TOXICITY AND BIOCHEMICAL IMPACTS OF SPINOSAD ON THE PINK CORN STEM BORER *SESAMIA CRETICA* LED. (LEPIDOPTERA: NOCTUIDAE)

M. A. M. Osman*, Y. Y. Mosleh* and M. F. Mahmoud*

* Plant Protection Department, Faculty of Agriculture, Suez Canal University 41522, Ismailia, EGYPT. E-mails: naeim70@hotmail.com, mfaragm@hotmail.com

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ABSTRACT: The toxicity of Spinosad to different larval instars of the pink corn stem borer, *Sesamia cretica* Led. (Lepidoptera: Noctuidae), was evaluated under laboratory and field conditions. The impact of Spinosad on the protein content and glycogen level of larvae was also taken into consideration. Data indicated that Spinosad had toxic activity against the tested larval instar and the mortality was in the order of first instar > second instar > third instar > fourth instar > fifth instar with respective LC_{50} values of 0.008, 0.016, 0.028, 0.044 and 0.159 ml/l after 7 days of treatment. Also, the field experiment showed high efficiency of Spinosad at the highest three concentrations down to 25% FR (0.125 m/l) against *S. cretica* larvae, which resulted in significant reduction in the number of plants containing either perforated stem or dead hearted case, number of larvae, tunnels and excavated areas inside infested plants. Regarding the biochemical parameters, results proved that the protein content and glycogen level in the treated larvae was significantly lower than that of the control at all concentrations of exposure. The highest rate of decreasing in total protein content was -54.5% for 2nd instar larvae exposed to 50% FR (0.25ml/l). Also, the decrease in glycogen level in the treated larvae was concentration-dependent and reached -55.8% to 2nd instar larvae exposure to 50% FR (0.25 ml/l.).

KEY WORDS: Spinosad, *Sesamia cretica*, toxicity, biochemical impacts, protein contents, glycogen level.

Maize *Zea mays* L., also called corn, is the third most important cereal crops in the world agricultural economy after wheat and rice. Maize occupies a crucial place since it used for human and livestock's consumption and as a source of industrial raw material for the production of oil, alcohol and starch. In Egypt, the cultivated area in 2012 stood approximately 750,000 hectares with a total grain yield of 7 M.T. (FAO, 2012). However, this crop is subjected to sever attack by several insect pests causing considerable damage estimated about 25% annually (Setamou et al., 2000). Stem borers are one of the major limiting factors to maize production in the world (Tende et al., 2005). In Egypt, maize is infested by three stem borer species: the pink borer *Sesamia cretica* Led. (Lepidoptera: Noctuidae), the striped stem borer *Chilo agamemnon* Blesz. and European corn borer *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae) (Moyal et al., 2002).

The pink borer, *Sesamia cretica* Led. (Lepidoptera: Noctuidae), is a key pest damaging corn mainly in the eastern Mediterranean countries, and is also spread in Africa and Asia (Onukogu, 1984; Moyal et al., 2002). In Egypt, attacks by *S. cretica* are usually high especially on early maize crops, sown between late March and mid-May, in which the borer may cause severe damage (Semeada, 1988).

Stem borers affect maize yields by reducing the photosynthetic area of the leaves. Also, crop losses due to death of the growing point, early leaf senescence, reduced translocation, lodging and direct damage to ears. Secondary losses have been documented due to infections by bacterial and fungal pathogens via entry points created by the stem borers within the plant tissues (Ndiritu, 1999). The

corn borer is estimated to cause significant and economic losses in yield production up to 20% in high infestation regions, where no insecticides are used (Bosque-Pérez, 1995).

