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Acute toxicity and field evaluation of spinetoram 12 SC against *Helicoverpa armigera* Hubner on tomato

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Abstract
A new biological insecticide molecule, spinetoram 12 SC was evaluated in the laboratory to determine acute toxicity on the third instar larvae of tomato fruit borer, *Helicoverpa armigera* (Hubner). The homogeneity response of *H. armigera* to different rates of spinetoram12 SC was observed and median lethal concentration (LC_{50}) was 5.20, 3.54 and 1.94 ppm after 24, 42 and 48 h after treatment. Field experiments were conducted and results revealed that application of spinetoram 12 SC at 45 g a.i./ha was significantly superior in minimizing the larval population (77.9 and 80.2% reduction) and fruit damage (80.3 and 81.2% reduction) of *H. armigera* and in increasing tomato fruit yield (100.5 and 94.4% increase) than indoxacarb 15 SC, novaluron 10 EC and quinalphos 25 EC. There was also minimum to moderate level of toxicity observed due to spinetoram at 45 g a.i./ha against coccinellids predators (9.6 and 5.5% reduction) of tomato ecosystem.

Keywords: Spinetoram, Acute toxicity, Field evaluation, *Helicoverpa armigera*, coccinellids

Introduction
India is the second largest producer of vegetables after China with an average annual production of 87.5 million tonnes from 5.9 million hectares, having a share of 14.4 percent to world production [1]. Tomato (*Lycopersicon esculentum* Mill) is the most popular and remunerative vegetable crop grown around the world for fresh consumption and processing. It was cultivated in an area of 5.1 lakh ha and yields to the extent of 8.8 million tonnes per annum in India averagely [1]. It is a rich source of vitamins like A, B_1, B_2 and C and tomato fruit is widely consumed as vegetable, pickles and as a salad. The average productivity is 17.7 q / ha, which is very low due to the attack of number of diseases and insect pests *viz*., tomato fruit borer (*Helicoverpa armigera* Hubner), tobacco caterpillar (*Spodoptera litura*), whitefly (*Bemisia tabaci* Gennadius) and leafhopper (*Amrasca devastans* Dist.) [2].

Among insect pests, the fruit borer, *H. armigera* is a potential and polyphagous pest which attacks cotton, groundnut, tomato, pulses, sunflower, millets, sorghum, maize *etc*., in India and causes extensive economic damage. *H. armigera* is causing extensive fruit damage to the tune of 50 to 60 percent and a single larva can destroy 2 to 8 fruits [2]. Synthetic insecticides provide dramatic effect initially, and hence chemical control methods are still largely in use among farmers. Earlier, conventional insecticides like endosulfan [3, 4], malathion and hostothion [5], chlorpyriphos [6], azadirachtin 1%, phosalone and quinalphos [7], synthetic pyrethroids and endosulfan alternatively with NSKE 4% [8] and fenvalerate, methomyl, azinphos methyl, carbaryl and pyrethrin/rotenone [9] were reported in management of pests on tomato.

In recent times, some new insecticide molecules offer manifold advantages over previous ones in terms of greater levels of safety, better performance and reduced environmental impact. One such new insecticide molecule is spinetoram, that has shown outstanding efficacy against 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 (*Liriomyza spp*), chilli thrips (*Scirtothrips dorsalis* Hood) [10], *Leucinodes orbonalis* in brinjal [11] and *Spodoptera litura* on tomato [12]. However, there are no reports on *in vivo* and field evaluation of spinetoram 12 SC against the *H. armigera* on tomato. So, this investigation was undertaken with the objectives to study the acute toxicity of spinosyn, spinetoram 12 SC against *H. armigera* in the laboratory and to evaluate their effectiveness for controlling the fruit borer in the field.

