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Lethal and sublethal impacts of some biopesticides against the black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae)

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Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae), a black cutworm, is a serious early-season soil pest of cotton, vegetables, and several field crops. Relatively little is known about the effectiveness of applying biopesticides against this pest. So, this research was conducted to evaluate the effectiveness of four biopesticides (Spinosad, Abamectin, Emamectin benzoate, and *Bacillus thuringiensis*) on the survival and growth rate of the black cutworm, *A. ipsilon*. The findings indicated significant differences in the impact of each biopesticide on the larvae, with Emamectin benzoate showing the highest efficacy in increasing mortality percentages, followed by Spinosad, Abamectin, and *B. thuringiensis* under laboratory conditions. In addition, the results of the pot experiment indicated that the Emamectin benzoate was more effective in protecting plants from damage compared to both the untreated control and the standard pesticide. Additionally, the growth rates of the black cutworm life stages were notably affected negatively; also, significant changes were found in the activity of estimated detoxifying enzymes and total protein in treated larvae compared to the control group. This indicates that the use of Emamectin benzoate not only reduces pest populations but also disrupts their biological processes, making it a promising option for sustainable pest control. Such findings highlight the potential for biopesticides to serve as effective tools in integrated pest management strategies.

Introduction

The black cutworm is a lepidopteran insect pest that has the ability to destroy several economic crops in many countries. The scientific name of the black cutworm is *Agrotis ipsilon* (Hufnagel), which belongs to the order Lepidoptera and family Noctuidae. The larvae of this insect cut the plant stem near the ground, which led to plant death in the early stage (Binning *et al.*, 2015). The black cutworm larva stage has six instars and

can consume 400 cm² of foliage during the larva stage (Amin *et al.*, 2019).

The selection of highly effective and safe insecticides is the main goal of integrated pest management strategies. There are few recorded insecticides in Egypt against *A. ipsilon*, such as cypermethrin, alpha-cypermethrin, lambda-cyhalothrin, and chlorpyrifos. All the recommended insecticides are synthetic chemical insecticides, which cause soil contamination.

There are no biopesticides recommended against *A. ipsilon*, such as *Bacillus thuringiensis*, spinosad, and abamectin and emamectin benzoate, according to recommendations of the Agricultural Pesticide Committee of the Ministry of Agriculture and Land Reclamation in 2020, 2021, 2022, 2023, and 2024 in Egypt. In addition, few studies were carried out on the effectiveness of biopesticides against black cutworm in Egypt. In spite of most biopesticides being effective pesticides against the order Lepidoptera insects. *B. thuringiensis* (Bt) is used to control lepidopteran insect pests such as *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Alfazairy *et al.*, 2013), *Tuta absoluta* (Lepidoptera: Gelechiidae) (Alsaedi *et al.*, 2017), and *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Karshanal and Kalia, 2023). Also, Spinosad is recommended to control many Lepidoptera insects like *S. frugiperda* (Meendez *et al.*, 2002), *S. littoralis* and *S. frugiperda* (Abdullah and Sukar, 2025), *S. littoralis* and *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Sukirno *et al.*, 2017), *Sesamia cretica* Lederer (Lepidoptera: Noctuidae) (Osman *et al.*, 2014), and *T. absoluta* (Bratu *et al.*, 2016). In addition, Emamectin benzoate has high toxic effects against several insects belonging to the order Lepidoptera, like *S. littoralis* (Moustafa *et al.*, 2018), *T. absoluta* (Gacemi and Guenaoui, 2012), *Mamestra brassicae* (Moustafa *et al.*, 2016), and *S. frugiperda* (Koffi *et al.*, 2022). Also, Abamectin is able to control many insects such as *T. absoluta* (Hanash, 2023), *Plutella xylostella* (Morsy and Elwan, 2021), *S. littoralis* and *S. frugiperda* (Abdullah and Sukar, 2025), and *S. frugiperda* (Attia *et al.*, 2023). There are many commercial formulations of these biopesticides in the Egyptian market, according to the Agricultural Pesticide Committee of the

Ministry of Agriculture and Land Reclamation. So, the aim of this study is to evaluate some biopesticide formulations such as Abamectin, Spinosad, Emamectin benzoate, and *B. thuringiensis* (Bt) against the black cutworm *A. ipsilon* and investigate their effects on the insect development rate and its detoxifying enzymes.