Current control of this pest in highly infested plantations has relied for a long time on the extensive use of traditional pesticides. Unfortunately, insects developed resistance to pesticides after several generations of exposure. Also, these pesticides have negative impacts on the environment, especially on the beneficial organisms. Thus, the need to environmentally friendly products for pest control is in continues increase. Spinosad is a mixture of tetracyclic macrolide neurotoxins, spinosyn A and D, produced through the fermentation of the soil actinomycete, *Saccharopolyspora spinosa* Mertz & Yao (Thompson et al., 2000). As such, it may be considered as a bioinsecticide (Copping & Menn, 2000). It is a broad-spectrum insecticide with a very low mammalian toxicity and a favorable environmental profile with low persistence and low toxicity to several natural enemies (Miles & Dutton, 2000; Williams et al., 2003). Spinosad exhibits a high degree of selective toxicity towards several classes of insects, especially lepidopterous larvae and has a unique mode of action involving the postsynaptic nicotinic acetylcholine and GABA receptors (Watson, 2001). It is an alternative reagent to classic pesticides, acts primarily as a stomach (Sparks et al., 1998), and contact poison (Toews & Subramanyam, 2003), and degrades rapidly in the environment (Cisneros et al. 2002). Due to its unique mode of action, high selectivity, low toxicity to mammals, beneficial arthropods, Spinosad is classified as reduced-risk product (Cisneros et al*.,* 2002). These advantages maximize its chance to be an integral part of the integrated pest management programs of certain key lepidopetrous pests (Thompson et al., 2000; Cisneros et al., 2002).

Lepidopteran larvae treated with Spinosad show unique symptoms of poisoning including feeding cessation, complete contraction paralysis and ultimately death (Tohnishi et al., 2005). Insecticides are reported to have the ability to influence the proportion of various biochemical components (Protein, Glycogen, lipids etc.) in the body of insects, thus disturbing the internal metabolism of the insect, causing their reduced activity or mortality. *S. cretica* represents a major lepidopteran pest of maize and extremely destructive if infestations exceed thresholds, it felt necessary to study the response of its protein and glycogen contents to such unique bioinsecticide.

Therefore, the objective of our study was to evaluate the efficacy of lethal and sub-lethal concentrations of Spinosad against the pink maize borer *S. cretica* under laboratory and field conditions. Also, determine the impact of this bioinsecticide on some biochemical aspects as the total protein contents and glycogen levels in different larval instars of *S. cretica*.

MATERIAL AND METHODS

Insect maintenance:

Larvae of maize borer, *Sesamia cretica* Led. were handily collected from untreated maize plants in Experimental Farm, Faculty of Agriculture, University of Suez Canal. The infested plants were detached and transferred to the laboratory to inspect and separate different larval instars of *S. cretica*. Maize borer *S. cretica* were reared for many generations under laboratory conditions of 27 ± 2^{o}C; 60 ± 10% RH and photoperiod of 14: 10 (L : D) h. The collected larvae were reared inside plastic boxes (20 × 60 cm) with screen lids, fed on untreated maize plants until pupal stage. Pupae were collected and transferred to Petri dish inside wood cages (60 × 60 × 60 cm) with three screen sides, and supplied with saturated

cotton piece by 10% sugar solution in another Petri dish. Upon emergence, adults of *S. cretica* were allowed to laying eggs on leaf sheathes of young maize plants (20-25 days old), which putted inside wood cages in the time of adults oviposition periods.

Bio-insecticide used:

A commercial formulation of Spinosad (Spinosad 12% EC) which was a gift from Dow Agro Science Inc, was used in all bioassays. It is registered in Egypt against several lepidopetran pests at a field rate of 0.5 ml/l (60 mg/l a.i). Solutions of tested compound in the present study were prepared in distilled water at the field rate concentration (0.5 ml/l.) 100% FR. The other tested concentrations of Spinosad were prepared via dilute the field rate with distilled water to serial concentrations of 50% FR, 25% FR, 12.5% FR, 6.25% FR, 3.12% FR and 1.56% FR using fresh concentrations prepared one hour prior to experiments.

Laboratory Bioassay:

As a result of preliminary tests, serial concentrations of Spinosad 12% EC were prepared by dilution of water and used for each test to get larval mortality ranged between ≥ 25 - ≤75% for the lowest and highest concentration, respectively. In this experiment, the effect of fresh preparations of the field rate (FR) (0.5 ml/l), 50%FR (0.25 ml/l.), 25% FR (0.125 ml/l.), 12.5% FR (0.06 ml./l.), 6.25% RF (0.03 ml./l.), 3.12% FR (0.016 ml./l.) and1.563% FR (0.007 ml./l.) of Spinosad was studied against 1st, 2nd, 3rd, 4th and 5th instar larvae of *S. cretica*. Each treatment was replicated 6 times with 3 larvae each. Small stem pieces of maize plants (3 cm length) were transected and allowed to dip into the different concentrations for 30 second. The stem pieces were kept fresh then placed on a paper towel for at least 2 hour or until they dried out before being used in the experiments. The tested larvae of *S. cretica* were starved for at least 4 hours prior experiment. Larvae were removed gently by fine camel-hair brush and placed into glass vials (2×10 cm), which supplied with treated maize stem pieces. Glass vials were closed and kept in the laboratory under the abovementioned laboratory conditions. Control treatments were also conducted with the same protocol using distilled water. Three days after treatment, the surviving larvae were fed on untreated maize stem pieces for the rest of the experimental period. To record mortality, vials were daily inspected till the larvae developed into pupae. Rates of mortality in *S. cretica* larvae were recorded 1, 3 and 7 days post treatments.

Field Bioassay:

The field experiment was conducted at the Experimental Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt to assess the field efficiency of Spinosad against *S. cretica*. The experimental field was grown during late summer season of 2011 with yellow corn hybrid plants and the normal agricultural practices were applied. Randomized complete block design was used in this experiment. The treatments were replicated four times. Each replicate contained 5 rows of corn plants (7 × 6 m square). Solutions containing different amounts of Spinosad (0.5, 0.25, 0.125, 0.06, 0.03, 0.016 and 0.007 ml/l) were sprayed two times. The first spray was done just two weeks after sowing, and the second spray was done after two weeks post the first one. The treated plants were investigated to record (the number of dead heart/50 plants, number of holes per plant, number of larvae per plant, number of tunnels inside stem per plant, percentage of excavated area of stem per plant) after 35 days of plants old.

Biochemical impacts:

Determination the total protein content and glycogen of *S. cretica* larvae

The biochemical parameters of 2nd, 3rd, 4th and 5th instar larvae of *S. cretica* were measured 72 hours after feeding on treated small corn stem pieces with sublethal concentrations (50, 25, 12.5, 6.25, 3.125 and 1.562% FR) of Spinosad. Total protein content of the supernatant was determined by dye binding method (Bradford, 1976) using bovine serum albumen as a standard. Glycogen level was determined using the method described by Carrol et al. (1956). Glycogen was separated from soluble sugars by precipitation in the presence of methanol. After centrifugation (15 min, 3000 rpm), precipitates were used for glycogen quantification with anthrone reagent according to the sulfuric acid method of Kemp & Heijningen (1954). Calibration was performed using standards of glucose ranging from 0 to 200 mg which received the same treatment as the samples.

Statistical analysis:

LC_{20}, LC_{50}, LC_{90} and slope values were calculated using the probit analysis program of Schoofs & Willhite (1984). All data were subjected to ANOVA (SAS Institute, 2009). If there were significant differences ($p \leq 0.05$), differences were compared using FLSD test.

RESULTS

Biological activity of Spinosad on different instars larvae of *S. cretica*:

Laboratory Bioassay:

Spinosad at its field rate (0.5 ml/l) showed high toxicity against first, second, third, fourth and fifth instar larvae of *S. cretica* (Table 1). Percent of larval mortality decreased gradually as Spinosad concentrations decreased. Moreover, mortality rates decreased as *S. certica* larvae aged, but increased with the increase of time post treatment. There were no significant differences among first three tested concentrations in their mortality rates in 1st, 2nd, 3rd, 4th and 5th instar larvae 1, 3 and 7 days post treatment.

A significant increase in percentages of mortality was observed in Spinosad treatments compared to control after 1 day ($F= 32.194$; $P< 0.0000$ for first instar, $F= 19.857$; $P< 0.0000$ for second instar, $F= 12.571$; $P< 0.0000$ for third instar, $F= 4.256$; $P< 0.0014$ for fourth instar, $F= 2.5$; $P< 0.0314$ for fifth instar). After 3 days ($F= 60.285$; $P< 0.0000$ for first instar, $F= 13.036$; $P< 0.0000$ for second instar, $F= 17.532$; $P< 0.0000$ for third instar, $F= 10.119$; $P< 0.0000$ for fourth instar, $F= 1.999$; $P< 0.0192$ for fifth instar), and after 7 days ($F= 13.809$; $P< 0.0000$ for first instar, $F= 23.771$; $P< 0.0000$ for second instar, $F= 16.547$; $P< 0.0000$ for third instar, $F= 14.513$; $P< 0.0000$ for fourth instar, $F= 12.455$; $P< 0.0000$ for fifth instar) after feeding on treated corn (Table 1).

