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Sublethal effects of different insecticides against *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae under laboratory conditions

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Abstract

One significant insect that negatively affects a variety of crops is the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). The purpose of this study was to examine, in a lab setting, how various insecticides affected the biological characteristics of *S. littoralis*. This study tested the following insecticides: spinosad (Tracer 24%) at 5.0 mg/L; pyridalyl (Pleo® 50% EC) produced by Sumitomo Chemical Co. at 0.5 cm/L; protecto (*Bacillus thuringiensis*) WP 9.4 at 4 g/L; profenofos (Diliron 72% EC) at 75 mg/L; and acetamiprid 22.7% + bifenthrin 27.3% (Robek® WP 50%) at 0.25 g/L. According to the results, *S. littoralis* responded favorably to every insecticide that was tested. However, acetamiprid 22.7% + bifenthrin 27.3% (Robek® WP 50%) at 0.25 g/L, pyridalyl (Pleo® 50% EC) at 0.5 cm/L, and protecto at 2 g/L were the most effective insecticides. Robek, at 0.25 g/L, had the greatest drop in the consumption index and growth rate for the fourth larvae, while pleo, at 0.5 cm/L, came in second. Larval duration, pupation percentage, normal larvae percentage, normal pupae percentage, pupa weight, emergence percentage, adult longevity, fecundity level, and hatchability percentage were all decreased by using robek at 0.25 g/L and pleo at 0.5 cm/L. Additionally, it generated the highest percentages of sterility, pupal mortality, malformed pupae, larval mortality, and malformed larvae.

Introduction

In Egypt, the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a major insect pest that seriously damages a variety of crops and vegetables, particularly cotton (Kandil *et al.*, 2003). *S. littoralis* larvae are polyphagous pests that cause a large financial loss for a variety of industrial, ornamental, and vegetable crops in both open fields and greenhouses. Conversely, a variety of insects, including a broad spectrum of agricultural pests around the globe, have acquired pesticide resistance (Gehig, 1983). According to Su and Sun (2014), *S. littoralis* has become resistant to a variety of insecticides, making insecticide resistance one of the main issues with its control. This has resulted in a decrease in its uptake and use, in addition to negative environmental repercussions. Chemical insecticides are still a useful tool in pest management programs, though. The use of bioinsecticides, whether they be bacterial, fungal, botanical, or insect growth regulators, is on the rise due to the emergence of resistance to various chemical pesticides (Georghiou, 1986; Ahmad *et al.*, 2008 and Mourad *et al.*, 2008). Compared to conventional compounds, these groupings exhibit distinct characteristics and mechanisms of action (Thompson *et al.*, 1999).

A highly powerful organophosphate pesticide, profenofos is used to control a variety of sucking and chewing pests in crops like vegetables and cotton. Larval and pupal duration were significantly increased by profenofos, but not by concentrations of mancozeb and bromoxynil. Every parameter investigated for the fourth-instar larvae of the cotton leafworm, *S. littoralis*, is significantly impacted by profenofos. A naturally occurring chemical called spinosad is produced by a soil bacterium and can be harmful to insects. Because it is a stomach and nerve poison, it kills bugs that come into contact with it as well as those that eat the foliage. According to Singh and Sohi (2007), spinosad insecticide increased the deterrent efficacy of feeding *S. litura* larvae at sub-lethal concentrations. According to Salem *et al.* (2023), the percentage of adult eclosion was reduced to 79% from 94% in the control group when the 4th instar *S. frugiperda* larvae were treated with LC_{50} spinosad. A broad-spectrum pesticide, mectin works well to control a variety of pests. The impact of bio-rational pesticides, such as mectin, on the biological characteristics of *S. littoralis* larvae was examined by Osman and Mahmoud (2008), who discovered that the tested insecticide increased mortality. It may be possible to boost the toxicity and accomplish more successful insect pest control by combining several pesticide groups. Combining pesticides may present intriguing options for controlling insects, particularly if there are interactions that make them more effective when used together (Yu, 2008) and give better insect control in both lab and field settings (Bhatti *et al.*, 2013). According to Ahmad (2004), combining insecticides with different mechanisms of action may help prevent pest populations from becoming resistant. This is due to the possibility that different insect populations may not have the resistance mechanisms required for each insecticide in the combination.

