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RESEARCH PAPER

Bioefficacy and dissipation studies of spiromesifen against mite, *Polyphagotarsonemus latus* banks on capsicum under field conditions

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Field experiments were conducted during 2013-14 and 2014-15 to evaluate the new insecticides for the management of mite, *Polyphagotarsonemus latus* banks and dissipation of effective insecticide on capsicum. Among the seven insecticides, mean of two seasons under poly house condition, population was less with spiromesifen (0.06 mites/ leaf) followed by diafenthiuron (2.21 mites/ leaf), triazophos (3.68 mites/ leaf) and thiamethoxam (5.30 mites/ leaf) which were significantly superior over untreated check (11.33). Spiromesifen residues were quantified through regular sampling till the residues are below determination level (BDL) of 0.05 mg kg^{-1} following the validated QuEChERS method. The qualitative and quantitative analysis of spiromesifen was performed on LC- MS/MS (PDA). Initial deposits of 1.61 mg kg^{-1} of spiromesifen detected at 2 hours after last spray, dissipated to BDL at 10^{th} day after spray. The half-life and safe waiting period for harvest was 2.09 and 10.00 days, respectively.

Key words : Capsicum, Mite, Spiromesifen, Dissipation

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INTRODUCTION

Capsicum [*Capsicum annuum* (L.) var. grossum Sendt.] is one of the most popular and highly remunerative annual herbaceous vegetable crop. Among the biotic factors, insect pests reduces the quality of produce and even a small blemish on the fruit will drastically reduce its market value. Butani (1976) reported over 20 insect species on chillies (*Capsicum* spp.) from India of which mite, *Polyphagotarsonemus latus* banks is the most damaging pest under field and poly house conditions (Barwal, 2004 and Kaur *et al*., 2010). Reddy and Kumar (2006) estimated crop loss of 40 to 60 tons per ha of capsicum when the crop was not subjected to insecticidal control. In order to control the mite and get higher market price, farmers are indiscriminately using insecticides. As capsicum is consuming fresh there is a need to estimate the residues and minimize the pesticide residues in marketable capsicum, hence, the present study was conducted to find effective insecticide to manage the mite on capsicum.

RESEARCH METHODOLOGY

Bioefficacy of insecticides against mite:

Field experiments were conducted in 2013-14 and 2014-15 at Horticultural Garden, Department of Horticulture, College of Agriculture, Professor

Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar, Hyderabad to evaluate the new insecticides for the management of mite, *Polyphagotarsonemus latus* Banks with leading popular capsicum variety Royal Wonder of Seminis Pvt. Ltd. The experiments were conducted in Randomized Block Design (RBD) with three replications.Capsicum seedlings raised in the nursery were transplanted at age of 40 days in the main field by adopting a spacing of 40 x 30 cm. Plot size was maintained 6 x 6m. All the recommended agronomical practices were implemented to raise sound crop except plant protection measures against mites. The selected insecticides belonging to different groups *viz.*, organophosphates (Triazophas @ 96 mla.i.ha^{-1}), neonicotinoids (Thiamethoxam @50 g.a.i.ha^{-1}), microbial insecticide (Spinosad @75 ml a.i.ha^{-1}), thiourea derivatives (Diafenthiuron @ 400 g. a.i.ha^{-1}), diamides (chlorantraniliprole @ 60 ml a.i.ha^{-1}), pthalic acid diamides (flubendiamide @ 60 ml a.i.ha^{-1}) and ketones (Spiromesifen @ 125 ml a.i.ha^{-1}) along with untreated control were evaluated for two years. First spray was initiated when the population reached its economic thresh hold levels (ETL) (mite –1 no./leaf) (Kumar *et al*., 2007) and second spray was given at 7 days after first spray. A total of three sprays were applied during the entire experimentation in both the seasons. Observations on insect populations mites, *Polyphagotarsonemus latus* banks, were recorded in ten randomly tagged plants, from five terminal leaves (2 from top, 2 from middle and 1 from bottom) per plant. Pre-count (1 day before spray) and post-count (1,3,5 and 7 days after spray) of the insects was recorded by using destructive sampling procedure. Per cent reduction over control was calculated by using the following formula (Flemming and Retnakaran, 1985).

$$\text{Per cent population reduction} = 1 - \frac{\text{Post - treatment population in treatment}}{\text{Pre – treatment population in treatment}} \times \frac{\text{Pre – treatment population in untreated control}}{\text{Post - treatment population in untreated control}} \times 100$$

Pre-count (1 DBS) and post count (mean of 1,3,5 and 7 DAS) population and per cent reduction over control were calculated after each spray. Cumulative mean of three sprays in 2013-14 and 2014-15 under poly house conditions and pooled mean of two years are represented in tables and discussed here under.

