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In-vivo and field evaluation of spinetoram 12 Sc against *Exelastis atomosa* on pigeonpea

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Abstract
A new biological insecticide molecule, spinetoram 12 SC was evaluated for acute toxicity on greenhouse environment reared *E. atomosa* population, and persistence on pigeonpea pods at laboratory conditions; and effect on *E. atomosa* on pigeonpea at field conditions during 2012 -2013 and 2013- 2014 seasons. Acute toxicity studies revealed that LC_{50}`s of spinetoram on third instar larvae after 24, 48 and 72 hours after treatment were 4.88, 1.98 and 1.31 ppm respectively. In persistence studies, the spinetoram 12 SC 27 g a.i./ha, emamectin benzoate 5 SG at 11 g a.i./ha and monocrotophos 36 SL at 500 g a.i./ha had no mortality at 14 DAT. Persistence for spinetoram 12 SC 27 g a.i./ha was upto 11 DAT and 14 DAT for spinetoram 12 SC 36 and 45 g a.i./ha and spinosad 45 SC 78 g a.i./ha. Results indicated that spinetoram 12 SC was significantly effective at 36 and 45 g a.i./ha when sprayed thrice at 15 days interval and minimized the incidence of *E. atomosa* on pigeonpea plants.

Keywords: spinetoram, pigeonpea, acute toxicity, persistence, field efficacy, *E. atomosa*

1. Introduction

Pigeonpea (*Cajanus cajan* L.) is an important pulse or grain legume crop in semi-arid tropical and subtropical areas of the world **[1].** Major constraint in the production of pigeonpea was the damage caused by insect pests with avoidable losses extending upto 78 per cent. The pod borers together damaged 57.07, 54.09 and 40.08 per cent pods and 34.79, 30.90 and 20.20 per cent seeds incurring the yield losses of 28.07, 21.01 and 15.02 per cent in early, medium and late maturing cultivars, respectively in pigeonpea [2]. The pod borers were considered to be the most important group causing crop loss to the tune of 80 to 100 per cent [3]. Estimated the infestation levels to range from 9 to 51 per cent under Bangalore conditions in pigeonpea[4]. Observed more larvae (52.3%) on flowers than on pods (37.8%) and leaves (9.9%) [5]. Larvae of *E. atomosa* (Walsingham) feed on buds, flowers and pods [6]. Synthetic insecticides provide dramatic effect initially, and hence chemical control methods are still in use among farmers. Earlier, conventional insecticides like malathion and hostathion [7], chlorpyriphos [8] were reported in management of pests on pigeonpea.

In recent times, new insecticide molecules offer advantages over earlier chemistry in terms of greater levels of safety, better performance and reduced environmental impact. One such new insecticide molecule is spinetoram, has shown outstanding efficacy against tomato caterpillar (*Spodoptera litura* Fabricius) [9], shoot and fruit borer (*Leucinodes orbonalis* Guenee) [10], codling moth (*Cydia pomonella* L.*)*, oriental fruit moth *(Grapholita molesta* Busck*)*, army worms (*Spodoptera spp*), cabbage looper (*Trichoplusia ni* Hubner), thrips such as western flower thrips (*Frankliniella occidentalis* Pergande) and onion thrips (*Thrips tabaci* Lindeman*)*, leaf miners (*Liriomyzaspp*), chillithrips (*Scirtothrips dorsalis* Hood), fruit borer (*H. armigera*) and many other pests [11]. However, there are no reports on *in-vivo* and field evaluation of spinetoram 12 SC against the *E. atomosa* on Pigeonpea. Therefore, this study was undertaken with the objectives to investigate the acute toxicity and persistence of spinosyn, spinetoram 12 SC and other insecticides against *E. atomosa* in the laboratory and to evaluate their effectiveness for controlling the pest in the field in two seasons.

2. Materials and Methods

2.1. Acute toxicity of spinetoram 12 SC against *E. atomosa* on pigeonpea

The acute toxicity experiment was conducted Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai, Tamil Nadu, India during the month of February and March 2013.