The estimated slope, LC_{20}, LC_{50} and LC_{90}, of Spinosad toward 1st to 5thinstar larvae of *S. cretica*are presented in Table (2). Data confirmed high toxicity of Spinosad against all tested larval instars of *S. cretica*. The steepest slope of 9.466 was observed in fifth instar larvae while the flattest one was recorded in first instar at 2.755. Regarding LC_{20}, LC_{50} and LC_{90}, the highest values were recorded in *S. cretica* fifth instar larvae, followed by fourth, third, second instars whereas the lowest values were observed in first instar larvae. The 1st instar larvae were the

most susceptible one to the toxic effect of Spinosad, where the respective values of LC_{20}, LC_{50} and LC_{90},s were 0.003, 0.008 and 0.030 cm/l, respectively.

These findings are in conformity with those reported earlier by Aydin & Gurkan (2006) and Elbarky et al. (2008), who found that Spinosad was very toxic effect to larvae of Cotton leafworm *Spodoptera littoralis*, and the highest toxicity was recorded against 2nd instar compared to 4th instar larvae. The same conclusion was reported by Mahmoud (2004) and Hussein et al. (2005) who observed that Spinosad was very toxic to earlier larval instars of Black cutworm *Agrotis ipsilon* compared to older ones. Also, Mandour et al. (2008) who confirmed high toxicity of Spinosad to the tested larval instars of Jasmine moth *Palpita unionalis* and mortality was in the order of first instar > third instar > fifth instar with respective LC_{50} values of 0.019, 0.025 and 0.040 ml/l. In the present study, mortality of *S. cretica* larvae increased with an increase in Spinosad concentration and the time after exposure. Such findings are consistent with those reported by Aydin & Gurkan (2006) who concluded that the third instar larvae of *S. littoralis* displayed a concentration-dependent response to Spinosad. Similar conclusion was reported by Mollaie et al. (2011) who revealed that the efficacy may vary by developmental stages of three stored product pests; red flour beetle *Tribolium castaneum*, Mediterranean flour moth *Ephestia kuehniella* and Indian meal moth *Plodia interpunctella,* and the mortality rate increased with an increase in Spinosad concentration and exposure time. Symptoms of poisoning in *S. cretica* larvae were consistent with typical effects of intoxication observed with insects including paralysis and cessation of feeding (Salgado, 1998). In all cases, no paralyzed or poisoned larvae were recovered.

Field Bioassay:

Efficacy of Spinosad on damage resulting from the larval activity:

The effect of different concentrations of Spinosad on the damage from the larval activity represented as number of corn plants with dead heart, number of holes per plant, number of larvae per plant, number of tunnels per plant and the excavation area of stem plant caused under natural infestation by larvae of *S. cretica* within maize plants are presented in (Table 3). Data indicated significant differences between Spinosad treatments and control in all investigated parameters.

On the percent of plants with dead heart:

Data in Table (3) indicated that the number of plants with dead heart under natural infestation considerably decreased with the increase of Spinosad concentrations. High level of reduction in the plants with dead heart (90.28) was recorded with the concentration of FR (0.5 ml/l), which decreased with decrease in concentrations of Spinosad. However, the reduction of plants with dead heart among the four highest treatment concentrations was not significantly different.

On the mean number of holes per infested plant:

All the insecticide treatments significantly decreased the mean number of holes. However the lowest mean number of holes per plant was 0.5 in treatment of field rate (0.5 ml/l), followed by 1.25, 2.5 and 2.75 in the treatment of 50, 25 and 12.5% FR, with no significant differences among them compared to control with an average of 6.25 holes per plant (Table 3).

On the mean number of larvae, tunnels and excavated area per infested plant:

Data presented in Table (3) showed that the mean number of larvae per plant varied from 1 to 5 larvae per infested plant. The plots treated with Spinosad in higher concentrations of 100, 50, 25% FR caused significantly decrease in the mean number of larvae per plant at 1, 1 and 2, respectively compared to control at 5 larvae per plant. Likewise, the mean number of tunnels formed by *S. cretica* larvae inside stem per plant and the percent of excavated area were significantly decreased as a result of decrease in the number of larvae in the three highest treatment concentrations of Spinosad.