To choose the optimal chemicals for managing this profitable bug in pest management programs, the current experiment sought to determine how well a number of insecticides worked against cotton leafworm.

Materials and methods

1. Chemicals:

Propenofos (Diliron 72% EC) at 75 mg/L, acetamiprid 22.7% + bifenthrin 27.3% (Robek® WP 50%) at 0.25 g/L, spinosad (Tracer 24%) at 5.0 mg/L, pyridalyl (Pleo® 50% EC) made by Sumitomo Chemical Co. at 0.5 cm/L, and protecto (*Bacillus thuringiensis*) WP 9.4 at 4 g/L were the insecticides used in this experiment (Table 1). At the Al-Marashda Agricultural Research Station in the Qena Governorate of Egypt, cotton was grown in order to collect insect rearing larvae of the cotton leaf worm (*S. littoralis*). For numerous generations, they were housed in a lab setting with controlled temperatures of 25 ± 2°C and 70 ± 10% relative humidity. The larvae were fed castor bean leaves every day (Marzouk *et al.*, 2012).

Table (1): Characteristics of the target insecticides evaluated in this study.

Common name	Commercial name	Formulation	Chemical group
Profenofos	Diliron	72 % EC	Organophosphate
Acetamiprid 22.7% + bifenthrin 27.3%	Robek	50 % WP	Neonicotinoid+ Pyrethroid
Spinosad	Tracer	24 % SC	Bio-insecticide
Pyridalyl	Pleo	50 % EC	Dihalopropenyloxybenzene
***Bacillus* thuringiensis**	Protecto	9.4 WP	Bio-insecticide

In this work, newly molted *S. littoralis* larvae in their fourth instar, weighing an average of 13–15 mg/larva, were employed. For every insecticide, there are three replicates of both treated and untreated larvae (20 larvae per replication). After being immersed in each of the utilized insecticides for 20 seconds, castor bean leaves were dried in a lab setting. In contrast, the leaves were submerged in water for the control treatment. Twenty larvae were placed in a 350 mL glass jar with the treated castor bean leaves for each replicate. Dead larvae were disposed of after 48 hours, and the remaining larvae were moved to sterile jars and fed new castor bean leaves constantly until pupation and adulthood. Every day, the number of survivors in each replicate correlated with the fresh weight of introduced leaves, untreated castor leaves, and survivors.

To calculate the natural moisture loss and adjust the weight of consumed leaves, fresh leaves were stored in identical glass jars under identical conditions. Waldbauer (1968) provided the following estimations for the nutritional indices used in this study. The amount of food consumed per unit of time in relation to the average weight of the larvae during the feeding period is measured by the consumption index, or C. I.

C. I. = C / [(T × A)]

Where A is the mean fresh weight of the larvae during the feeding time, T is the length of the feeding period, and C is the fresh weight of the leaf devoured. The amount of weight gained per unit of time in relation to the mean weight of larvae throughout the feeding session is determined by the growth rate (GR).

GR is equal to G / (T x A).

Where G is the larvae's fresh weight increase. Male and female couples from either treated or untreated larvae were moved to glass jars with muslin coverings and filter paper for mating when the moths emerged as adults. A piece of cotton that had been dipped in a 10% sugar solution was used to feed the emerging moths. Fresh *Nearium oleander* (L.) leaves were provided for the glass jars, which were used as oviposition sites. Every day, deposited eggs were gathered and tallied. The hatchability percentage was calculated by counting the number of newly hatched larvae. In accordance with Toppozada *et al.* (1966), the sterility % was calculated as follows:

1 - [a x b / A × B] × 100 is the sterility percentage.

b = Treatment-related hatchability percentage. A is the number of eggs laid per control female. B is the hatch/female percentage in control. The following formula was used to determine the percentage of pupation, the percentage of malformed pupation, and the percentage of emergence:

Percentage of emergence = [Number of moths / Total number of larvae] × 100%.