Acute toxicity of spinetoram 12 SC against plume moth, *E. atomosa* was conducted by pigeonpea pod dip bioassay technique [12]. Uniform medium sized early matured pigeonpea pod were surface sterilized in sodium hypochlorite (0.5%), rinsed in sterile water and shade dried on a filter paper in open air. The pods were dipped in various concentrations (1.2 ppm, 2.4 ppm, 3.6 ppm, 4.8 ppm, 6.0 ppm and 7.2 ppm) for 60 seconds and left to dry. Spinetoram treated pods of 30 numbers were kept fresh by placing on piece of wet cotton in a plastic container. Greenhouse condition reared (under natural environment) 30 third instar *E. atomosa* were allowed to feed on treated pods in each container. Three replications were maintained per treatment. The acute toxicity experiments, observations on larval mortality were fixed till 72 hours of exposure as spinetoram 12 SC tested was lepidoptericide characterized by stomach action showing slower mortality [13]. The cumulative mortality data were observed till 72 h at 24 h interval and corrected by Abbott's formula.

2.2. Persistence of spinetoram 12 SC against *E. atomosa* under laboratory condition

The Persistence toxicity experiment was conducted Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai, Tamil Nadu, India during the month of February and March 2013. Sixty days old potted pigeonpea plants were used for the study. Insecticidal solutions were prepared by dissolving 0.45 ml, 0.6 ml and 0.75 ml of spinetoram 12 SC, 0.44 g of emamectin benzoate 5 SG, 0.34 ml of spinosad 45 SC, 2.5 ml of monocrotophos 36 SL in one litre of water which was equivalent to the field doses. Potted pigeonpea plants (cv. CO 7) were sprayed with the insecticides at the respective concentrations at 70 days after sowing (DAS) by using a hand operated sprayer to the point of run-off.

After application, treated pigeonpea pods were collected separately from the plants starting from first day after treatment (DAT) (2 h after spray) and continued on 3, 5, 7, 9, 11, 14 DAT and till the mortality due to insecticides on *E. atomosa* declined to practically negligible level. In each treatment, treated pods samples were placed separately and natural environment under greenhouse condition reared *E. atomosa* of 30 numbers were released on treated pods. There were three replications for each insect. In all the four cases, larval mortality was observed at 24 h interval till zero per cent mortality observed or pupation of the larvae [14].

Persistence toxicity = Average residual toxicity x Period for which the toxicity persisted (days)

The number of days for which the toxicity persisted was recorded as 'P'. The average of the mortality percentage constituted the residual toxicity 'T'. The product of 'P' x 'T' was calculated. Based on the 'PT' value the order of relative efficacy (ORE) was worked out. Here, greater the 'PT' value, better was the treatment. The procedures of Saini (1959) [15] and elaborated further by Pradhan [16] and Sarup *et al.* [17] were adopted to calculate the persistent toxicity.

2.3. Field evaluation of spinetoram 12 SC against *E. atomosa* on pigeonpea

Two field experiments were conducted at farmer's field at Jadhi Goundanpatti, Attur Block, Dindigul district, Tamil Nadu, India during September to March months of 2012-13 and 2013-14 in a randomized block design with a plot size of 5 x 5m. Pigeonpea (var. CO1) was raised as per recommended package of practices except insect pest management practices. Effect of seven insecticidal treatments comprising spinetoram 12 SC @ 45, 36 and 27 g a.i. ha-^{1} along with emamectin benzoate 5 SG @ 11 g a.i. ha-^{1}, spinosad 45 SC @ 78 g a.i. ha^{-1} and monocrotophos 36 SL @ 500 g a.i. ha^{-1} was determined and each treatment was replicated thrice. Three sprays of each insecticide were applied with the help of knapsack hand sprayer up to the point of runoff at fortnightly intervals starting from 50 per cent flower initiation. Observations on the larval population on number basis per plot from ten randomly selected plants were recorded at one day before first spray and on 1, 3, 7 and 10 days after each spray. Grain yield was also taken after harvest and represented as yield per ha.