Materials and Methods

Test insects

From the farmers field nucleus culture of *H. armigera* was collected and reared on chickpea based diet at suitable environmental conditions (28±1 ºC, RH 65±5% and photoperiod 16:8 h scoto/photo regime) in the Insectary of the Department of Agricultural Entomology, Agricultural College and Research Institute, Madurai of Tamil Nadu Agricultural University in the year of 2013. The pupae were collected and placed in emergence cages with moist saw dust to facilitate pupation. Emerged adults were sexed and allowed for oviposition with black cloth substrate and 10% honey solution [13]. Laboratory cultures were kept away from insecticidal contamination and maintained for five generations before using for bioassay experiments.

Acute toxicity experiment

Research on acute toxicity of spinetoram 12 SC against tomato fruit borer, *H. armigera* by fruit dip bioassay technique was conducted in the laboratory condition. Serial concentrations of spinetoram 12 SC (0.01 ml (1.2 ppm), 0.04 ml (4.8 ppm), 0.07 ml (8.4 ppm), 0.1 ml (12.0 ppm), 0.13 ml (15.6 ppm), 0.16 ml (19.2 ppm), 0.19 ml (22.8 ppm), 0.22 ml (26.4 ppm) and 0.25 ml (30.0 ppm) were prepared. Uniform medium sized tomato fruits were surface sterilized in sodium hypochlorite (0.5%), rinsed in sterile water and shade dried. The fruits were dipped in various concentrations for 60 seconds and left to dry. Ten spinetoram - treated fruits were kept fresh by placing on piece of wet cotton in a plastic container and 30 third instar *H. armigera* were allowed to feed on treated fruits in each container. For control, fruits treated with water alone were used. Three replications were maintained for each concentration. The larvae were considered dead if they became desiccated with shortened body and dark cuticle, and/or unable to move in a coordinated manner when disturbed with a needle. In this acute toxicity experiment, 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 [14]. The cumulative mortality data were observed till 72 h at 24 h interval and corrected by Abbott's formula.

Field experiments

Field experiments were conducted in farmers' field in Madurai District, Tamil Nadu, India during two kharif seasons at Pannikundu, Thirumangalam block and Kokkulam, Chekkanoorani block. Plots were planted to tomato var. PKM 1 with a plot size of 7 x 9 m. Insecticidal treatments were arranged in a randomized block design with three replications. Other crop management practices recommended by the Tamil Nadu Agricultural University were used to maintain tomato plots in a consistent manner. The treatments included new molecule spinetoram 12 SC (36, 45 and 54 g a.i/ha), indoxacarb 14.5 SC (75 g a.i/ha), novaluron 10 EC (75 g a.i/ha), quinalphos 25 EC (250 g a.i/ha) and untreated control. Pre-treatment observation across the experimental area indicated the occurrence of 3 or more *H. armigera* larvae and >20% fruit borer damage per plant during fruiting stage. Insecticide treatments were applied with a high-volume sprayer calibrated to deliver 200 L per ha through hollow cone nozzles up to the point of runoff. Insecticides were applied three times at 20 days intervals during the experimental period.

Effectiveness of treatments was determined on 1, 3, 7 and 10 d after treatment (DAT) by observing larval population and fruit damage of *H. armigera* from 10 randomly selected plants from each plot. Observations were also made on the total number of grubs and adults of coccinellids observed *Chilomenes sexmaculatus* and *Coccinella septumpunctata* to assess the effect of treatments on the beneficial insects. Tomato fruit yield was estimated and represented as yield/ha.

Statistical analysis

The statistical analysis of the data on mortality was subjected to the Abbott formula [15] for correction wherever required. Probit analysis was used to calculate LC_{50} and LC_{95} values [16] through software computer programme. Field data were transformed with square root, arcsine and logarithmic transformations wherever appropriate before analysis. These data were analyzed using RBD analysis of variance (ANOVA). Means were separated and ranked by using Duncan's Multiple Range Test [17] and original values are given in Tables. In all the data, percent reduction was estimated [18].

