Effects of Spinosad as a Bioinsecticide on the Corn Stem Borer, *Sesamia cretica* Led. (Lepidoptera: Noctuidae)

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Abstract: The corn stem borer, *Sesamia cretica* Led. (Lepidoptera: Noctuidae) is one of the most economically important pests of maize crop in Egypt. The pest is generally controlled by repetitive application of chemicals, resulting in environmental pollution and resistance in pest population. In this study, efficacy of spinosad against different larval instars of *S. cretica* was evaluated under laboratory and field conditions. The impact of spinosad on larval protein content and glycogen level were also assessed. Data indicated that spinosad had exerted some toxic effect against the tested larval instars and the mortality was in the order of first instar > second instar > third instar > fourth instar > fifth instar, with respect to the LC_{50} values estimated as 0.008, 0.016, 0.028, 0.044 and 0.159 ml/l for the prementioned instars respectively, 7 days after treatment. The field experiment showed high efficiency of spinosad against *S. cretica* at the highest three concentrations down to 25% field rate (FR); inducing significant reduction in the number of plants containing either perforated stems or dead hearted cases, 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 were significantly lower those that of the control at all concentrations. The highest reduction in protein content, -54.5% was recorded in 2nd instar larvae exposed to 50% FR (0.25ml/l). Similarly, the highest reduction in glycogen level, -55.8%, was recorded in 2nd instar larvae exposed to 50% FR (0.25 ml/l), meanwhile, it was also noted that impact was concentration dependent.

Keywords: Spinosad, *Sesamia cretica*, Toxicity, Biochemical impacts, Protein contents, Glycogen level

INTRODUCTION

Maize *Zea mays* L., is the third most important cereal crop in the world agricultural economy after wheat and rice. Maize occupies a crucial place since it was 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 around 750.000 hectares, with a total grain yield of 7 MT (FAO, 2012). However, this crop is subjected to severe attack by several insect pests causing considerable damage estimated that amount to 25% of the total production 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 corn borer *Sesamia cretica* Led. (Lepidoptera: Noctuidae), the striped stem borer *Chilo agamemnon* Blesz., and the European corn borer *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae) (Moyal *et al.*, 2002).

The corn stem borer, *S. cretica*, 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 in early maize crops, which are sown between late March and mid-May, in which the stem borer may cause severe damage (Semeada, 1988).

Stem borers affect maize yields by reducing the photosynthetic area of the plant leaves. Moreover, crop losses are caused due to death of the growing point, early leaf senescence, reduced translocation, lodging, beside direct damage to ears. Secondary losses have been documented as a result of infections by bacterial and fungal pathogens via entry points created by the stem borers within the plant tissues (Ndiritu, 1999). The corn borers cause significant economic losses in production amount to about 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, many insects developed resistance to pesticides after several generations of successive exposure. Also, these pesticides have negative impacts on the environment, especially on the beneficial organisms. Thus, the need to environmental friendly products for pest control is in continuous 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 and 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 and 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 and 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 elemental part of the integrated pest management programs of certain

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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 proportional balance 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 is extremely destructive if its infestation exceeds thresholds. Hence, it was quite imperative to study the response of protein and glycogen of treated *S. cretica* to such this bioinsecticide.

Therefore, the present research had the objective of evaluating the effects of spinosad as a bioinsecticide on the corn stem borer *S. cretica* under laboratory and field conditions. The study has also meant to determine the impact of this bioinsecticide on some biochemical constituent such as the total protein contents and glycogen levels in different larval instars of this pest.

MATERIALS AND METHODS

Insect maintenance:

Larvae of maize borer, *S. cretica* were manually collected from untreated maize plants in the experimental farm, Faculty of Agriculture, University of Suez Canal. The infested plants were detached and transferred to the laboratory to inspect and separate the different larval instars of *S. cretica* that were reared for several generations under laboratory conditions of 27 ± 2ºC; 60 ± 10% RH and photoperiod of 14: 10 (L : D) h. Collected larvae were reared inside plastic boxes (50 × 50 × 20 cm) with screen lids, fed on untreated maize plants until pupal stage. Pupae were collected and transferred to Petri dishes inside wood cages (60 × 60 × 60 cm) with three screen sides, and supplied with saturated cotton piece by 10% sugar solution. Upon emergence, adults were allowed to lay eggs on leaf sheathes of young maize plants (20-25 days old), inside the wood cages in the time of adults oviposition periods.

Bio-insecticide used:

A commercial formulation of spinosad (Spinosad12%EC), a gift from Dow Agro Science Inc. was used in all bioassays. Spinosad is registered in Egypt against several lepidopetran pests at a field rate of 0.5 ml/l (60 mg/l a.i.). Solutions of this compound were prepared in distilled water at the field rate concentration (0.5 ml/l.) 100% FR. Other tested concentrations were prepared by diluting 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 application.