Materials and methods

1. Insect breeding procedure:

Third instar larvae of *A. ipsilon* were kindly obtained from the Plant Protection Department, Faculty of Agriculture, Cairo University. Larvae were reared on castor leaves until the pupation stage at 27°C and 65% RH. The pupae were transferred to glass jars (30 cm height and 20 cm diameter) that were covered by muslin until the adult emergence. The obtained adults were fed on a 10% sucrose solution until egg-laying. Tissue paper strips were put in jars for egg-laying. Eggs were collected every day and transferred to glass jars (20 cm height and 20 cm diameter) until hatching. After hatching, the larvae were fed on clean castor leaves. A thin layer of sawdust was put in the bottom of the larvae jars. The larvae were reared to obtain the suitable instar for experiments as described by Ismail (2021).

2. The tested biopesticides:

Commercial formulations of Spinosad 24% SC, Abamectin 1.8% EC, Emamectin benzoate 2.3% EC, *Bacillus thuringiensis* 9.4% WP, and Alpha-Cypermethrin 10% EC were supplied by the Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

3. Lethal impact procedures of tested biopesticides:

3.1. Bioassay under laboratory conditions:

Five serial concentrations of each tested pesticide were prepared, based on all concentrations being less than the field-recommended rate of each pesticide. Twenty larvae of the 2nd instar, as well as the same number of the 4th instar, were transferred to

jars (20 cm * 20 cm) with a thin layer of sawdust under the larvae. The leaf-dipping bioassay method was applied to evaluate the toxicity of the tested biopesticides against black cutworm. Pieces of castor leaves were dipped in the pesticide solution for 30 seconds and left to air dry. The treated leaves were transferred to larvae jars and covered by muslin. Alpha-cypermethrin was used as the standard pesticide; water was used to treat the control group. Each treatment was replicated four times. The dead larvae were recorded every day. The mortality percentages were counted and corrected by Abbott's formula (Abbott, 1925).

3.2. Bioassay under semi-field conditions:

Sixty plastic pots (size 40 cm) were filled with agricultural soil. Ten cotton seeds (Giza 86) were planted in each pot. After the seeds germinated and five true leaves appeared, poison bait was prepared (1 kg of wheat bran, 100 gm of molasses, 500 ml of water, and the LC₉₀ of biopesticide) and applied in equal quantities near the plants grown in the pots. The control group was treated with the baits without pesticide. Ten of the 4th instar larvae were added to each pot (A larva/plant). Ten plastic pots were used for each pesticide and control. Three days after the treatment, healthy and infected plants were counted, and the infection rate in each treatment was calculated. The experiment was conducted outdoors on the farm of the Dakahliah Agricultural Directorate.

4. Sublethal impact procedures of tested biopesticides:

4.1. Determination of the insect development rate after treatment:

The sublethal concentration (LC₂₅) of each tested pesticide was prepared. Fifty larvae of the 2nd instar of the same size were selected and transferred to ten jars (5 larvae per 0.5L jar). Pieces of castor leaves were dipped in the pesticide solution and left to air dry. The treated leaves were presented to the larvae in the treated jars group; also,

untreated leaves were presented to the larvae in the control jars group. The larvae were allowed to feed on treated leaves for three days, after which untreated leaves were provided to larvae in all jars until the end of the experiment. The larval period, pupation percentage, pupae with deformities, pupae period, pupae weight, adult emergence, sex ratio, and adults with deformities were recorded as described by Ismail (2024).