Leaf curl index (LCI) was recorded one day before and 10 days after each spray following the methodology of Kumar *et al*. (1996) (Table A).

Dissipation of spiromesifen:

Chemicals and reagents:

Certified reference materials (CRMs) of spiromesifen was obtained from Dr. Erhenstorfer, Germany were used to prepare primary standards. Intermediary and working standards were prepared using acetone and hexane as solvents (1:9 ratio). Working standards of spinosad was prepared in the range of 0.01 ppm to 0.5 ppm in 10 ml calibrated graduated volumetric flask using distilled n-hexane as solvent. All the standards were stored in deep freezer maintained at -40^{0}C. For sample preparation primary secondary amine (Agilent), magnesium sulfate anhydrous (Emsure grade of Merck), sodium sulfate anhydrous (Emparta ACS grade of Merck), acetonitrile (LC MS gradient grade of Merck), acetic acid glacial (LC MS grade of Merck), acetone (Emplure grade of Merck), n-hexane (LC MS grade of Merck) were used during the study. Spiromesifen 22.9 SL was procured from local market.

Analytical instruments and limits of detection:

The working standards of spiromesifen was injected in liquid chromatograph with photo diode array (PDA). The detector for estimating the lowest quantity of above insecticides which can be detected under standard operating parameters are given in Table B.

Under LC operational parameters given in Table B, the retention time of spiromesifen is 3.84 minutes. Working standards of above insecticide (0.05 ppm, 0.075 ppm,

Table A : Scoring procedure for sucking pests damage

Sr. No.	Score	Symptom
1.	0	No symptoms
2.	1	1-25% leaves/plant showing curling
3.	2	25-50% leaves/plant showing curling, moderately damaged
4.	3	51-75% leaves/plant showing curling, heavely damaged, malformation of growing points and reduction in plant height
5.	4	>76% leaves/plant showing curling, severe and complete destruction of growing points, drastic reduction in plant height, defoliation and severe malformation

0.10 ppm, 0.25 ppm and 0.50 ppm) were injected six times and the linearity lines were drawn.

For confirmatory analysis, samples were also injected in LC-MS/MS. The LC operating parameters for spiromesifen detection and estimation are presented in Table B.

Based on the response of the detector (PDA) to different quantities (ng) of CRM standards injected under the LC- MS/MS operational parameters given in Table B, it was found that the LOD (limit of detection) for spiromesifen was 0.05 ng and the linearity was in the range of 0.05 ng to 0.10 ng , respectively as given in Fig A.

Method validation :

Prior to pesticide application and field sample analysis, the residue analysis method was validated following the SANCO document (12495/2011). The capsicum fruits (5 kg) collected from untreated control plots were brought to the laboratory and the stalks were removed prior to samples preparation. The sample was homogenized using robot coupe blixer (High volume homogenizer) and homogenized sample of each 15 g was taken into 50 ml centrifuge tubes. The required quantity of spiromesifen intermediate standards prepared from CRM were added to each 15 g sample to get fortification levels of 0.05 ppm, 0.25 ppm and 0.5 ppm in three replications each. These foritifcation levels were selected to know the suitability of the method to detect and quantify pesticides in capsicum below maximum residue limits (MRLs) of codex alimentarius commission (CAC).