Percentage of distorted pupation = [Number of deformed pupae / Total number of pupae] × 100%.

Percentage of pupation = [Number of pupae / Total number of larvae] × 100.

By looking at a number of important factors, the biological effects of the five tested insecticides on *S. littoralis* were evaluated (Aly *et al.*, 2023). Pupation rate, duration of the pupal stage, weight of pupae, percentage of adult emergence, adult longevity, fertility (Proportion of hatched eggs), fecundity (The total number of eggs deposited by a female), and larval development period after treatment were among these parameters.

2. Statistical analysis:

Analysis of variance (ANOVA) was performed on the data, and Duncan's means were used to separate the data (Duncan, 1995).

Results and discussion

1. The effect on the consumption index and growth rate:

The effects of tested insecticides, including profenofos, robek, spinosad, pleo, and protecto, on the growth rate and consumption index of *S. littoralis* larvae in their fourth instar were displayed in Table (2). The results showed that, in comparison to the control, the tested insecticides had a substantial impact on the consumption index and growth rate features. When compared to the control, Robek showed the largest decrease in these features for the fourth larva at 0.25 g/L, followed by pleo at 0.5 cm/L and protecto at 2 g/L. robek, pleo, and protecto had consumption indices of 0.92, 1.17, and 1.20 and growth rates of 0.20, 0.21, and 0.23 at 2 g/L, respectively.

Table (2): Consumption index (C.I.) and growth rate of 4th instar *Spodoptera littoralis* fed on leaves treated with tested insecticides (Profenofos, robek, spinosad, pleo, and protecto).

Treatments	Consumption index (C.I)	Growth rate
Control	2.70	0.46
Profenofos at 75 mg/L	1.39	0.24
Robek at 0.25 g/L	0.92	0.20
Spinosad at 5.0 mg/L	1.36	0.25
Pleo at 0.5 cm/L	1.17	0.21
Protecto at 2g/L	1.20	0.23
LSD 5%	0.04	0.02

2. The effect on larval duration:

Statistically, it was revealed that the different tested insecticides have a significant reduction in larval duration of *S. littoralis* when compared to the control (Table 3). Applying robek at 0.25 g/L, followed by pleo at 0.5 cm/L, and protecto at 2 g/L showed minimum larval durations of 9.40, 9.50, and 9.57 days, while the maximum ones of 10.60 and 10.23 days were associated with the control and spinosad at 5.0 mg/L, respectively. Also, applying profenofos at 75 mg/L as an insecticide led to a decrease in the larval duration of *S. littoralis* compared to the control, as the larval duration was 9.67 days.

3. The effect on pupation:

The influences of different insecticides on the pupation percentage of *S. littoralis* were significant, as presented in Table (3). It is obvious from the results that applying robek at 0.25 g/L, followed by pleo at 0.5 cm/L and protecto at 2 g/L, produced the lowest values of pupation percentage, as it was 43.33, 50.0, and 50.0% for the three insecticides, respectively. Also, applying spinosad at 5.0 mg/L and profenofos at 75 mg/L led to a decrease in the pupation percentage of *S. littoralis* compared to the control, as it was 55.0% and 60.0% for both insecticides, respectively. The effect on larval mortality %. Statistically, it was proven that applied insecticides have a significant effect on larval mortality % (Table 3). Robek at 0.25 g/L, followed by pleo at 0.5 cm/L and protecto at 2 g/L, showed maximum larval mortality of 50.0, 45.0, and 41.67%, while the lowest ones of 1.67, 35.0, and 36.67% were associated with the control, profenofos at 75 mg/L, and spinosad at 5.0 mg/L, respectively. The effect on normal larvae.