4.2. Estimation of some detoxifying enzymes' activity after treatment:

The median lethal concentration (LC₅₀) of each pesticide was prepared. Pieces of castor leaves were dipped in the pesticide solutions for 30 seconds and then allowed to air dry. Twenty larvae of the fourth instar were transferred to jars (1L). The treated castor leaves were presented to larvae in the treatment jars, and untreated castor leaves were used for larvae in the control jars. After four days, the live larvae were collected from each treatment and weighed. Phosphate buffer (pH 7) was added to larvae (1 gm: 5 ml), and the mixture was homogenized and centrifuged. One ml of the supernatant was transferred to an Eppendorf tube and frozen under -20°C until enzyme estimation. The enzyme kits of the bio-diagnostic company, Egypt, were used to estimate the detoxifying enzymes. The acetylcholine esterase (AChE) was estimated according to Simpson *et al.* (1964) at 515 nm, the glutathione S-transferase (GST) was estimated according to Pan *et al.* (2016) at 540 nm, alanine transaminase (ALT) and aspartate transaminase (AST) were estimated according to Reitman and Frankel (1957) at 520 nm, acid phosphatase (ACP) was estimated according to Powell and Smith (1954), and the total protein was estimated according to Gornal *et al.* (1949) at 550 nm.

5. Statistical analysis:

Data from the bioassay experiment were calculated and corrected by Abbott's formula (Abbott, 1925). The lethal and sublethal

concentrations (LC₂₅, LC₅₀, and LC₉₀) at a 95% confidence limit and slope values were calculated by the Finney method (Finney, 1971) using LDP-line software. The Sun equation was used to calculate the toxicity index according to Sun (1950). The results of enzyme activities and insect development rate were subjected to analysis of variance (ANOVA) by using SAS software (SAS, 1997).

Results and discussion

According to the recommendations of the Pesticides Committee of the Ministry of Agriculture and Land Reclamation, there are no recommended biopesticides against black cutworm, *A. ipsilon*. In addition, few studies were conducted to evaluate the biopesticides against *A. ipsilon*. Therefore, this study was concerned with evaluating the efficacy of four biopesticides (Spinosad, Abamectin, Emamectin benzoate, and *B. thuringiensis*) against black cutworms and comparing their efficacy to one recommended pesticide (Alpha-Cypermethrin). The lethal and sublethal concentrations of tested pesticides were used to determine the impact of these biopesticides on the survival of larvae, the activity of specific detoxification enzymes, and the rate of insect development.

1. Lethal impacts of tested biopesticides:

1.1. Toxicity of biopesticides tested under laboratory conditions:

Tables (1 and 2) display the toxicity of tested pesticides against the 2nd and 4th instar larvae of *A. ipsilon* after two days of treatment under laboratory conditions. The second and fourth instar larvae of *A. ipsilon* were found to be more sensitive to the biopesticide Emamectin benzoate than other tested pesticides. The toxicity index values illustrate that the most effective pesticide (100%) was Emamectin benzoate, where its LC₅₀ was 1.14 ppm for the 2nd instar and 1.48 ppm for the 4th instar larvae. Emamectin benzoate was a highly effective pesticide compared to the standard pesticide (Alpha-Cypermethrin) and other tested pesticides. In addition, the toxicity of Emamectin benzoate against the 2nd and 4th

instar larvae was almost equal. The Spinosad pesticide was approximately equal to the standard pesticide in the toxicity effect against the 2nd instar larvae. The 4th instar larvae were less sensitive to Spinosad compared to the standard pesticide. Abamectin and *B. thuringiensis* were fewer effective pesticides compared to other tested pesticides against the 2nd and 4th instar larvae. The results in Tables (1 and 2) illustrate that the 2nd instar larvae were more sensitive to all tested pesticides than the 4th instar larvae, except with Emamectin benzoate; the toxicity was mostly equal.

2.1. Efficacy of tested biopesticides under semi-field conditions:

The pot experiment was carried out outdoors by using the calculated concentration (LC₉₀) of each pesticide based on laboratory bioassays against the fourth instar larvae of *A. ipsilon*. The damage percentages of cultivated cotton plants were decreased in the treated pots by Emamectin benzoate (6%), Alpha-Cypermethrin (14%), Spinosad (22%), Abamectin (34%), and *B. thuringiensis* (38%) compared to the plants in the control group (82%), as shown in Figure (1). This suggests that Emamectin benzoate is more effective in protecting plants from damage compared to both the untreated control and the standard pesticide.