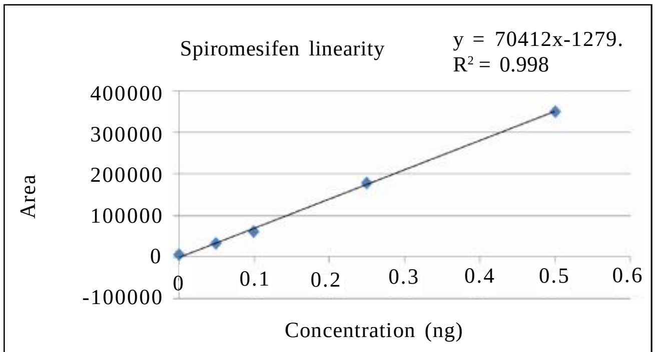

Fig. A : Calibration curve for spiromesifen (Concentration vs area)

The AOAC official method 2007.01 (Pesticide residues of foods by acetonitrile extraction and partitioning with magnesium sulphate) was slightly modified to suit to the facilties available at the laboratory and the same was validated for estimation of LOQ (Limit of quantitation) in capsicum matrix. The final extract of the sample was evaporated using turbovap and made upto 1 ml (equal to 1 g sample) using suitable solvent [n-Hexane: Acetone (9:1)] for LC analysis, filtered 1 ml final extract (equal to 0.5 g sample) was directly injected in LC and the residues of pesticides recovered from fortified samples were calculated using the following formula.

$$\text{Residues (mg kg}^{-1}) = \frac{a \times b \times c \times d}{e \times f \times g} \times R$$

Table B : Details of LC-MS/MS operating parameters for the analysis of spiromesifen

LC-MS/MS		SHIMADZU LC-MS/MS 8040		
Detector		Mass spectrophotometer		
Column		KINETEX, 100 x 3, 2 um		
Column oven temperature		40°C		
Retention time (RT)		5.1		
Nebulizing gas		Nitrogen		
Nebulizing flow gas		2.0 lit.min^{-1}		
Pump mode/ flow		Gradient/ 0.4 ml. min^{-1}		
Retention time,		Spiromesifen – 3.84		
LC programme		Ammonium formate in water		
		Ammonium formate in methanol		
	Insecticide	Time	Methanol	Water
	Spiromesifen	10.00	95	5
Precursor ion and quantifier ion	Insecticide	Precursor ion	Quantifier ion	
	Spiromesifen	371.00	273.10	

Table C : Recovery of spiromesifen residues in capsicum samples

Details	Fortified level					
	0.05 mg kg^{-1}		0.25 mg kg^{-1}		0.50mg kg^{-1}	
	Residues recovered (mg kg^{-1})	Recovery %	Residues recovered (mg kg^{-1})	Recovery %	Residues recovered (mg kg^{-1})	Recovery %
R^1	0.045	89.78	0.242	96.76	0.516	103.25
R^2	0.045	90.73	0.222	88.72	0.527	105.39
R^3	0.046	91.56	0.247	98.79	0.522	104.39
Mean		90.69		94.76		104.34
SD		0.89		5.33		1.07
RSD		0.98		5.62		1.02

R^1, R^2 and R^3 : Replications, Standard deviation (SD), Replicated standard deviation (RSD)

where,
a : Sample peak area
b : Concentration of standard (ppm)
c : µl standard injected
d : Final volume of the sample
e : Standard peak area
f : Weight of sample analysed
g : µl of sample injected
R : Recovery factor.

$$\textbf{Weight of the sample analysed} = \frac{\textbf{Sample weight (15g) x aliquot taken}}{\textbf{Volume of acetonitrile (30 ml)}}$$

Capsicum samples fortified with spiromesifen at 0.05 mg kg^{-1}, 0.25 mg kg^{-1} and 0.5 mg kg^{-1}, respectively were analyzed and the mean recovery of the residues using the method was 95.85 per cent, 95.16 per cent and 95.27 per cent, respectively (Table C). The results show that the method is suitable for the analysis of spiromesifen residues upto 0.05 mg kg^{-1} and the limit of quantitation (LOQ) is 0.05 mg kg.

Calculation methods :

Capsicum fruit samples were collected at regular intervals *i.e.* 0, 1, 3, 5, 7, 10, 15 and 20 days after last spray for dissipation studies. Qualitative and quantitative analysis of residues of spiromesifen were done following validated methods explained in .3 using the analytical instruments given in 2. Half -life and TBDL (Time required for residues to reach below determination level) were calculated as per Hoskins (1961) from first order dissipation kinetics. OECD (Organization for Economic Co-operation and Development) MRL calculator is used for calculation of MRL and chronic hazard risk analysis was performed using TMDI (Theoretical Maximum Daily Intake) for arriving at MRL for recommendation.

