential-35 V -Entrance potential-6 V, CEP-Collision cell

entrance potential-28 V, CE-Collision energy-16 V, CXP-Collision cell exit potential-3.0 V. Under these operating conditions the retention time of spiromesifen was found to be 4.31. The MRM transitions used for the quantitative estimation of spiromesifen was m/z 371.2→273.2 and for qualitative estimation m/z 371.2→255.1 (Fig. 1).

Field experiment

Chilli crop was raised in a field located at Kalliyoor, Thiruvanthapuram, Kerala and maintained as per the Package of Practices Recommendations of Kerala Agricultural University to conduct the study. The trial was laid out in randomized block design replicated thrice with a plot size of 2 x 2 m^2. Single spray of spiromesifen at the rate of 96 and 192 g ai ha^{-1} was given during the fruiting stage.

Tender fruits (500 g) were collected randomly on 0, 1, 3, 5, 7, 10, 15, 21 and 27 days after insecticide spray, pooled, extracted, and cleaned of spiromesifen as per the modified QuEChERS method (Xavier *et al*.,2014) and quantification was done by UPLC-MS/MS analysis for estimating the residues of spiromesifen present.

The fresh sample of the chilli fruits were sun dried and crushed well using a blender from which five replicates of three gram representative samples of the fruits were taken in 50 ml centrifuge tubes and spiked with 0.01 ml, 0.05, 0.1 ml and 0.5 ml of 10 µg ml^{-1} working standard mixtures of the insecticides.. After mixing of the sample with 10 ml distilled water, the samples were extracted using 20 ml acetonitrile. The extraction and clean up was done as described above and quantified using UPLC-MS/MS.

Soil samples were collected from 0 to 10 cm depth using a stainless steel soil tube drill. The samples collected from 3 randomly selected spots in each treatment were pooled, air dried, grinded and then passed through 2 mm sieve. The extraction and clean up was done as described by Asensio-Ramos *et al*., (2010) and quantified using UPLC-MS/MS.

The residue data obtained at different intervals were subjected to statistical analysis to determine the half-life values on the treated substrate as per the procedure outlined by Hoskins (1961) which was done using Micrsoft Excel spread sheet 2007.

Decontamination studies

The effect of different household practices on removal of residues of spiromesifen from chilli fruits was assessed by dipping the fruits collected from sprayed fields at two hours and on the third day after application in solutions of common salt 2% (20 g of common salt dissolved in one liter of water), tamarind 2% (20 g of preserved tamarind pulp extracted in one liter of water), vinegar 2% (20 ml of vinegar diluted in one liter of water), slaked lime 2% (20 g of hydrated lime dissolved in one liter of water), turmeric 1% (10 g of turmeric powder dissolved in one liter of water) for 20 minutes followed by washing for 2 minutes in running water. Scrubbing for 2 minutes followed by washing for 2 minutes in running water was also inclued as a treatment. The estimation of residues in the processed chilli samples were done as described above. The per cent reduction of residues was worked out by comparing the residue data in processed fruits with unprocessed fruits.

RESULTS AND DISCUSSION

Efficiency of the method

The method validation studies of spiromesifen on fresh and dry chilli fruits indicated good recovery and high sensitivity. The response function was found to be linear with a good coefficient of determination (R^2) higher than 0.99 in chilli and soil matrix standard solutions for spiromesifen. The fortification at levels of 0.01, 0.05 0.10 and 0.5 µg g^{-1} gave a recovery of 84.7 to 95.1% for fresh chilli and 76.5 to 86.8 % for dried chilli (Table 1). The relative standard deviation ranged from 6.5 to 13.10 and 5.80 to 12.50 respectively for fresh and dried chilli satisfying the acceptance criteria of the method. In the case of soil, recovery ranged from 78.6 to 84.8% and relative standard deviation from 8.6 to 11.90. The limit of detection (LOD), determined with a signal to noise ratio (S/N) ≥ 3, was found to be 0.003 µg g^{-1}, 0.005 µg g^{-1} and 0.004

Table 1. Recovery of spiromesifen residues in soil, fresh and dried chilli fruits

No.of replications	Fortification Level (µg g-1)	Soil		Fresh chilli fruits		Dried chilli fruits	
		Mean % recovery	RSD	Mean % recovery	RSD	Mean % recovery	RSD
5	0.01	78.6	10.3	90.5	13.1	76.5	12.5
5	0.05	80.3	11.9	84.7	10.4	81.4	11.2
5	0.1	84.8	9.2	95.1	6.5	83.2	9.50
5	0.5	81.5	8.60	85.2	7.9	86.8	5.80