The above mentioned results revealed that Spinosad at concentrations down to 25% FR showed high efficacy against *S. cretica* under field conditions, in which there were significant reduction in the number of plants containing either perforated stem or dead hearted case, number of larvae, tunnels and excavated areas inside infested plants. These findings are in agreement with those of Ahmed et al. (2002) who studied the field efficacy of some biopesticides (Spinosad one of them) against Jower stem borer *Chilo partellus* (Pyralidae: Lepidoptera) and found that in Spinosad treated plots, the infestation was reduced from 10.72% before spray to 3.05% after seven days of first spray and to 0.74% on seventh day of second spray, which was done one week after first spray. Also, Sabbour & Abdel-Rahman (2013) recorded significantly decreased in the infestation number of corn pests when treated with the Spinosad under laboratory and field conditions. Moreover, Abd El-Mageed & Elgohary (2007) suggested the possibility of replacing the conventional insecticides with safety environmental compounds as Spinosad for controlling two corn borers *S. cretica* and *Ostrinia nubilalis*.

Biochemical activity of Spinosad on different instars larvae of *S. cretica*:

Effect of Spinosad on total protein content:

In control larvae, the concentration of soluble protein remained stable throughout the experiments (1450.34 ± 24.34 µg.g FW-1 to 70.45 ± 16.98 µg.g FW-1 Table 4). In exposed larvae, the protein content was significantly ($P<0.05$) lower than that of the control at all concentrations of exposure (Table 4). The highest rate of decreasing was -54.5% for 2nd instar larvae exposed to 50% FR (0.25ml/l). The significantly decrease of total protein contents were also reported in earlier studies on the 6th instar larvae of *Spodoptera littoralis* when treated with pyrethroid (Shaaban et al., 1985), cypermethrin and spinosad compounds (El-Sheikh, 2012). The reduction of protein content may be ascribed to a catabolism of protein in response to larvae energy demand as suggested for an isopod in response to parathion (Ribeiro et al., 2001). Several authors have shown that the reduction of worm protein content was one of the primary toxic effects of various pesticides; this decrease of protein content appeared to be an early defense reaction to the pesticides stress in insects. Mosleh et al. (2003) found that the reduction of total protein of earthworms (*Aporrectodea caliginosa*) might be the primary effect of chlorfluazuron, while it comes as a secondary effect for other pesticides (cypermethrin, aldicarb, profenofos, atrazine and metalaxyl). The decrease in protein content might be due to a mechanical lipoprotein formation, which will be used to repair damaged cells, tissues, and organs (Saravana Bhavan & Geraldine, 2001; Ribeiro et al., 2001; Mosleh et al., 2003).

Effect of Spinosad on glycogen content:

Similarly to protein, the glycogen level in the treated larvae was significantly lower than those in control larvae which were approximately 11.4 ± 0.09 µg.g FW-1, this decrease was concentration-dependent and reached -55.8% to 2nd instar larvae exposed to 50% FR (0.25 ml/l) (Table 4). Similar results were obtained by Elbarky et al. (2008) who estimated the reduction in carbohydrate contents of 4th instar larvae of *S. littoralis* after treatment by LC_{50} of Spinosad (Radiant) by -65.06%, and -26.7% as compared to untreated control. A decrease in glycogen in response to pesticides was also observed in isopods (Vink et al., 1995; Ribeiro et al., 2001), albino mice (Ksheerasagar & Kaliwal, 2003), and snails (Rambabu & Rao, 1994). The depletion of glycogen may be due to direct utilization of this compound for energy generation, as a result of pesticide-induced hypoxia (Saravana Bhavan & Geraldine, 2001). Glycogen is rapidly catabolized, resulting in an important decrease in this energy reserve.