Table (3) represented the mean value of normal larvae % of *S. littoralis* as affected by the tested insecticides. Analysis of variance showed significant differences among different insecticides in normal larvae %. The lowest values of normal larvae % of 90.0, 91.67, and 93.33% were registered with robek at 0.25 g/L, pleo at 0.5 cm/L, and protecto at 2 g/L, while the highest ones of 100.0 and 96.67 % were noticed with the control and profenofos at 75 mg/L, respectively. The effect on malformed larvae (%). Data in Table 3 presented the effect of the tested insecticides on malformed larvae of *S. littoralis*. Obviously, tested insecticides significantly affected the malformed larvae percentages of *S. littoralis* during the experiment. The highest malformed larvae percentages (10.0, 8.33, and 6.67%) were obtained from applying robek at 0.25 g/L, pleo at 0.5 cm/L, and protecto at 2 g/L, with a significant increase compared to other insecticides. Meanwhile, the lowest malformed larvae percentages (3.33 and 5.0%) resulted from applying profenofos at 75 mg/L and spinosad at 5.0 mg/L when compared to the other insecticides, respectively.

Table (3): Effect of tested insecticides (Profenofos, robek, spinosad, pleo, and protecto) on larval duration (Days), pupation (%), larval mortality (%), normal larvae (%) and malformed larvae (%) of *Spodoptera littoralis.*

Treatments	Larval duration (Days)	Pupation (%)	Larval mortality (%)	Normal larvae (%)	Malformed larvae (%)
Control	10.60	95.00	1.67	100.00	0.00
Profenofos at 75 mg/L	9.67	60.00	35.00	96.67	3.33
Robek at 0.25 g/L	9.40	43.33	50.00	90.00	10.00
Spinosad at 5.0 mg/L	10.23	55.00	36.67	95.00	5.00
Pleo at 0.5 cm/L	9.50	50.00	45.00	91.67	8.33
Protecto at 2g/L	9.57	50.00	41.67	93.33	6.67
LSD 5%	0.25	5.67	7.97	6.57	6.57

4. The effect on pupal duration (Days):

The percentage of pupal duration rose dramatically after being exposed to several insecticides, as seen in Table (3). In S. *littoralis* larvae that were not treated, the pupal period lasted 9.60 days. After receiving robek at 0.25 g/L, pleo at 0.5 cm/L, and protecto at 2 g/L, the pups' mean pupal duration was 10.50, 10.45, and 10.30 days, respectively. This was a considerable increase over their control period. Table (4) shows that the pupal length was 10.20 days for profenofos at 75 mg/L and 9.80 days for spinosad at 5.0 mg/L.

5. The effect on normal pupae (%):

The mean percentage of *S. littoralis* normal pupae was considerably lower after treatment with the investigated insecticides. In comparison to 100% in their respective control insects, normal pupae percentages after the robek at 0.25 g/L, pleo at 0.5 cm/L, and protecto at 2 g/L treatments were 50.0, 60.0, and 65.0%, as shown in Table (4). Additionally, after being exposed to spinosad at 5.0 mg/L, the percentage of normal pupae dropped dramatically to

67.0% in comparison to the control. Similarly, when *S. littoralis* larvae were exposed to 75 mg/L of profenofos, the normal pupae percentage (75.0%) was significantly lower than that of untreated flies.