2.4. Statistical Analysis

2.4.1. Laboratory experiments

The data from various laboratory experiments were scrutinized by CRBD analysis of variance (ANOVA) after getting transformed into √x+0.5 and arcsine percentage values where appropriate [18]. The per cent mortality in laboratory studies was corrected using Abbot's formula [19],

$$\text{Per cent corrected mortality} = \frac{\text{Per cent test mortality} - \text{per cent control mortality}}{100 - \text{per cent control mortality}} \times 100$$

Median lethal concentrations (LC$_{50}$) of spinetoram 12 SC and other insecticides were determined by Finney's Probit analysis [20] and confirmed in EPA Probit analysis version 3.1.

2.4.2. Field experiments

The data from various field experiments were scrutinized by RBD analysis of variance (ANOVA) after getting transformed into √x+0.5, logarithmic and arcsine percentage values where appropriate [18]. Critical difference values were calculated at five per cent probability level and treatment mean values were compared using Duncan's Multiple Range Test (DMRT) [21]. The corrected per cent reduction over untreated check in field population was calculated by [22]

$$\text{Corrected per cent reduction} = \left\{ 1 - \frac{T_a \times C_b}{T_b \times C_a} \right\} \times 100$$

where,

T_a - number of insects in the treatment after spraying

T_b - number of insects in the treatment before spraying

C_a - number of insects in the untreated check after spraying

C_b - number of insects in the untreated before spraying

3. Results and Disscussion

3.1. Acute toxicity of spinetoram 12 SC against *E. atomosa* on pigeonpea

Toxicity test on a laboratory strain of the third instar larva of *E. atomosa* was carried out at different concentrations after 24, 48 and 72 h after treatment. The data in Table 1 indicates that percentage mortality of larvae showed positive correlations with the spinetoram concentrations, which ranged from 10.0, 32.2 and 54.43 to 98.86 per cent after 24 and 48 h after treatment respectively. The response of larvae to different concentrations was represented by straight regression lines, indicating homogeneity of the population to the tested concentrations. LC$_{50}$\`s of spinetoram 12 SC in third instar larvae after 24, 48 and 72 h treatment were 4.88, 1.98 and 1.31 ppm respectively and LC$_{95}$\`s of spinetoram 12 SC in third instar larvae after 24, 48 and 72 h treatment were 17.49, 6.79 and 6.64 ppm respectively. The probit regression line for spinetoram 12 SC were Y = 3.16x + 2.72, Y = 4.108x + 3.25 and Y = 4.51x + 2.98 at 24, 48 and 72 h after treatment respectively. Spinetoram 12 SC had the least slope of 2.72 with 24 h after exposure, while slope values were 3.25 and 2.98 with 48 and 72 h after exposure respectively. The results are in rationale with the reports of LC_{50} value of spinosad was 0.557 µg/ml against fall army worm, *S. frugiperda* using diet-incorporated bioassay and was less toxic than spinetoram 12 SC [23]. Spinetoram 12 SC showed better results than its analogue spinosad about ten times in topically and feeding bioassays in laboratory conditions [24]. The present investigation clearly elicited that spinetoram 12 SC has high insecticidal activity and are far better than other insecticides. Spinosad was the most toxic against *Earias vittella* (Fab.) when compared to emamectin benzoate, abamectin and indoxacarb and the order of toxicity in terms of LD_{50} (µg /larva) to *E. vittella* was spinosad (0.00188) > abamectin (0.00264) > emamectin benzoate (0.00266) > indoxacarb (0.09270), respectively) [25]. LC_{50} of spinosad was 0.0004 per cent for five day old larvae of *E. vittella* [26]. Differences in the observed values may be due to difference in the age of the larvae used and method of application. The 0.058 and 9.85 µg per larvae as LD_{50} and LD_{99} for spinosad against *H. armigera* and suggested 10 µg per larvae as the diagnostic dose for resistance monitoring [27]. LC_{50} of spinosad for *H. armigera* was 2.944 µg per ml [28] and less than equal to 5.0 ppm against *H. zea* [29]. Thus the toxic values of spinetoram had significant effect on insects.