2. Sublethal impacts of tested biopesticides:

2.1. Effect of tested biopesticides on insect development rate:

There are significant changes in all growth stages of *A. ipsilon* among treatments compared to the control group, as shown in Table (3). All tested pesticides caused negative changes in the measured biological aspects of *A. ipsilon* at LC₂₅. Emamectin benzoate was a more effective pesticide on all measured biological aspects compared to the control group and other tested pesticides. The total duration of larvae and pupa was increased in the larvae treated by Emamectin benzoate (60.5 days), more than other tested pesticides, followed by Alpha-Cypermethrin (54.5 days) and *B. thuringiensis* (53 days), compared to 41.5 days in the control group, as shown in Figure (2).

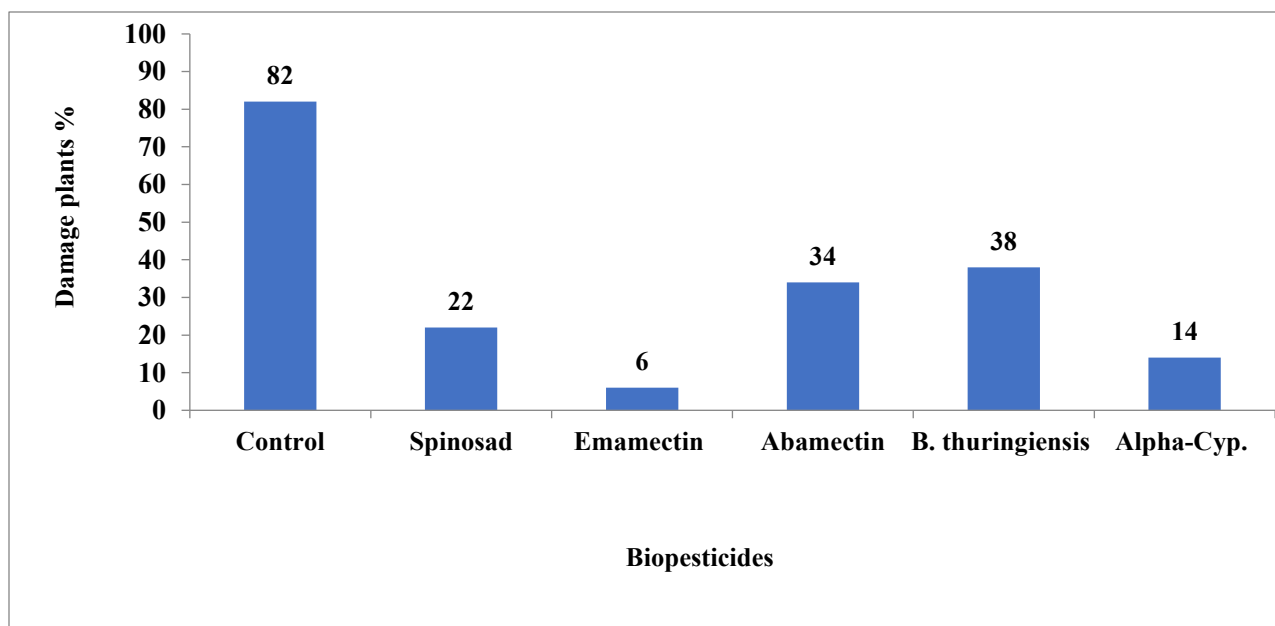


Figure (1): The percentage of plants damaged by *Agrotis ipsilon* larvae in the pots experiment after 3 days of treatment.

Table (1): Toxicity of tested biopesticides against the 2nd instar of *Agrotis ipsilon* larvae after 2 days of treatment under laboratory conditions.

Treatments	LC ₂₅ (ppm) (C.I. 95%)	LC ₅₀ (ppm) (C.I. 95%)	LC ₉₀ (ppm) (C.I. 95%)	Slope ± SE	Chi square (x ²)	Toxicity Index (%)
Spinosad	9.9 (7.54 -12.08)	19.52 (16.64-22.49)	70.7 (56.47-97.16)	2.29 ± 0.23	5.14	5.84
Emamectin benzoate	0.29 (0.03 - 0.63)	1.14 (0.69-1.54)	9.33 (6.66-16.55)	1.14 ± 0.21	2.61	100
<i>Bacillus thuringiensis</i>	42 (28 - 55)	105 (85-126)	606 (433-1023)	1.68 ± 0.21	5.49	1.09
Abamectin	14.21 (11.21-17.46)	38.71 (29.9-57.64)	259 (139-771)	1.55 ± 0.22	1.07	2.94
Alpha-Cypermethrin	6.07 (2.92-9.27)	18.79 (13.43-23.78)	161 (107-327)	1.37 ± 0.20	0.13	6.07