Table 2.Dissipation of spiromesifen residues in/on chilli fruits

Days after spraying (DAS)	96 g a.i.ha^{-1}				Fresh chilli (192 g a.i.ha^{-1})	
	Fresh chilli		Dried chilli			
	Mean residue ± SD(µg g^{-1})	Dissipation %	Mean residue ± SD(µg g^{-1})	Processing factor	Mean residue ± SD(µg g^{-1})	Dissipation %
0 (2 hr after spraying)	0.62 ± 0.052		0.9 ± 0.07	1.45	1.20 ± 0.104	
1	0.43 ± 0.032	30.65	0.71± 0.06	1.65	0.91 ± 0.069	24.17
3	0.31 ± 0.025	50.00	0.66 ± 0.07	2.12	0.71 ± 0.051	40.83
5	0.23 ± 0.018	62.90	0.45 ± 0.04	1.95	0.55 ± 0.043	54.17
7	0.135 ± 0.01	78.23	0.24 ±0.02	1.77	0.26 ± 0.020	78.33
10	0.11 ± 0.015	82.26	0.18 ±0.013	1.64	0.15 ± 0.013	87.50
15	0.03 ± 0.004	95.16	0.04 ±0.03	1.33	0.08 ± 0.009	93.33
21	BDL		BDL		0.01±0.001	99.17
28	BDL		BDL		BDL	
$T_{½}$(days)	3.65				3.19	
$T_{tol\ /\ WP}$(days)	0.74				4.49	
Integrated first order rate equation	LogCt=1.75-0.189t/2.303				LogCt = 2.12 -0.217t/2.303	

BDL – Below detectable limit, SD – Standard deviation

$T_{1/2}$ -half life , T_{tol} /(WP)-Waiting period
LogCt –Logarithm of concentration of the residue at time (t), t-time in days

µg g^{-1} for fresh, dry chilli and soil respectively. The limit of quantitation (LOQ), determined with a signal to noise ratio (S/N) ≥ 10, was found to be 0.01 µg g^{-1} in fresh chilli, dry chilli and soil.

Persistence of spiromesifen in chilli and soil

The dissipation pattern of spiromesifen in chilli fruits, following application of spiromesifen (Oberon 240

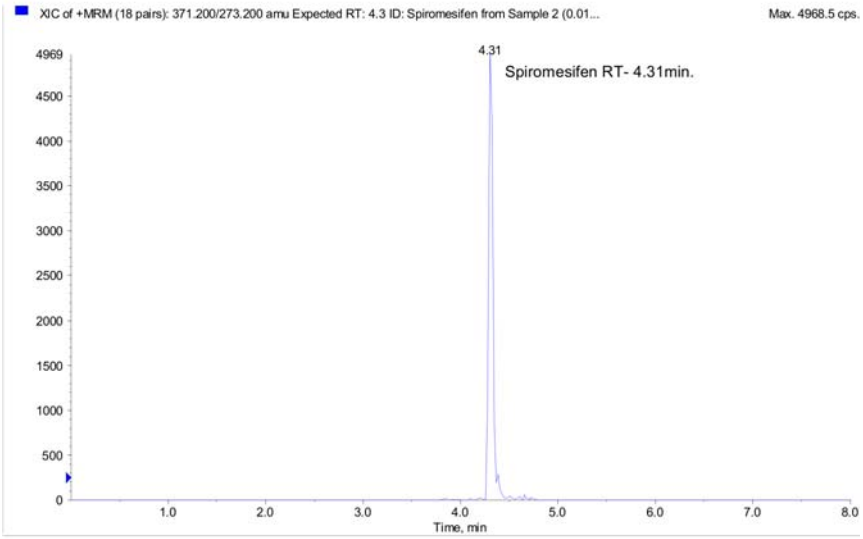

Fig. 1 The LC-MS/MS MRM chromatogram of spiromesifen in standard solution of 0.01 µg ml^{-1}