3.2. Persistence of spinetoram 12 SC against *E. atomosa* on pigeonpea

Spinetoram 12 SC was applied at 27, 36 and 45 g a.i./ha on pigeonpea plants with pods. Cent per cent mortality of third instar larvae of *E. atomosa* was observed upto 3 DAT and 5 DAT in spinetoram 12 SC at 36 and 45 g a.i./ha respectively (Table 2). Spinosad 45 SC 78 g a.i./ha, emamectin benzoate 5 SG at 11 g a.i./ha, spinetoram 12 SC 27 g a.i./ha and monocrotophos 36 SL at 500 g a.i/ha had 90.0, 75.5, 65.5 and 61.1 per cent mortality, respectively at 5 DAT. In the case of spinetoram 12 SC 27 g a.i./ha, emamectin benzoate 5 SG at 11 g a.i./ha and monocrotophos 36 SL at 500 g a.i./ha had no mortality at 14 DAT. Persistence for spinetoram 12 SC 27 g a.i./ha was upto 11 DAT and 14 DAT for spinetoram 12 SC 36 and 45 g a.i./ha and spinosad 45 SC 78 g a.i./ha. There was a reduction in the mortality of *E. atomosa* larvae as the time increased and the toxicity persisted for 11 days in emamectin benzoate 5 SG at 11 g a.i./ha and monocrotophos 36 SL at 500 g a.i./ha. The order of relative efficacy (ORE) of the insecticides based on the persistent toxicity index (PTI) values was spinetoram 12 SC 45 g a.i./ha > spinetoram 12 SC 36 g a.i./ha > spinosad 45 SC 78 g a.i./ha > emamectin benzoate 5 SG at 11 g a.i./ha > spinetoram 12 SC 27 g a.i./ha > monocrotophos 36 SL at 500 g a.i./ha. Present findings are in conformity [30] who found on the basis of PT values, spinosad 0.005% (1026.2) was effective against 1st instar larvae of *E. vittella* and the highest PT value was obtained for spinosad 0.005% (764.9) against 3rd instar larvae of *E. vittella* on okra fruits. Persistence of spinosad was comparably high in the laboratory (100% nymphal mortality at 6-9 days post application), but in the greenhouse a faster decline of activity was evident by increased egg deposition, egg hatch and reduced rates of immature mortality[31]. Spinosad persisted in cabbage and cauliflower up to 7 and 10 days, respectively, following spinosad application at lower and higher dosages [32]. However, spinetoram 12 SC has translaminar activity, thereby providing a relatively prolonged residual action [33]. The persistent toxicity of nine insecticides, *viz.*, fenitrothion, methyl parathion, malathion, endosulfan, monocrotophos, quinalphos, phosphamidon, carbaryl and dimethoate against neonate larvae of *Leucinodes orbonalis* Guen[34]. Among these insecticides, phosphamidon proved extremely persistent based on PT index (PT = 12528.00) while malathion was the least persistent insecticide (PT = 5376.36).

3.3. Field evaluation of spinetoram 12 SC against *E. atomosa* on pigeonpea

E. atomosa larval population varied from 4.9 to 6.5 per plant during first season before imposing treatments (Table 1) and crossed the economic threshold level (ETL). Mean larval population ranged from 1.6 to 7.6 per plant due to various treatments. Spinetoram 12 SC 45 g a.i./ha and spinetoram 12 SC 36 g a.i./ha were significantly effective in minimizing the population to 1.6 and 2.0 per plant along with 78.9 and 73.6 per cent reduction respectively when compared to 7.6 per plant of untreated plot. Spinosad 45 SC 78 g a.i./ha, spinetoram 12 SC 27 g a.i./ha and emamectin benzoate 5 SG at 11 g a.i./ha were next treatments, which achieved population of 2.4, 2.8 and 3.1 per plant along with 68.4, 63.1 and 59.2 per cent reduction respectively. Monocrotophos 36 SL at 500 g a.i./ha was the least effective which achieved only 53.0 per cent reduction with larval population of 3.5 per plant