Laboratory Bioassay:

As a result of preliminary tests, serial concentrations of spinosad 12% EC were prepared and used for each test to get larval mortality ranging between ≥ 25 to ≤ 75% for the lowest and highest concentrations, 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.) and 1.56% 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 9 larvae each. Small stem pieces of maize plants (5 cm length) were transected and dipped into the different concentrations for 10 seconds. The stem pieces were placed on a paper towel for at least 2 hours or until they dry out before being used in the experiments. The tested larvae of *S. cretica* were starved for at least 4 hours before the experiment. Larvae were removed gently by fine camel-hair brush and placed into glass vials (3×10 cm), supplied with the 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 larval mortality 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 concentrations of spinosad (0.5, 0.25, 0.125, 0.06, 0.03, 0.016 and 0.007 ml/l) were sprayed twice. The first spray was applied just two weeks after sowing, and the second was after two weeks post the first one. 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) at 35 days-old plants.

Biochemical impacts:

Determination of the total protein and glycogen contents 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 stem pieces with (50, 25, 12.5, 6.25, 3.125 and 1.563% 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 and Heijningen (1954). Calibration was performed using standards of glucose ranging from 0 to 200 mg/dl 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 and 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 AND DISCUSSION

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

Laboratory Bioassay:

Spinosad at field rate level (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 as a function of post treatment time increase. There were no significant differences between first three tested concentrations in their mortality rates among the five instar larvae at 1, 3 and 7 days post treatment.

Significant increase in mortality was observed in spinosad treatments compared to control after 1 day of feeding (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 data were (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) (Table 1).

The estimated slope, LC_{20}, LC_{50} and LC_{90}, of spinosad toward 1st to 5th instar larvae of *S. cretica* are presented in Table (2). Data confirmed the high toxicity of spinosad against all tested larval instars. The steepest slope of 9.466 was observed in the fifth instar larvae while the flattest one was recorded for the first instar at 2.755. Regarding LC_{20}, LC_{50} and LC_{90}, the highest values were recorded for the fifth instar larvae, followed by the fourth, third, second instars, whereas the lowest values were observed in first instar larvae. The 1st instar larvae were the most susceptible 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 and Gurkan (2006) and Elbarky *et al.* (2008), who found that spinosad was very toxic to cotton leaf worm *Spodoptera littoralis*, larvae and the highest toxicity was recorded against 2nd instar compared to 4th instar larvae. The same conclusion was reported also by Mahmoud (2004) and Hussein *et al.* (2005) who observed that spinosad was very toxic to earlier larval instars of the black cutworm *Agrotis ipsilon* compared to older ones. Mandour *et al.* (2008) have also reported high toxicity of spinosad to the tested larval instars of Jasmine moth *Palpita unionalis,* and also reported that 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 the increase of spinosad concentration and the time after application. Such findings are consistent with those reported by Aydin and Gurkan (2006) who concluded that the third instar larvae of *S. littoralis* displayed a concentration-dependent response to spinosad. Similar conclusion was also 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*. They also reported that mortality rate increased with the 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.

On the percent of plants with dead heart:

Data in Table (3) indicated that the number of plants with dead hearts have considerably decreased with the increase of spinosad concentrations. High level of dead hearts reduction (90.28) was recorded with the concentration of FR (0.5 ml/l), and also decreased as spinosad concentration decreased. However, the reduction of plants with dead hearts 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 in treated plants. However, the lowest mean number of holes per plant was 0.5 in the treatment of field rate (0.5 ml/l), followed by 1.25, 2.5 and 2.75 in the treatments of 50, 25 and 12.5% FR, with no significant differences among them, compared to control with the 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 at concentration level of 100, 50, 25% FR have significantly decreased the mean number of larvae per plant to 1, 1 and 2, respectively compared to 5 larvae per plant in the control plants. Likewise, the mean number of tunnels formed by *Sesamia* larvae inside a plant stem and the percent of excavated area were significantly decreased as a result of decrease in the number of larvae in the three aforementioned concentrations of spinosad.