6. The effect on malformed pupae (%):

Exposure of *S. littoralis* larvae to all insecticides under study led to a significant increase in malformed pupae percentage when compared to the control (Table 4). The highest malformed pupae percentages were associated with robek at 0.25 g/L (50.0%), followed by pleo at 0.5 cm/L insecticides (40.0%) as compared to the other insecticides. Malformed pupae percentages were increased to 35.0% with spinosad at 5.0 mg/L treatment compared to the control. Also, protecto at 2 g/L treatment resulted in an increase of malformed pupae percentage (33.0%) when compared to the control. The effect on pupa weight (g). The pupae not treated with any insecticides recorded the highest weight value (353.0 g), while the lowest values of 275, 288.33, and 290.0 g were produced with exposure of *S. littoralis* larvae to robek at 0.25 g/L, followed by pleo at 0.5 cm/L and profenofos at 75 mg/L, respectively. Also, treating *S. littoralis* larvae with spinosad at 5.0 mg/L led to a reduced pupa weight of *S. littoralis* compared to the control, as it was 310.0 g. Treating *S. littoralis* larvae with the insecticide protecto at 2 g/L resulted in a significant reduction of pupa weight (325.0 g) compared to the control.

7. The effect on pupal mortality (%):

Data in Table (4) showed the effect of tested insecticides on the pupal mortality percentage of *S. littoralis*. Obviously, the insecticides tested significantly affected pupal mortality percentages of *S. littoralis* during this study. The highest pupal mortality percentages (40.0, 35.0, and 30.0%) were registered with applying robek at 0.25 g/L, pleo at 0.5 cm/L, and protecto at 2 g/L, with a significant increase compared to the other insecticides. Meanwhile, the lowest pupal mortality percentages (25.0 and 28.0%) resulted from applying spinosad at 5.0 mg/L and profenofos at 75 mg/L when compared to the other insecticides, respectively. The effect on emergence. Statistically, it was indicated that the used insecticides have significant effects on emergence % (Table 4). Robek at 0.25 g/L, followed by pleo at 0.5 cm/L and protecto at 2 g/L, showed minimum emergence percentages (60.0, 65.0, and 70%), while the highest ones (75.0 and 75.0%) were associated with spinosad at 5.0 mg/L and profenofos at 75 mg/L, respectively.

Table (4): Effect of tested insecticides (Profenofos, robek, spinosad, pleo and protecto) on pupal duration (Days), normal pupae (%), malformed pupae (%), pupa weight (g), pupal mortality (%) and emergence (%) of *Spodoptera littoralis*.

Treatments	Pupal duration (Days)	Normal pupae (%)	Malformed pupae (%)	Pupa weight (g)	Pupal mortality (%)	Emergence (%)
Control	9.60	100.00	0.00	353.00	0.00	100.00
Profenofos at 75 mg/L	10.20	75.00	25.00	290.00	25.00	75.00
Robek at 0.25 g/L	10.50	50.00	50.00	275.00	40.00	60.00
Spinosad at 5.0 mg/L	9.80	67.00	35.00	310.00	28.00	72.00
Pleo at 0.5 cm/L	10.45	60.00	40.00	288.33	35.00	65.00
Protecto at 2g/L	10.30	65.00	33.00	325.00	30.00	70.00
LSD 5%	0.26	8.94	8.94	9.05	8.94	8.94

8. The effect on adults:

The findings indicated that robek at 0.25 g/L, pleo at 0.5 cm/L, and protecto at 2 g/L significantly impacted the adult longevity, fecundity level, hatchability percentage, and sterility percentage of *S. littoralis* when compared to the insecticides under study. Robek at 0.25 g/L, pleo at 0.5 cm/L, and protecto at 2 g/L were linked to the greatest decrease in adult longevity (6.40, 7.20, and 7.53 days), respectively. Also, robek at 0.25 g/L, pleo at 0.5 cm/L, and protecto at 2 g/L showed the greatest decrease in fecundity levels (461.67, 510.0, and 620.0 eggs/female). The decrease in hatchability percentage and increase in sterility percentage showed the same pattern (Table 5).