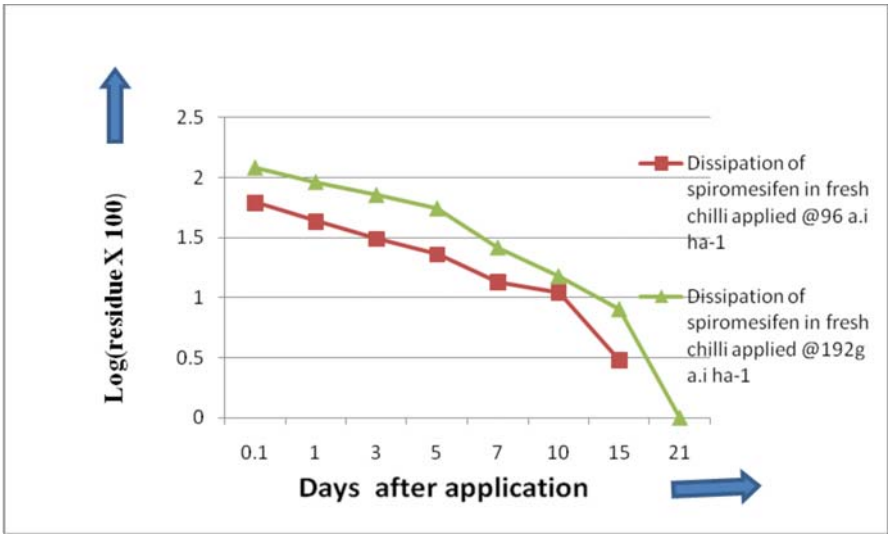

Fig. 2 Semi logarithm-graph showing dissipation kinetics of spiromesifen 240 SC in chilli

SC) at 96 and 192 g ai ha^{-1} are presented in Table 2 and Fig. 2. The mean initial deposit estimated in chilli fruits were found to be 0.62 and 1.20 µg g^{-1} respectively which reached below detectable level on the twenty first and twenty seventh day for single and double dose respectively. Conversely, the residue was found to be higher ie., 0.90 µg g^{-1} in dried chillies collected 2 h after the spray of spiromesifen @ 96 g a.i. ha^{-1} and the processing factor recorded was 1.45. On fifteenth day, the residue became 0.04 µg g^{-1} for dry chillies with the processing factor calculated being 1.33. Similarly, the initial residue was found to be 0.28 µg g^{-1} in the soil collected 2 h after the spray of spiromesifen @ 96 g a.i. ha^{-1} and the residues remained up to 10 days only (Table 3).The degradation half-life of spiromesifen, at single and double dose in the fresh fruits were 3.65 and 3.19 days respectively and the waiting period calculated was 0.74 and 4.49 days respectively which corroborated with the

Table 3. Persistence of spiromesifen residues in/on soil

Days after spraying (DAS)	Single dose	
	Mean residue± SD(μg g^{-1})	Dissipation %
0 (2 hr after spraying)	0.28 ± 0.03	
1	0.12 ± 0.015	57.14
3	0.06 ± 0.008	78.57
5	0.03 ± 0.004	89.28
7	0.01 ± 0.001	96.42
10	BDL	
Half life (days)	1.55	
Integrated first order rate equation	LogCt = 1.380-0.4470t/2.303	

BDL – Below detectable limit, SD – Standard deviation

findings of George *et al*., 2014 who recorded a waiting period of 3 days followed by application of spiromesifen at 150 g a.i. ha^{-1} in Tomato. Persistence data of spiromesifen was fitted into first-order dissipation kinetics with correlation coefficient value of greater than 0.99 for both doses. In the studies by Sharma *et al*., 2007 spiromesifen (Oberon 240 SC) when sprayed at 96 g a.i. ha^{-1} in chilli, the initial residues were in the range of 0.51-0.56 μg g^{-1} and the half life values of spiromesifen in chilies were calculated as 2-2.5 days. Varghese *et al*., 2011 also reported spraying of spiromesifen @ 100 g a.i ha^{-1} on chilli fruits deposited an initial average residue of 0.609 μg g^{-1} and the residues reached below detectable level on the tenth day.

Persistence of spiromesifen on chilli was low as the residues dissipated below EU MRL value of 0.5 μg g^{-1} on 0.74 days for the single dose application whereas it took 4.49 days in the case of double dose. Although adoption of safe waiting period is a means of reducing the risk of the residue problems, methodical processing with simple household practices further ensures safety of the vegetable to the consumers.

Effect of decontamination techniques

All the decontaminating treatments were found effective in removing spiromesifen residues substantially from chilli fruits. The initial deposits of 0.62 μg g^{-1} of spiromesifen on chilli samples were reduced appreciably to the range of 0.197 to 0.231 μg g^{-1} as a result of scrubbing, treatment with slaked lime 2 % and tamarind 2% solution, thus accounting a residue loss of more than 85 per cent, two hours after spraying. Same scenario was also found in case of samples treated with 2 % tamarind solution and scrubbing with more than 68 per cent reduction three days after spraying. Wisam *et al*., 2004 reported washing the cucumber fruit, Oberon residue obtained in first and second spray after 3 days was 0.21 μg g^{-1} and 4 days was 0.2 μg g^{-1}. Also, treatment of peeling, saline solution and pickling reduced spiromesifen residue in first spray to MRL after two days [0.21, 0.23 and 0.22] μg g^{-1} respectively, while in second spray, residues dropped to MRL after 2 days [0.2, 0.17 and 0.18] μg g^{-1} respectively for previous treatment. It is clear from the data that various processing methods are very useful for dislodging of spiromesifen residues. High amount of pesticide residues in spices especially chilli strengthens the need for simple domestic practices that will eliminate harmful pesticide residues in these fruits (Table 4).