Results and Discussion

Acute toxicity of spinetoram 12 SC against third instar larvae of *H. armigera*

Acute toxicity studies were conducted in laboratory and results shows that the percent mortality of third instar *H. armigera* larvae indicates positive correlations with spinetoram concentrations, which ranged from 25.1, 27.6 and 41.5 to 100.0 percent after 24 h, 48 h and 72 h treatment respectively. The initial LC_{50} was 5.20 ppm which decreased with succeeding hours after treatment and the final LC_{50} value after 72 h of spinetoram exposure was 1.94 ppm. Similarly, LC_{95} was 94.4, 54.1 and 22.2 ppm due to 24, 48 and 72 h after treatment (Table 1).

Table 1: Acute toxicity of spinetoram 12 SC at different concentrations against third instar larvae of *Helicoverpa armigera* on tomato.

Dose (ml/l)	Concent-ration (ppm)	After 24 h of treatment		After 48 h of treatment		After 72 h of treatment	
		Mean dead larvae ± SE	Mortality %	Mean dead larvae ± SE	Mortality %	Mean dead larvae ± SE	Mortality %
0.01	1.2	7.53 ± 0.51	25.10	8.27 ± 0.47	27.57	12.45 ± 0.30	41.50
0.04	4.8	13.97 ± 0.72	46.57	18.41 ± 0.41	61.37	21.77 ± 0.22	72.57
0.07	8.4	16.43 ± 0.79	54.77	19.97 ± 0.40	66.57	23.63 ± 0.31	78.77
0.10	12	18.97 ± 0.38	63.23	21.43 ± 0.32	71.43	24.98 ± 0.47	83.27
0.13	15.6	20.00 ± 0.88	66.67	22.87 ± 0.29	76.23	27.03 ± 0.48	90.10
0.16	19.2	22.71 ± 0.52	75.70	23.95 ± 0.51	79.83	28.65 ± 0.34	95.50
0.19	22.8	25.37 ± 0.77	84.57	27.17 ± 0.58	90.57	29.73 ± 0.51	99.10
0.22	26.4	28.00 ± 0.57	93.33	30.00 ± 0.0	100	30.00 ± 0.0	100
0.25	30	30.00 ± 0.0	100	30.00 ± 0.0	100	30.00 ± 0.0	100
Untreated		0	0	0	0	0	0
LC_{50} and Fiducial limit		5.20 (3.63 – 7.45)		3.54 (2.36 – 5.30)		1.94 (1.19 – 3.16)	
LC_{95} and Fiducial limit		94.39 (43.77 – 145.56)		54.10 (29.85 – 98.04)		22.24 (14.45 – 34.21)	
Slope		1.07		1.15		1.43	
Regression equation		Y = 4.206 + 1.066x		Y = 4.372 + 1.146x		Y = 4.610 + 1.425x	

The LC_{50} showed higher values at 24 h after treatment and decreased with the 48 h and 72 h after treatment. Thus *H. armigera* showed more sensitive to spinetoram 12 SC at 24 h than 48 and 72 h after exposure. The response of *H. armigera* larvae to different rates of spinetoram 12 SC was represented by straight regression lines (Fig. 1) indicating homogeneity. As for slope values of the regression line, spinetoram had the least slope of 1.07 with 24 h after exposure, while slope values were 1.15 and 1.43 with 48 h and 72 h exposure respectively (Table 1). The results are in rationale with the reports of scientist [19] who estimated LC_{50} values of spinetoram 12 SC on fourth instar larvae of *Spodoptera littoralis* (Boisd) as 6.67 and 2.86 ppm after 24 and 48 h after treatment and researcher found 0.066 µg/ml after 96 h exposure [20]. However, LD 50 was 59.88 µg a.i. per g of larval body weight of fourth instar *S. littoralis* [21]. Thus the toxic values of spinetoram had significant effect on insects.