In conclusion, results of the present study highlighted the toxicity and biochemical impacts of Spinosad to the pink corn borer. Results showed that the target insect pests were susceptible to treatments with different concentrations of Spinosad. The high efficacy of the sublethal concentrations of Spinosad indicated its high biological activity and possibility for the reduction of the recommended concentrate. Under field conditions, the percentages of infestation were significantly decreased among the plots treated with different concentrations of Spinosad down to 25% FR, which merits further attention toward more cost saving in control management. Based on the biochemical studies, Spinosad at the sub-lethal concentrations altered some biochemical cycles, the level of carbohydrate (glycogen) was reduced and the protein content was found to be decreased in the treated larvae of *S. cretica*. This fact, in turn, can confirm the reasons that adversely affect the growth, and development, thus the expected damage of this serious pest.

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Table 1. Mortality percentage of *Sesamia cretica* larvae fed on corn stem treated with serial concentrations of Spinosad one, three and seven days post treatment.

Concentration	% Mortality														
	1st instar			2nd instar			3rd instar			4th instar			5th instar		
	Post 1 day	Post 3 days	Post 7 days	Post 1 day	Post 3 days	Post 7 days	Post 1 day	Post 3 days	Post 7 days	Post 1 day	Post 3 days	Post 7 days	Post 1 day	Post 3 days	Post 7 days
FR*	88.89 a	100 a	100 a	77.78 a	77.78 a	100 a	77.77 a	100 a	100 a	44.44 a	66.67 a	88.89 a	11.11 a	22.22 a	66.67 a
% FR	88.89 a	100 a	100 a	66.67 a	77.78 a	100 a	44.44 b	55.55 b	100 a	33.33 ab	55.55 a	88.89 a	0 a	11.11 a	55.55 ab
25% FR	77.78 a	100 a	100 a	66.67 a	66.67 a	100 a	33.33 bc	44.44 bc	88.89 a	33.33 ab	44.44 ab	66.67 ab	0 a	11.11 a	55.55ab
12.5% FR	44.43 b	88.89 a	100 a	33.33 b	44.44 ab	88.89 a	11.11 cd	22.22 cd	77.77 a	22.22 ab	22.22 bc	44.44 bc	0 a	11.11 a	33.33 bc
6.25% FR	22.22 c	33.33 b	88.89 a	0 c	22.22 bc	77.78 a	11.11 cd	11.11 d	33.33 b	11.11 ab	22.22 bc	44.44 bc	0 a	0 a	22.22 cd
3.125% FR	11.11 c	33.33 b	66.67 ab	0 c	11.11 c	33.33 b	0 d	11.11 d	33.33 b	0 b	11.11 c	33.33 c	0 a	0 a	11.11 cd
1.563% FR	11.11 c	11.11 c	55.56 b	0 c	0 c	33.33 b	0 d	0	22.22 b	0 b	11.11 c	22.22 cd	0 a	0 a	11.11 cd
Control	0 c	0 c	11.11 c	0 c	0 c	11.11 b	0 d	0	0 b	0 b	0 c	0 d	0 a	0 a	0 d
F	32.194	60.285	13.809	19.857	13.036	23.771	12.571	17.532	16.547	4.256	10.119	14.513	2.5	1.999	12.455
P	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0014	0.0000	0.0000	0.0314	0.0192	0.0000
LSD	18.788	15.879	24.599	22.457	26.57	21.304	22.457	23.552	27.503	24.599	21.304	23.553	7.101	14.203	20.085

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; P≤0.05)
* = Field Rate 0.5 ml/l (60 mg/l a.i.).

Table 2. The toxic effect of Spinosad to different developmental stages of *Sesamia cretica*.

Larval instars of *Sesamia cretica*	Slope	LC20 (95% CI)*	LC50 (95% CI)	LC90 (95% CI)
1st instar larvae	2.775	0.003 (0.001-0.008)	0.008 (0.005-0.013)	0.030 (0.018-0.048)
2nd instar larvae	2.716	0.007 (0.004-0.012)	0.016 (0.012-0.033)	0.059 (0.036-0.094)
3rd instar larvae	3.262	0.010 (0.006-0.017)	0.028 (0.020-0.039)	0.129 (0.076-0.219)
4th instar larvae	7.411	0.008 (0.003-0.019)	0.044 (0.027-0.071)	0.577 (0.212-1.568)
5th instar larvae	9.466	0.024 (0.011-0.049)	0.159 (0.086-0.293)	8.74 (0.589-14.017)

- Confidence interval cm/l
- Data for larval instars are based on the mortality rates 7 days post treatment.