ACKNOWLEDGMENTS

The authors wish to express their gratitude to the Manonmaniam Sundaranar University, Tirunelveli, Scott Christian College, Nagercoil, and All India Network Project on Pesticide Residues, College

Table 4. Extent of removal of spiromesifen residues from chilli fruits in the treatments

Treatments	0 day		3 day	
	Mean residue ± SD (îg g^{-1})	% residue removal	Mean residue ± SD (îg g^{-1})	% residue removal
Unprocessed	0.62 ±0.041	-	0.31 ±0.028	-
Scrubbing	0.073 ±0.008	88.18	0.099 ±0.001	68.04
Tamarind	0.086 ±0.001	86.14	0.090 ±0.0016	71.00
Vinegar	0.138 ±0.001	77.68	0.138±0.010	55.46
Slaked lime	0.076 ±0.005	87.76	0.124 ±0.004	59.87
Turmeric	0.083 ±0.004	86.61	0.121 ±0.008	60.96

SD – Standard deviation

of Agriculture Vellayani Centre for providing the necessary research facilities for the conduct of the study.

REFERENCES

Asensio-Ramos M., Hernández-Borges J., Ravelo-Pérez L.M. and Rodríguez-Delgado M.A. (2010) Evaluation of a modified QuEChERS method for the extraction of pesticides from agricultural, ornamental and forestal soils. Analytical and Bioanalytical Chemistry**,** 396(6):2307-19

Xavier, G., Chandran, M., George, T., Beevi , S. N. Mathew, T. B , Paul , A., Arimboor, R., Vijayasree ,V., Pradeepkumar, G. T., and Rajith, R. (2014). Persistence and effect of processing on reduction of fipronil and its metabolites in chilli pepper (*Capsicum annum* L.) fruits. Environmental Monitoring and Assessment*,* 186:5429–5437.

HoskinWM (1961) Mathematical treatment of the rate of loss of pesticide residues. FAO Plant Protection Bulletin, 9: 163-168.

George T., Beevi S. N., Mathew T. B., Paul A., Xavier G. and Ravi P.K. (2014) Dissipation and safety of spiromesifen Oberon 240 SC on Tomato. Pesticide Research Journal, 26(2): 160-163.

The United Kingdom (2004) Draft assessment report on the active substance spiromesifen prepared by the rapporteur Member State (RMS) United Kingdom in the framework of Council Directive 91/414/EEC, February 2004.

Nauen R., Bretschneider T., Bruck E., Elbert A., Reckmann U. and Wqchendorff U. (2002) BSN 2060 – A novel compound for whitefly and spidermite control,' Proc Internat Conf the BCPC – Conf: Pest and Diseases, pp. 39–44, November 18–21, 2002, Brigton, UK.

Rao SC (2005) Pesticide Residues- Impact on Indian Agricultural Exports in WTO Era. Proceedings of the National Seminar on Pesticide Residues and their Risk Assessment. Hyderabad, pp 54-57.

Wisam A., Al-Mashhadany., Saleh H., Abid S. and. Ali A.J.(2004) Dissipation and determination of spiromesifen 240SC residues in cucumber and effect of food processing on residue. Iraqi Journal of Science*,* ijs.scbaghdad.edu.iq

Varghese T.S., Mathew T. B., George T., Beevi S. N. and Xavier G. (2011) Dissipation of Propargite and Spiromesifen in/on Chilli Fruits. Pesticide Research Journal, 23(2): 135-139.

Sharma, K. K., Rao S.C., Dubey J. K., Patyal S. K., Parihar N. S., Battu R. S. , Sharma V., Gupta P., Kumar A., Kalpana, Jaya M., Singh B., Sharma I. D. , Nath A. and Gour T.B. (2007) Persistence and dissipation kinetics of spiromesifen in chili and cotton. Environmental Monitoring and Assessment. 132:25–31.

(Received 20 December 2015; Accepted 10 May 2016; Published 30 June 2016)