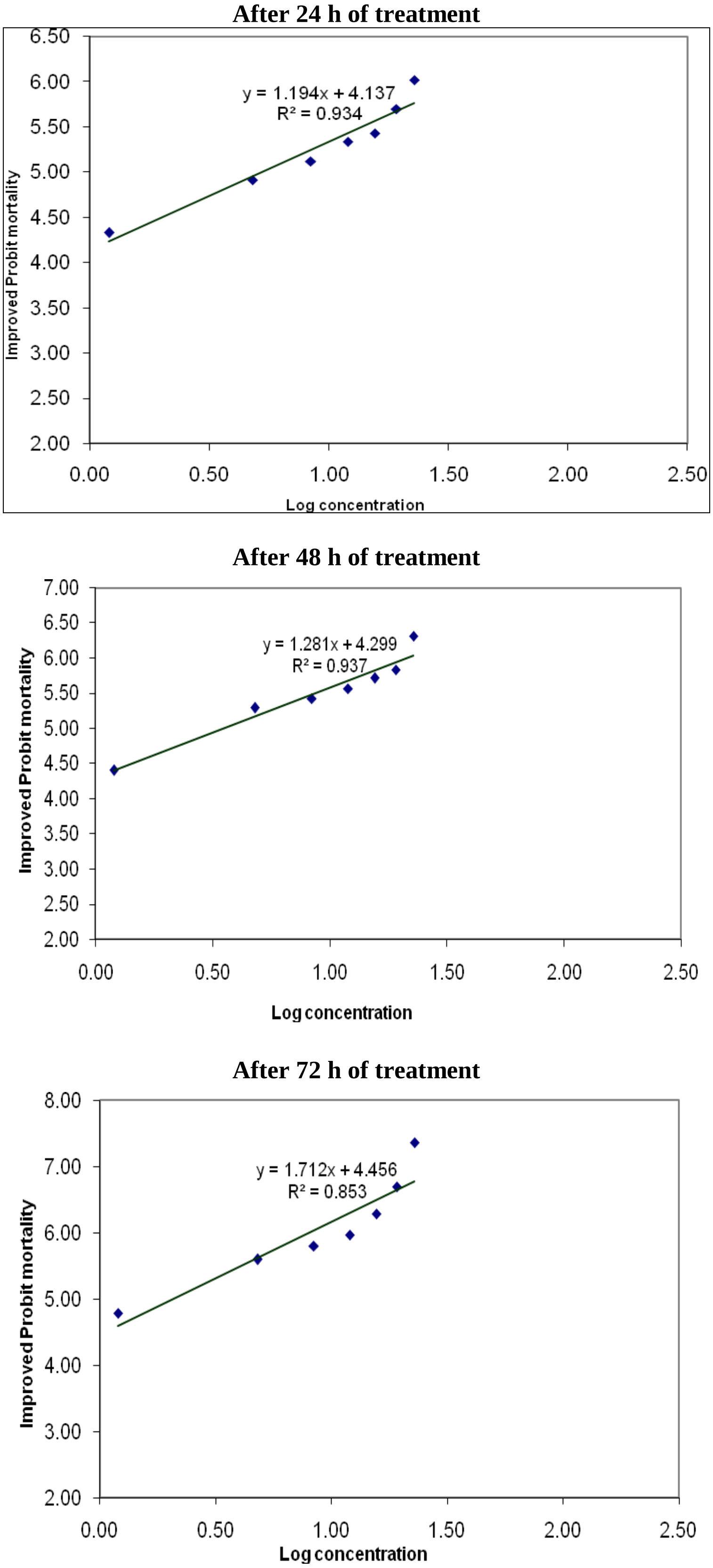

Fig 1: Lethal Dose - Probit mortality response of *H. armigera* to spinetoram.

Field evaluation of spinetoram 12 SC against *H. armigera* on tomato

In the first and second year field experiments, tomato fruit borer larval population varied from 4.0 to 6.0 and 7.0 to 8.0 per plant respectively before imposing treatments and the observations recorded on 1, 3, 7 and 10 DAT on both the seasons are given in Table 2. Spinetoram 12 SC at 45 and 54 g a.i./ha exhibited significantly high mortality after 3 DAT and mortality rate reduced after 10 DAT in both the seasons. Thus residual activity of spinetoram got reduced with time. However, novaluron 10 EC 75 g a.i./ha, indoxacarb 15 SC 75 g a.i./ha and quinalphos 25 EC 250 g a.i./ha contributed residual toxicity up to 7 DAT. Residual toxicity data represents that spinetoram 12 SC is toxic up to 10 days than indoxacarb and novaluron.