C.I. 95% = Confidence limit at 95%. The units of LC₅₀ and LC₉₀ is part per million (ppm). SE = Standard error

Toxicity index = (LC₅₀ of the most effective pesticide / LC₅₀ of other pesticide) *100

Table (2): Toxicity of tested biopesticides against the 4th instar of *Agrotis ipsilon* larvae after 2 days of treatment under laboratory conditions.

Treatments	LC ₂₅ (ppm) (C.I. 95%)	LC ₅₀ (ppm) (C.I. 95%)	LC ₉₀ (ppm) (C.I. 95%)	Slope ± SE	Chi square (x ²)	Toxicity Index (%)
Spinosad	32.32 (26 - 38)	73.6 (61 - 94)	351 (229 - 689)	1.88 ± 0.22	4.33	2.01
Emamectin benzoate	0.38 (0.15 - 0.75)	1.48 (0.47 - 2.46)	31.65 (18 - 112)	0.96 ± 0.20	4.4	100
<i>Bacillus thuringiensis</i>	48 (23 -73)	137 (98 - 172)	1731 (1065 - 4135)	1.49 ± 0.22	0.525	1.08
Abamectin	20.97 (12 - 29)	112 (81 - 182)	2718 (1043 - 15651)	0.92 ± 0.14	0.18	1.32
Alpha-Cypermethrin	7.26 (5 - 10)	30.89 (24 - 41)	483 (245 - 1492)	1.07 ± 0.14	1.92	4.79

C.I. 95% = Confidence limit at 95%. The units of LC₅₀ and LC₉₀ is part per million (ppm). SE = Standard error

Toxicity index = (LC₅₀ of the most effective pesticide / LC₅₀ of other pesticide) * 100

Table (3): Sublethal effects of tested biopesticides on the properties of development stages in the third instar larvae of *Agrotis ipsilon*

Properties of development stages	Control	Spinosad	Abamectin	Emamectin benzoate	<i>B. thuringiensis</i>	Alpha-Cypermethrin	LSD (0.05)	F. value	P. value
Larvae period (days)	19.5 ^e	24.5 ^c	22.5 ^d	29.5 ^a	25 ^c	27.5 ^b	0.91	142 ***	0.0000
Pupation rate (%)	93 ^a	74 ^b	94 ^a	54 ^c	24 ^d	76 ^b	2.74	886 ***	0.0000
Deformities Pupae (%)	1 ^f	11 ^d	19 ^b	23 ^a	16.7 ^c	3 ^e	2.06	187 ***	0.0000
Pupae period (days)	22 ^d	27 ^b	24 ^c	31 ^a	28 ^b	27 ^b	2.10	22 ***	0.0000
Pupae weight (gm) ♂	0.4034 ^a	0.3866 ^b	0.3381 ^e	0.2899 ^f	0.3648 ^d	0.3766 ^c	0.0019	4061 ***	0.0000
Pupae weight (gm) ♀	0.4459 ^b	0.4271 ^c	0.3843 ^e	0.3001 ^f	0.4691 ^a	0.3928 ^d	0.0016	11976 ***	0.0000
Adult emergence (%)	97.5 ^a	87.8 ^b	89.5 ^b	67 ^d	80 ^c	81 ^c	2.66	144 ***	0.0000
Sex ratio ((♀/(♀ + ♂)) (%)	46 ^c	48.5 ^c	47 ^c	51 ^b	70 ^a	35 ^d	2.43	208 ***	0.0000
Adults with deformities (%)	3 ^e	76 ^{ab}	59 ^c	78 ^a	75 ^b	53 ^d	2.71	1044 ***	0.0000

Duncan test at significance level 0.05. The same letters in the row mean no significant.