Data pertaining to larval population of *E. atomosa* during second season for 1, 3, 7 and 10 DAT after three sprays are presented in Table 3. Mean larval population of 1, 3, 7 and 10 DAT ranged from 2.0 to 11.4 larvae per plant due to treatments. Spinetoram 12 SC 45 g a.i/ha was on par with 36 g a.i./ha and were significantly higher in minimizing the population to 2.0 and 2.0 per plant along with 82.4 and 80.7 per cent reduction respectively when compared to 11.4 per plant of untreated plot. Spinetoram 12 SC 27 g a.i./ha and spinosad 45 SC at 78 g a.i./ha were next best treatments, which achieved population of 2.5 and 2.7 per plant along with 77.3 and 76.3 per cent reduction respectively. Emamectin benzoate 5 SG at 11 g a.i./ha and monocrotophos 36 SL at 500 g a.i./ha were next effective treatments which achieved only 73.6 and 68.4 per cent reduction with larval population of 3.0 and 3.6 per plant.

These results are in accordance with spinosad was effective against third instar larvae of *M. vitrata*. Spinosad 45 SC @ 90 g a.i./ha was the most potent insecticide in reducing the larval population (0.29 larvae/plant), pod damage (5.62%), grain damage (22.85%) and producing highest grain yield of 1681 kg/ha [35]. It was followed by flubendiamide 20 WDG @ 50 g a.i./ha and novaluron 10 EC @ 75 g a.i./ha [36]. *M. vitrata* can be managed effectively with new chemicals, spinosad and indoxacarb (82 and 72% reduction in population) within two days after application [31]. Since the reduction in larval population is faster with these chemicals as compared to other conventional chemicals and also registered lowest seed damage (3.9/plant) and highest grain yield (795 kg/ha). It would be worth keeping these new chemicals as one of the best options in IPM (Integrated Pest Management) module for effective management of this species. Insecticide Tracer remained the most effective against the pest activity and resulted in the maximum reduction percentage of larval population of pod borer and the pods damage percentage was also decreased as comparison to other insecticides in lentil crop [38]. Tracer (spinosad) was the most effective insecticide in reducing the population of pod borer and pod damage, followed by Lorsban and Thiodan. Curacron was found to be the least effective insecticide. The maximum increase in seed yield per hectare was obtained with Tracer, whereas Thiodan resulted in minimum increase seed yield over control [39].

The treatment of spinosad 2.5 SC (25 g a.i./ha) and endosulfan 35 EC (0.07%) were the most promising treatments in terms of least mean per cent pod damage of 11.85 and 17.80, respectively by the pod borer complex of pigeonpea[40]. Spinosad 90 g a.i./ha was most effective against pigeon pea pod borers in reducing the pod infestation and grain damage [41]. Tracer (spinosad) 240SC @ 60 ml was the most effective treatment in restricting the pest infestation followed by Steward and Lanante[42]. The application of spinosad 0.005 per cent was most effective against *M. vitrata* Fabricius recording highest mean per cent larval reduction (63.99%) over untreated control in urdbean [43].

Table 1: Acute toxicity of spinetoram 12 SC at different concentrations against *Exelastis atomosa* on pigeonpea

Dose (ml/l)	**Concent-ration (ppm)**	**After 24 h of treatment**		**After 48 h of treatment**		**After 72 h of treatment**	
		***Mean no. of dead larvae**	**Mortality (%)**	***Mean no. of dead larvae**	**Mortality (%)**	***Mean no. of dead larvae**	**Mortality %**
0.02	2.4	3.66	12.2	6.33	21.1	14.0	46.66
0.04	4.8	8.0	26.6	11.33	37.76	17.0	56.66
0.06	7.2	11.0	36.66	14.66	48.86	20.0	66.66
0.08	9.6	14.00	46.66	21.66	72.20	22.33	74.43
0.10	12.0	20.33	67.76	24.33	81.10	25.0	83.33
0.12	14.4	22.33	74.43	25.33	84.43	29.0	96.66
0.14	16.8	24.33	81.10	27.33	91.10	29.66	98.86
LC_{50} and Fiducial limit		9.16 (7.86-10.66)		6.31 (5.32- 7.50)		3.71 (2.74 -5.01)	
LC_{95} and Fiducial limit		35.77 (23.53-54.39)		25.2(17.88 -35.84)		21.91 (14.19- 33.83)	
Slope		2.59		2.60		2.14	
Regression equation		Y=2.49X+2.59		Y=2.92X+2.60		Y=3.78X+2.14	