Mean data of all days of observations revealed that *H. armigera* larval population ranged from 1.6 to 8.1 larvae / plant I season and 1.7 to 10.1 larvae / plant during II season. Spinetoram 12 SC 45 g a.i./ha contributed significant reduction of larval population to 1.8 and 2.0/plant and to an extent of 77.9 and 80.2 percent reduction respectively during two consecutive kharif seasons. Indoxacarb 15 SC at 75 g a.i./ha however registered 2.8 and 3.5 larvae/plant (with moderate reduction of 65.6 and 65.3% respectively) followed by novaluron 10 EC at 75 g a.i./ha (3.0 and 3.6 larvae/plant with 63.3 and 64.4% reduction respectively). However, quinalphos 25 EC registered higher larval population of 3.7 (lower reduction of 54.5%) and 4.2/plant (58.4% reduction) (Table 2). Most apparent action of spinetoram 12 SC is probably by the increased activity of acetylcholine esterase, one scientist who found that spinetoram 12 SC at LC_{50} increased the level of Ach.E in 2nd larval instars of *S. littoralis* [19]. The present finding corroborate with results of many researchers who reported the applications of new spinetoram improved the higher biological activity towards the management of *H. virescens* [22-26].

Table 2: Effect of spinetoram 12 SC against *Helicoverpa armigera* larval population and fruit damage on tomato – (Season I & II)

Treatments and doses (a.i. /ha)	*Helicoverpa armigera*				Fruit damage by *H. armigera*			
	Mean population per plant		Percent reduction over control		Mean percent fruit damage		Percent reduction over control	
	I Season	II Season	I Season	II Season	I Season	II Season	I Season	II Season
Spinetoram 12 SC 36 g a.i./ha	2.9^{b}	3.3^{c}	64.3	67.3	6.9^{c}	7.9^{b}	71.2	70.3
Spinetoram 12 SC 45 g a.i./ha	1.8^{a}	2.0^{ab}	77.9	80.2	4.7^{b}	5.0^{a}	80.3	81.2
Spinetoram 12 SC 54g a.i./ha	1.6^{a}	1.7^{a}	80.3	83.2	3.9^{a}	4.1^{a}	83.7	84.6
Indoxacarb 15 SC 75 g a.i./ha	2.8^{b}	3.5^{cd}	65.6	65.3	7.0^{c}	8.4^{bc}	70.7	68.4
Novaluron 10 EC 75 g a.i./ha	3.0^{b}	3.6^{d}	63.3	64.4	7.9^{d}	9.7^{c}	66.9	63.5
Quinalphos 25EC 250g a.i/ha	3.7^{c}	4.2^{e}	54.5	58.4	9.7^{e}	11.7^{d}	59.5	56.0
Untreated check	8.1^{d}	10.1^{f}	-	-	23.9^{f}	26.6^{e}	-	-
CD at 0.05%	0.08	0.07	-	-	0.40	1.68	-	-
SEd	0.04	0.03	-	-	0.18	0.77	-	-

Data are mean values of three replications

Figures were transformed by square root transformation for population data, arc sine transformation for percent data and the original values are given

Means within columns lacking common bold upper case superscript are significantly different ($p<0.05$)