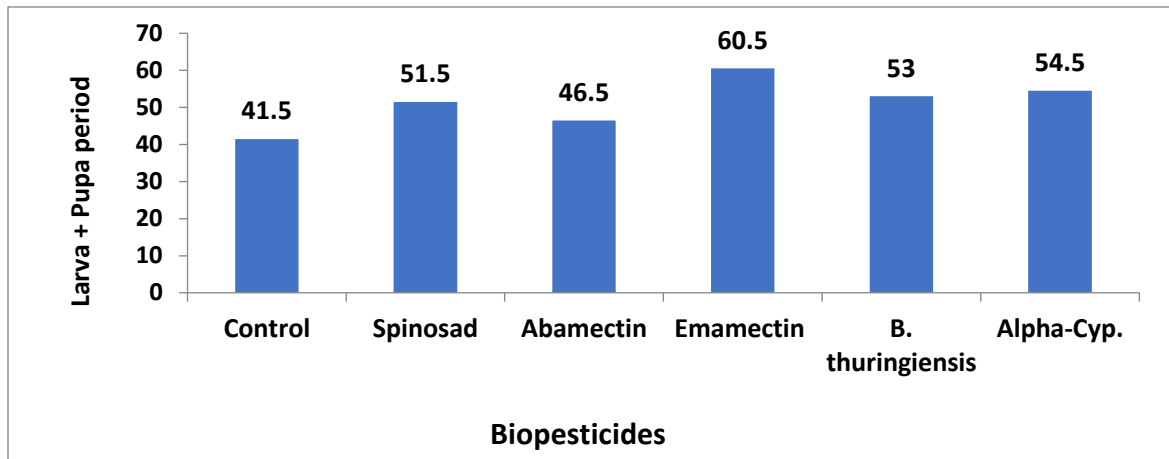


Figure (2): Effect of the tested pesticides on the total duration of larvae and pupa stages in *Agrotis ipsilon*

2.2. Effect of biopesticides on some detoxifying enzyme activity:

Some detoxifying enzymes and total proteins were estimated in the hemolymph of the treated 4th instar of *A. ipsilon* larvae. The obtained results in Table (4) display that significant activations of AchE and total protein were found in treated larvae by all tested pesticides compared to the larvae in the control group. On the other hand, all tested

pesticides led to inhibition in the GS-T except Alpha-Cypermethrin, which caused activation. Additionally, all tested pesticides caused activation in the GPT, except Abamectin, which led to inhibition. While the inhibition effect was observed in the GOT by all tested pesticides, except with Alpha-Cypermethrin, it had no effect on GOT. Also, ACP was inhibited in treated larvae by all tested pesticides, as shown in Table (4).

Table (4): Activity of some detoxifying enzymes and total protein in treated 4th instar larvae of *Agrotis ipsilon* after four days of treatment by tested pesticides.

Treatments	Enzyme activity					Total Protein (gm/dl)
	AchE (U/L/min.)	GS-T (U/L)	GPT (U/L)	GOT (U/L)	AcP (U/L)	
Control	23 ^f	1397 ^b	55 ^d	230 ^a	72 ^a	0.58 ^e
Spinosad	78 ^c	930 ^c	106 ^b	169 ^c	27 ^c	2.33 ^a
Abamectin	62 ^c	1220 ^c	28 ^c	221 ^b	56 ^b	0.97 ^d
Emamectin benzoate	70 ^d	1161 ^d	56 ^d	190 ^c	42 ^d	1.55 ^c
<i>B. thuringiensis</i>	86 ^b	902 ^f	70 ^c	175 ^d	48 ^c	1.55 ^c
Alpha-Cypermethrin	132 ^a	1698 ^a	112 ^a	230 ^a	42 ^d	1.89 ^b
LSD (0.05)	2.44	9.86	5.05	3.97	3.19	0.021
F. value	1924***	8602***	361***	488***	211***	7762***
P. value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Duncan test at significance level 0.05. The same letters in the column mean no significant.