Table 2: Persistent toxicity of spinetoram 12 SC against *Exelastis atomosa* on pigeonpea

Treatments and doses	**Per cent larval mortality (days after treatment)**								**P**	**T**	**PTI**	**ORE**
	1	**3**	**5**	**7**	**9**	**11**	**14**	**21**				
Spinetoram12 SC 30 g a.i/ha	91.1	86.6	71.1	60.0	35.5	21.1	0.0	0.0	11	60.9	669.9	4
Spinetoram12 SC 36 g a.i/ha	100	93.3	80.0	72.2	42.2	31.1	13.3	0.0	14	61.7	864.2	2
Spinetoram12 SC 45 g a.i/ha	100	100	91.1	78.8	45.5	32.2	15.5	0.0	14	66.1	926.2	1
Emamectin benzoate g a.i/ha	88.8	87.7	78.8	65.5	*48.86*	23.3	11.1	0.0	11	57.7	634.9	5
Spinosad 45 SC 120 g a.i/ha	100	88.8	80.0	71.1	41.1	22.2	10.0	0.0	14	59.0	826.4	3
Fipronil 80 WG 40 g a./ha.	78.8	73.3	68.8	60.0	31.1	16.6	0.0	0.0	11	54.7	602.4	6
Thiamethoxam 25 WG 62.5 g a.i/ha	74.4	65.5	52.2	35.5	22.2	10.0	0.0	0.0	11	43.3	476.3	7

P – Period of toxicity persistence (days)
T – Mean per cent mortality
PTI – Persistent toxicity index
ORE – Order of relative efficacy

Table 3: Effect of spinetoram 12 SC against *Exelastis atomosa* on pigeonpea

Treatments and doses (g a.i. /ha)	***Exelastis atomosa* larval population (larva/ plant)**					
	I season (Aug 2012 – Mar 2013)			**II season (Aug 2013 – Mar 2014)**		
	Precount	**Over all Mean**	**Per cent reduction over control**	**Precount**	**Over all Mean**	**Per cent reduction over control**
Spinetoram 12 SC 27 g a.i./ha	4.9	2.8^{d}	63.1	6.7	2.5^{b}	77.3
Spinetoram 12 SC 36 g a.i./ha	5.6	2.0^{b}	73.6	6.9	2.2^{a}	80.7
Spinetoram 12 SC 45 g a.i./ha	6.1	1.6^{a}	78.9	5.8	2.0^{a}	82.4
Emamectin benzoate 5 SG 11 g a.i./ha	5.2	3.1^{d}	59.2	6.1	3.0^{c}	73.6
Spinosad 45 SC 78 g a.i./ha	6.5	2.4^{c}	68.4	6.8	2.7^{b}	76.3
Monocrotophos 36 SL 500 g a.i./ha	5.6	3.5^{f}	53.0	6.3	3.6^{d}	68.4
Untreated check	5.5	7.6^{g}		5.9	11.4^{e}	
CD (0.05%)	-	0.17		-	0.21	
SEd	-	0.08		-	0.10	

Data are mean values of three replications
Figures were transformed by square root transformation and the original values are given
Means within columns lacking common upper case superscript are significantly different ($P<0.05$)

4. Conclusion

The present investigation, it can be concluded that LC_{50}`s of spinetoram on third instar larvae after 24, 48 and 72 hours after treatment were 4.88, 1.98 and 1.31 ppm respectively. Persistence for spinetoram 12 SC 27 g a.i./ha was upto 11 DAT and 14 DAT for spinetoram 12 SC 36 and 45 g a.i./ha and spinosad 45 SC 78 g a.i./ha. spinetoram 12 SC was significantly effective at 36 and 45 g a.i./ha when sprayed thrice at 15 days interval and minimized the incidence of *E. atomosa* on pigeonpea plants.

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