RESEARCH FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Efficacy of insecticides against mite :

Pooled mean of 2013 -14 and 2014-15 :

The results with regards to overall cumulative mean efficacy of the treatments against mite, *P. latus* during the two years under open field conditions are presented in Table 1. Mean mite population in pre-count ranged from 5.46 to 15.88 and post-count population was lower with spiromesifen (0.62 mites/ leaf) followed by diafenthuiron (4.08 mites/ leaf), triazophos (5.73 mites/ leaf) and thiamethoxam (9.43 mites/ leaf) which were significantly superior over rest of the treatments and untreated check (17.60 mites/ leaf). The descending order of efficacy of the other treatments were chlorantraniliprole (12.84 mites/leaf), flubendiamide (14.69 mites/leaf) and spinosad (15.21 mites/leaf) which were found to be at par with over untreated check (17.60 mites/ leaf).

The highest per cent reduction of mite population was recorded in spiromesifen (97.29 %) followed by diafenthiuron (71.32 %), triazophos (61.31 %) and thiamethoxam (43.45 %) which were at par with each other and significantly superior over rest of the treatments and untreated check. The other treatments that followed in the ascending order of efficacy were chlorantraniliprole (20.60 %), flubendiamide (13.42 %) and spinosad (11.21%) which were found to be significantly superior over untreated check.

The mean LCI of two years revealed that, LCI at one DBS (1.53) was significantly reduced to 0.69 at 10 DAS in spiromesifen treated plants followed by diafenthiuron (1.78 to 1.09) and triazophos (2.03 to 1.72). Where as LCI was significantly increased from one DBS to 10 DAS in flubendiamide (2.48 to 2.71), chlorantraniliprole (2.49 to 2.70), thiamethoxam (2.50 to 2.67), spinosad (2.52 to 2.73) and untreated control (2.72 to 3.00) (Table 1).

The results obtained from both years of open field experiment showed that, spiromesifen was significantly superior over rest of the treatments by recording lower mean no.of mites per leaf (0.62) and mean reduction of mite population (97.29 %). Spiromesifen is a tetraonic acid derivative insecticide and acaricide effective against *P. latus* (Elbert *et al.*, 2005).

The present results are in concurrence with Varghese and Mathew (2013) who tested certain insecticides and acaricides against chilli mite, *P. latus*. Spiromesifen 45 SC @ 100 g a.i.ha^{-1} and propargite 57 EC @ 570 g a.i. ha^{-1} were found to be effective in reducing chilli mite

Table 1 : Cumulative efficacy of certain insecticide molecules against mite, *P. latus* on capsicum under open field conditions during 2013-14 and 2014-15 (Pooled mean of 2013-14 and 2014 -15)

T. No.	Treatments	Dose (g or ml ha^{-1})	Mean of 2013-14 and 2014-15: Pre count (Mean no. of mites/ leaf) (1 DBS)*	Post count (Mean of 1,3,5,7 DAS)*	Per cent reduction$	Leaf curl index (LCI): 1 DBS	10 DAS
T_1	Spinosad 45 SC	125	14.75 (3.96)	15.21 (4.02)^{ab}	11.21 (19.55)^{d}	2.52(1.87)	2.73(1.93)a
T_2	Flubendiamide 480 SC	200	13.46 (3.80)	14.69 (3.96)^{ab}	13.42 (21.48)^{d}	2.48(1.86)	2.71(1.92)a
T_3	Chlorantraniliprole 20 SC	200	12.09 (3.61)	12.84 (3.72)^{ab}	20.60 (26.48)^{d}	2.49(1.86)	2.70(1.92)a
T_4	Diafenthiuron 25 WP	750	8.18 (3.03)	4.08 (2.25)^{d}	71.32 (57.59)^{b}	1.78(1.66)	1.09(1.44)b
T_5	Spiromesifen 22.9 SL	750	5.46 (2.54)	0.62 (1.27)^{e}	97.29 (80.49)^{a}	1.53(1.59)	0.69(1.30)b
T_6	Thiamethoxam 25 WG	150	11.95 (3.39)	9.43 (3.23)^{bc}	43.45 (41.22)^{c}	2.50(1.87)	2.67(1.91)a
T_7	Triazophos 40 EC	1250	9.40 (3.22)	5.73 (2.59)^{cd}	61.31 (52.78)^{c}	2.03(1.74)	1.72(1.64)ab
T_8	Untreated check	--	15.88 (3.97)	17.6 (4.19)^{a}	0.00e	2.72(1.87)	3.00(1.88)a
		S.E.±	0.23	0.48	3.38	0.20	0.41
		C.D. (P= 0.05)	0.73	1.42	11.73	0.44	1.25
		C.V. (%)	11.04	14.51	16.80	15.67	12.68