Table 3. Effect of various concentrations of Spinosad on the damage caused by the infestation activity *S. critica* larvae.

Spinosad concentrations	Dead heart/50 plants		No. of holes/infested plant	No. of larvae/ infested plant	No. of tunnels/infested plant	% Excavated area of stem/infested plant
	Average	% reduction				
% FR	1.75±0.48 d	90.28 a	0.5±0.29 e	1.0±0.41 b	0.75±0.48 c	5.0±1.19 c
25% FR	2.25±0.25 d	87.50 a	1.25±0.25 de	1.0±0.41 b	1.0±0.00 c	6.11±0.65 c
12.5% FR	4.25±0.75 d	76.39 a	2.5±0.65 cd	2.0±0.41ab	1.75±0.25bc	7.77±1.22 c
6.25% FR	5.0±0.71 d	72.22 a	2.75±0.25 cd	3.25±0.65ab	2.75±0.25ab	11.66±1.71 c
3.125% FR	10.75±1.65 c	40.28 b	4.25±0.25bc	3.25±0.48ab	3.25±0.48 a	20.55±2.33 b
1.563% FR	13.5±0.65bc	25.00 b	4.25±0.75bc	4.25±0.85 a	4.0±0.58 a	18.88±1.73 b
% FR	14.5±1.04 b	19.44 b	5±0.82 b	5.0±0.91 a	4.0±0.71 a	27.5±3.42 a
Control	18.0±1.47 a	-	6.25±0.48 a	5.0±1.08 a	4.0±0.00 a	31.94±2.53 a
F	39.93	19.06	15.71	5.57	10.46	20.34
P	0.000	0.000	0.000	0.001	0.000	0.000
	***	***	***	***	***	***
LSD	2.873	20.56	1.512	2.031	1.228	5.909

Means followed with the same letters (column wise) are not significantly different (Tukey' HSD; P≤0.05.

Table 4. Effect of different concentrations of Spinosad on the total soluble protein (µg.g FW-1) and glycogen level (µg.g FW-1) concentrations on 2nd, 3rd, 4th and 5th instar larvae of *Sesamia cretica*.

Concentrations	2nd instar		3rd instar		4th instar		5th instar	
	Total Soluble Protein (µg.g FW-1)	Glycogen (µg.g FW-1)	Total Soluble Protein (µg.g FW-1)	Glycogen (µg.g FW-1)	Total Soluble Protein (µg.g FW-1)	Glycogen (µg.g FW-1)	Total Soluble Protein (µg.g FW-1)	Glycogen (µg.g FW-1)
% FR	659.56 ± 15.34	5.03 ± 0.03	723.56 ± 15.65	6.21 ± 0.12	731.12 ± 14.21	6.56 ± 0.56	757.34 ± 9.12	7.02 ± 0.38
25% FR	723.34 ± 21.23	5.65 ± 0.07	759.21 ± 21.12	7.25 ± 0.10	792.21 ± 9.34	7.12 ± 0.67	789.21 ± 7.98	7.79 ± 0.21
12.5% FR	769.45 ± 14.43	6.78 ± 0.09	823.23 ± 15.34	8.45 ± 0.93	821.31 ± 11.78	8.49 ± 0.98	821.54 ± 12.11	8.69 ± 0.76
6.25% FR	887.56 ± 17.34	7.56 ± 0.15	873.43 ± 21.12	9.13 ± 1.01	891.16 ± 11.21	9.38 ± 0.67	878.32 ± 9.98	9.49 ± 0.58
3.125% FR	885.56 ± 14.32	8.54 ± 0.45	859.56 ± 15.34	10.56 ± 0.98	932.76 ± 10.65	10.49 ± 0.95	921.12 ± 21.10	10.69 ± 0.93
1.563% FR	922.45 ± 153	10.94 ± 0.83	920.12 ± 12.13	11.43 ± 1.26	989.34 ± 15.53	11.79 ± 0.74	1012.98 ± 18.87	12.28 ± 1.3
Control	1450.34 ± 24.34	11.4 ± 0.09	1467.34 ± 0.32	12.12 ± 0.12	1521.34 ± 9.87	12.98 ± 0.34	1565.45 ± 9.98	13.87 ± 0.54