Table (5): Effect of tested insecticides (Profenofos, robek, spinosad, pleo and protecto) on adult longevity (Days), fecundity (Number eggs/female), hatchability (%) and sterility (%) of *Spodoptera littoralis.*

Treatments	Adult longevity (Days)	Fecundity	Hatchability (%)	Sterility (%)
Control	8.63	1140.00	96.00	0.00
Profenofos at 75 mg/L	8.30	725.00	65.67	30.00
Robek at 0.25 g/L	6.40	461.67	47.33	57.33
Spinosad at 5.0 mg/L	8.33	781.67	67.67	37.33
Pleo at 0.5 cm/L	7.20	510.00	56.67	47.67
Protecto at 2g/L	7.53	620.00	63.00	40.33
LSD 5%	0.28	15.19	4.50	4.62

However, acetamiprid 22.7% + bifenthrin 27.3% (Robek® WP 50%) at 0.25 g/L, pyridalyl (Pleo® 50%EC) at 0.5 cm/L, and protecto at 2 g/L were the most effective insecticides. Robek, at 0.25 g/L, had the greatest drop in the consumption index and growth rate for the fourth larvae, while pleo, at 0.5 cm/L, came in second. Larval duration, pupation percentage, normal larvae percentage, normal pupae percentage, pupa weight, emergence percentage, adult longevity, fecundity level, and hatchability percentage were all decreased by using robek at 0.25 g/L and pleo at 0.5 cm/L. Additionally, it generated the highest percentages of sterility, pupal mortality, malformed pupae, larval mortality, and malformed larvae. The consumption index, growth rate, larval duration, pupation, larval mortality, normal larvae, malformed larvae, adult longevity (Days), fecundity (Number of eggs/female), hatchability, and sterility of *S. littoralis* were among the biological effects of the insecticides that were tested, according to the study. In general, it was also discovered that robek (0.25 g/L), pleo (0.5 cm/L), and protecto (2 g/L) are the insecticides that have the greatest effects on these traits. Additionally, the treatment of spinosad and profenofos had a major impact on the insect's biological characteristics. According to Gad (2023), pyridalyl (pleo) reduced the number of *S. littoralis* and *C. carnea*.

Marzouk *et al.* (2012) previously documented the beneficial effects of the pesticide profenofos on the consumption index and growth rate of *S. littoralis*. They found that profenofos was the most effective chemical on the larvae of *S. littoralis* in their fourth instar when compared to the control, and it also provided the greatest reduction in consumption index at all tested concentrations. Since profenofos are advised for controlling this insect, it provides the strongest strength. With these treatments, the larvae's ability to convert ingested food into bodily components was reduced, which resulted in a slower growth rate. The results were consistent with those of Fergani *et al.* (2024), who found that robek and pleo insecticides significantly reduced the number of *S. littoralis* larvae, demonstrating long-term efficacy. Pyridalyl 50% EC and Robek® WP 50% (acetamiprid 22.7% + bifenthrin 27.3%) demonstrated notable effectiveness in managing *S. littoralis* larvae populations (Abou El-Ghar, 1994). Our findings demonstrated that the pesticide spinosad had a beneficial effect on lowering the growth rate and consumption index of *S. littoralis* larvae. When compared to the other insecticides studied, Moustafa *et al*. (2024) demonstrated that spinosad and emamectin benzoate were the most hazardous to sensitive populations of *S. frugiperda* larvae.

The results obtained were consistent with those of El-Saleh *et al.* (2025), who assessed the efficacy of various insecticides in managing *S. littoralis*. They demonstrated that lufenuron and emamectin benzoate were the most effective for spinosads. Additionally, they stated that the most effective combination was emamectin benzoate and lufenuron, which achieved over 90% efficacy, whereas the least effective combination was emamectin benzoate and spinosad. According to Korrat *et al.* (2012), the most effective insecticides in this regard were spinosad, profenofos, and emamectin benzoate. Spinosad and emamectin benzoate were found to have an indirect ovicidal impact on *S. littoralis* by Abd-El-Aziz and Sayed (2014). Attia *et al.* (2023) revealed that spinosad was found effective in reducing *S. frugiperda* and *S. cretica* larvae populations and protecting maize plants compared with the control.

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