#No.of mites/leaf, mean of five leaves per plant, ten plants per replication, three replications per treatment
* Figure in the parenthesis are square root transformed values. $ Figure in the parenthesis are arc-sin transformed values
DBS : Days before spray, DAS : Days after spray., NS : Non-significant
DOS :Ist Spray :6-12-2013; IInd Spray 13-12-2013 and IIIrd Spray :21-12-2013
DMRT : Means followed by a common letter are not significantly different (P= 0.05)

Table 2 : Dissipation of spiromesifen in capsicum in field conditions

Days after spray	Residues of spiromesifen (mg kg^{-1}): R^1	R^2	R^3	Average	Dissipation %
0	1.32	1.26	1.28	1.29	0.00
1	0.58	0.66	0.62	0.62	51.93
3	0.15	0.18	0.16	0.16	87.59
5	0.05	0.06	0.04	0.05	96.12
7	BDL	BDL	BDL	BDL	100.00
10	BDL	BDL	BDL	BDL	--
15	BDL	BDL	BDL	BDL	--
20	BDL	BDL	BDL	BDL	--
Regression equation			Y = 3.086 + (-0.282)X		
R^2			0.997		
Half life			1.06 days		
Safe waiting period			7 days		

population.

Similarly the efficacy of spiromesifen 45 SC at 100 g a.i.ha^{-1} in reducing chilli mite in comparison to other insecticides was reported by Nagaraju *et al*. (2007). The efficacy of spiromesifen 45 SC at 120 g a.i.ha^{-1} in reducing chilli mite in comparison to dicofol 18.5 EC @ 185 g a.i.ha^{-1} was reported by Kavitha *et al*. (2006). Spiromesifen 45 SC @ 120 g a.i.ha^{-1} showed long lasting efficacy by reducing the leaf curl damage from 41.8 per cent to 12.5 per cent without producing any phytotoxicity. These reports are in line with the present findings.

The present findings are also in conformity with the findings of Seal and Klassen (2006) who reported the effectiveness of spiromesifen @ 300-400 ml ha^{-1} in reducing the incidence of chilli thrips in Scotch Bonnet variety of chilli.

The insecticides spiromesifen, diafenthiuron, thiamethoxam and triazophos reduced the incidence of mite population upto seven days after spraying, while in others an increase the mite population after spray.

Dissipation of spiromesifen :

Initial deposits of 1.29 mg kg^{-1} of spiromesifen was detected at 2 hours after last spray, dissipated to 0.62, 0.16 and 0.05 mg kg^{-1} by 1, 3 and 5 days after last spray, respectively under open field conditions. The residues reached BDL at 7^{th} day after spray. The dissipation pattern showed decrease of residues from first day to 7^{th} day and residues dissipated by 51.93, 87.59, 96.12 and 100.00 per cent at 1, 3, 5 and 7 days, respectively (Table 2). The regression equation is Y = 3.086 + (-0.282) x with R^2 of 0.997. The half - life value was worked out by using linear semi-logarithmic regression analysis (Hoskins, 1961) and found to be 1.06 days. The safe harvest period was 7.00 days after third spray of spiromesifen when sprayed @ 125 ml ha^{-1} in open field conditions.

Sharma *et al*. (2005) 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 $\mu g.kg^{-1}$ at recommended dose, respectively. Raj *et al*. (2012) 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 upto 3^{rd} and 5^{th} day at lower and higher dose. The residues fell below quantification limit of 0.01 $\mu . g^{-1}$ on the 5^{th} and 7^{th} day at standard and double the dose.

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