Fig. 2 depicts the mean data of post treatment observations on fruit damage at 1, 3, 7 and 10 DAT during both seasons. Mean data of all days of observation indicated that fruit damage varied from 3.9 to 23.9 percent during I season and from 4.1 to 26.6 percent during II season. In both the seasons, spinetoram 54 and 45 g a.i/ha were equally effective and superior in reducing the fruit damage to 3.9 percent (83.7% reduction) and 4.7 percent (80.3% reduction) during I season; and 4.1 percent (84.6% reduction) and 5.0 percent (81.2% reduction) during II season. The present findings on the effectiveness of spinetoram 12 SC is in accordance with scientist who reported that spinosad 45 SC new A:D ratio @ 100 g a.i/ha recorded the lowest fruit damage of 3.19 percent which was on par with all lower dosages and the standard check spinosad @ 75 g a.i/ha [27]. Another scientist also reported that percent damage on chilli fruits due to *H. armigera* was lower due to spinetoram 12 SC treatments @ 60, 56 g, 46 g and spinosad 45 SC @ 73 g a.i/ha [10].

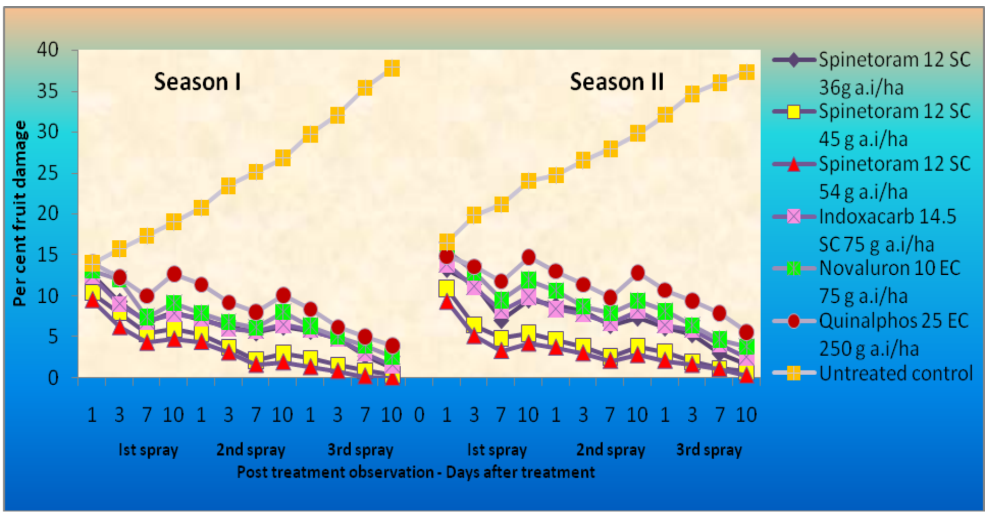

Fig 2: Effect of spinetoram 12 SC on tomato fruit damage by *H. armigera*.

Effect of spinetoram 12 SC on coccinellids and yield

Before imposing treatments, population of *C. sexmaculaus* and *C. septumpunctata* varied from 3.4 to 4.0 and from 5.0 to 5.4 grubs and adults per plant in first and second experimental periods respectively (Table 3). Post- treatment mean data for first and second season revealed predators' population range of 2.9 to 5.2 and 3.1 to 5.5 per plant respectively. Maximum number of predators was observed in untreated check (5.2 and 5.5/plant in I and II season respectively). Lower dose of spinetoram 12 SC @ 36 g a.i (4.8 and 5.2/plant with percent reduction of only 7.7 and 5.5%), spinetoram 12 SC @ 45 g a.i / ha (4.7 and 5.2/plant with percent reduction of 9.6 and 5.5%) and spinetoram 12 SC @ 54 g a.i (4.2 and 5.0/plant with percent reduction of 19.2 and 9.1%) for first and second season respectively. However, lower population of predators was observed due to novaluron 10 EC @ 75 g a.i (2.8 and 3.8/plant with percent reduction of 46.2 and 30.9%), indoxacarb 15 SC 75g a.i/ha (2.9 and 3.7/plant with percent reduction of 44.2 and 32.7%) and quinalphos 25 EC 250g a.i/ha (3.0 and 3.1/plant with percent reduction of 42.3 and 43.6%) in first and second season respectively.