Table (1): Mortality percentage of *S. cretica* larvae fed on corn stem treated with serial concentrations of spinosad at one, three and seven days post treatment

Spinosad concentrations	% 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*	88.89a	100a	100a	77.78a	77.78a	100a	77.77a	100a	100a	44.44a	66.67a	88.89a	11.11a	22.22a	66.67a
٥٠% FR	88.89a	100a	100a	66.67a	77.78a	100a	44.44b	55.55b	100a	33.33ab	55.55a	88.89a	0a	11.11a	55.55ab
25% FR	77.78a	100a	100a	66.67a	66.67a	100a	33.33bc	44.44bc	88.89a	33.33ab	44.44ab	66.67ab	0a	11.11a	55.55ab
12.5% FR	44.43b	88.89a	100a	33.33b	44.44ab	88.89a	11.11cd	22.22cd	77.77a	22.22ab	22.22bc	44.44bc	0a	11.11a	33.33bc
6.25% FR	22.22c	33.33b	88.89a	0c	22.22bc	77.78a	11.11cd	11.11d	33.33b	11.11ab	22.22bc	44.44bc	0a	0a	22.22cd
3.125% FR	11.11c	33.33b	66.67ab	0c	11.11c	33.33b	0d	11.11d	33.33b	0b	11.11c	33.33c	0a	0a	11.11cd
1.563% FR	11.11c	11.11c	55.56b	0c	0c	33.33b	0d	0d	22.22b	0b	11.11c	22.22cd	0a	0a	11.11cd
Control	0c	0c	11.11c	0c	0c	11.11b	0d	0d	0b	0b	0c	0d	0a	0a	0d
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

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

Table (2): Toxicity of spinosad against different larval instars of *S. cretica*.

Larval instars of *S. cretica*	Slope	LC20 (95% CI)*	LC50 (95% CI)	LC90 (95% CI)
1st instar	2.775	0.003 (0.001-0.008)	0.008 (0.005-0.013)	0.030 (0.018-0.048)
2nd instar	2.716	0.007 (0.004-0.012)	0.016 (0.012-0.033)	0.059 (0.036-0.094)
3rd instar	3.262	0.010 (0.006-0.017)	0.028 (0.020-0.039)	0.129 (0.076-0.219)
4th instar	7.411	0.008 (0.003-0.019)	0.044 (0.027-0.071)	0.577 (0.212-1.568)
5th instar	9.466	0.024 (0.011-0.049)	0.159 (0.086-0.293)	٢8.74 (0.589-14.017)

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

Table (3): Effect of various concentrations of spinosad on the larval activity of *S. cretica*.

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
٥٠% 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
25% 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
12.5% 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
6.25% 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
3.125% 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
1.563% 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

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.)

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 including spinosad against the 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, then dropped to 0.74% on the seventh day of second spray, which was done one week after first spray. Also, Sabbour and Abdel-Rahman (2013) recorded a significant decrease in numbers of corn pests when treated with spinosad under laboratory and field conditions. Moreover, Abd El-Mageed and Elgohary (2007) suggested the possibility of replacing the conventional insecticides with safety environmental compounds as spinosad for controlling the two corn borers *S. cretica* and *Ostrinia nubilalis*.

Biochemical activity of spinosad on different larval instars of *S. cretica*:

Effect of spinosad on total protein content:

In the control larvae, the concentration of soluble protein remained stable throughout the experiments (1450.34 ± 24.34 µg.g FW-1 (fresh weight, FW) to 70.45 ± 16.98 µg.g FW-1 Table 4). While, in treated larvae, the protein content was significantly (P<0.05)

lower than that of the control at all concentrations (Table 4). The highest rate of protein content drop was -54.5% recorded in the 2nd instar larvae exposed to 50% FR (0.25ml/l). The significant decrease of total protein contents was also reported in earlier studies on the 6th instar larvae of *Spodoptera littoralis* when treated with synthetic pyrethroids insecticide cypermethrin (Shaaban *et al.,* 1985), and spinosad compounds (El-Sheikh, 2012). The reduction of protein content may be ascribed to a catabolism of protein in response to larval 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*) may 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 may be due to a mechanical lipoprotein formation, which will be used to repair damaged cells, tissues, and organs (Bhavan and Geraldine, 2001; Ribeiro *et al.*, 2001; Mosleh *et al.*, 2003).

Effect of spinosad on glycogen content:

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 as compared to untreated control. A decrease in glycogen in response to pesticides was also observed in isopods (Ribeiro *et al.,* 2001), albino mice (Ksheerasagar and Kaliwal, 2003), and snails (Rambabu and 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 (Bhavan and Geraldine, 2001). Glycogen is rapidly catabolized, resulting in an important decrease in this energy reserve.

CONCLUSION

Results of the present study highlighted the toxicity and biochemical impacts of spinosad to the corn borer, *S. cretica*. Results showed that the target pest was susceptible to treatments with different concentrations of spinosad. The high efficacy of the sublethal concentrations of spinosad indicated its high biological activity and offering the possibility for cutting down its current recommended rate. 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 has 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 (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 *S. cretica*

Spinosad 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)
50% 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

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