A. ipsilon, commonly known as the black cutworm, poses a significant threat as an early-season soil pest affecting cotton,

vegetables, and various field crops. Its ability to cut the stems of plants results in the need for replanting, leading to increased

agricultural costs. Relatively little is known about the effectiveness of applying biopesticides against the black cutworm in Egypt. This lack of usage regarding biopesticides highlights the need for further research to explore their potential as an alternative pest management strategy. Understanding their effectiveness could lead to more sustainable agricultural practices and reduced reliance on chemical insecticides (Liu *et al.*, 2023). So, this research was conducted to evaluate the effectiveness of four biopesticides (Spinosad, Abamectin, Emamectin benzoate, and *B. thuringiensis*) on the survival and growth rate of the black cutworm, *A. ipsilon*, under laboratory and field conditions.

The findings indicated significant differences in the impact of each biopesticide on the larvae, with Emamectin benzoate showing the highest efficacy in reducing survival rates under laboratory conditions and in pot experiments. Additionally, the growth rates of the black cutworm were notably affected; also, significant changes were found in the activity of estimated detoxifying enzymes and total protein in treated larvae compared to the control group. This suggests that these biopesticides could be viable alternatives for pest management in agricultural practices. It is also noted that tested biopesticides have a strong toxic effect compared to the standard pesticide (Alpha-Cypermethrin) recommended for cutworms. Also, the tested biopesticides have toxic effects against many lepidopterans' insect pests. Several studies reported that Emamectin benzoate was the more effective biopesticide against many species of lepidopteran, like *Spodoptera littoralis*, *Spodoptera exigua*, *Spodoptera frugiperda*, *Heliothis virescens*, *Plutella xylostella*, *Tuta absoluta*, and *Mamestra brassicae* (Gacemi and Guenaoui, 2012; Bengochea *et al.*, 2014; El-Sheikh, 2015; Moustafa *et al.*, 2016, and Moustafa *et al.*, 2018).

Abd El-Samei *et al.* (2019) evaluated the effectiveness of two biopesticides (Spinosad and *B. thuringiensis*) against the third and fifth-instar larvae of *S. littoralis*. They found that the cumulative percentage mortality increased gradually by increasing concentration; also, *B. thuringiensis* was less potent than Spinosad. More than 30 nations have registered Spinosad as a promising bio-insecticide for the control of Lepidoptera, Coleoptera, Diptera, and Thysanoptera (Williams *et al.*, 2004). In addition, Abamectin and Spinosad were evaluated against *S. frugiperda* and *S. cretica* in the laboratory and field experiments by Attia *et al.* (2023). They found that Spinosad was less effective for both insects than Abamectin. Also, they found that Abamectin had insecticidal activity against *S. frugiperda* and *S. cretica* in the laboratory and field conditions, more than other tested treatments. Also, Abdullah and Sukar (2025) mentioned that Spinosad and Abamectin had a high toxic effect against *S. littoralis* and, *S. frugiperda*. The LC₅₀ values of Spinosad and Abamectin were 6.23 and 27.75 ppm, respectively, against *S. frugiperda* and they were 20 and 30 ppm, respectively, against *S. littoralis*.

Using biopesticides as poison bait to control cutworms will help maintain soil fertility by preserving the microorganisms present in the soil, unlike traditional chemical pesticides that pollute the soil and eliminate the microorganisms present in the soil. This approach not only promotes a healthier ecosystem but also supports sustainable agricultural practices. By integrating biopesticides into pest management strategies, farmers can enhance crop yields while protecting the vital soil health necessary for future generations.

The compelling evidence surrounding Emamectin benzoate underscores its potential as a superior pesticide, particularly in the battle against the black cutworm, *A. ipsilon*, in this study. Not only does it

outperform untreated controls and traditional pesticides, but it also significantly impairs growth rates across various life stages of this pest. The alterations observed in detoxifying enzyme activity and total protein levels among treated larvae further illustrate its effectiveness, suggesting that Emamectin benzoate disrupts critical physiological processes within the organism. As agricultural practices continue to seek sustainable and efficient pest control methods, embracing Emamectin benzoate could provide a promising alternative that enhances crop protection while mitigating reliance on conventional pesticides.

This study suggested using Emamectin benzoate and Spinosad to manage black cutworm (*A. ipsilon*) because they were more effective at killing the pests in lab and semi-field tests than the recommended pesticide, Alpha-cypermethrin.

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