Spinetoram, an improved chemistry of spinosad, is a biological green insecticide which may be the reason for lower toxicity towards predators on tomato ecosystem. Present study also supports with spinosad which is safe to the natural enemies (parasitoids and predators) found on egg plant crop. The population of parasitoid *Encarsia lutea* and predators *Chrysoperla zastrowi sillemi* and lady bird beetles in spinosad treated plots were at par with untreated control. All the chemical insecticides were toxic to natural enemies since the numbers of all natural enemies in these insecticides were found to be significantly lesser than control [28].

Marketable yield of tomato fruits ranged from 66.8 to 102.1 q/ ha and from 70.7 to 100.3 q / ha in first and second season experiments due to all treatments respectively (Table 3). Highest yield was registered due to spinetoram 12 SC @ 54 g ai / ha (102.1 and 100.3 q/ha) followed by spinetoram 12 SC @ 45 g a.i /ha (97.6 and 99.9 q/ ha) and spinetoram 12 SC @ 36 g a.i / ha (89.7 and 94.1 q / ha) in both the seasons respectively. The next best treatments were indoxacarb 15 SC @ 75g a.i/ha (89.7 and 95.2 q/ ha), novaluron 10 EC @ 75 g a.i /ha (89.5 and 94.0 q/ ha), and quinalphos 25 EC @ 250 g a.i/ha (79.0 and 82.1 q / ha), compared to untreated check which registered only 66.8 and 70.7 q/ha of tomato fruits during two seasons respectively.

Table 3: Effect of spinetoram 12% SC against coccinellids on tomato and fruit yield – (Season I & II).

Treatments and doses (a.i. /ha)	Coccinellids				Yield			
	Mean number of grub and adult per 10 plants		Percent reduction over control		Fruit yield q / ha		Percent increase over control	
	I Season	II Season	I Season	II Season	I Season	II Season	I Season	II Season
Spinetoram 12 SC 36 g a.i./ha	4.8^{b}	5.2^{ab}	7.69	5.46	89.7^{c}	94.1^{b}	33.7	32.7
Spinetoram 12 SC 45 g a.i./ha	4.7^{b}	5.2^{ab}	9.61	5.46	97.6^{b}	99.9^{a}	45.4	40.8
Spinetoram 12 SC 54g a.i./ha	4.2^{c}	5.0^{b}	19.23	9.10	102.1^{a}	100.3^{a}	52.2	41.5
Indoxacarb 15 SC 75 g a.i./ha	2.9^{d}	3.7^{c}	44.22	32.73	89.7^{c}	95.2^{b}	33.6	34.3
Novaluron 10 EC 75 g a.i./ha	2.8^{d}	3.8^{c}	46.16	30.92	89.5^{c}	94.0^{b}	33.4	32.6
Quinalphos 25EC 250g a.i/ha	3.0^{d}	3.1^{d}	42.31	43.64	79.0^{d}	82.1^{c}	17.7	15.7
Untreated check	5.2^{a}	5.5^{a}	-	-	66.8^{e}	70.7^{d}	-	-
CD at 0.05%	0.07	0.07	-	-	0.02	0.02	-	-
SEd	0.03	0.03	-	-	0.009	0.008	-	-

Data are mean values of three replications

Figures were transformed by square root transformation for population data, arc sine transformation for percent data and the original values are given

Means within columns lacking common bold upper case superscript are significantly different ($p<0.05$)

Conclusion

In conclusion, spinetoram 12 SC was fairly toxic green insecticide to third instar *H. armigera* larvae treated with LC_{50} (1.94 to 5.20ppm) in the laboratory. Field application of spinetoram 12 SC at 45 to 54 g a.i./ha was also better than indoxacarb 15 SC and novaluron 10 EC for the management of *H. armigera.* This clearly explicited that spinetoram 12 SC has high insecticidal activity and far better than any other conventional insecticide. Thus spinetoram 12 SC to be an integral component of pest management programs due to the continual need to deal with a multitude of arthropod pests associated with vegetable cropping systems and moderately safe to natural enemies.

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