Journal of Desert and Environmental Agriculture, 3 (1): 62-75, 2023 JDEA
DOI: 10.21608/jdea.2023.207241.1018

Article

Effectiveness of a biopesticide and chitin synthesis inhibitor on the biological and biochemical traits of Agrotis ipsilon (H.) (Lepidoptera: Noctuidae)

Mohamed R. Abo Elela,

Department of Vegetable, Medicinal, Aromatic and Ornamental Plant Pests, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt.

Article info.

Received on:25-4-2023

Accepted on: 2-5-2023

Published on: 6-2023

Open Access

Abstract

The use of synthetic chemical insecticides with a high control efficiency against the black cutworm Agrotis ipsilon (Hufnagel) (Lepidoptera: Noctuidae) has a negative influence on human health, farmland, and natural enemies because of chemical residue, environmental pollution, and the development of insect resistance to these chemical insecticides. The present study, investigate the effect of the entomopathogenic fungi (EPF), Beauveria bassiana (Bio-Bower) and chitin synthesis inhibitor lufenuron (Wormatin) on the fourth instar larvae of the black cutworm Agrotis ipsilon (Lepidoptera: Noctuidae) under laboratory conditions of 27± 1 0C and 65% ± 10% R.H.

The findings showed that for the black cutworm A. ipsilon larvae exposed to various concentrations of Bio-Bower and lufenuron, the LC50 values obtained were 1.0677 and 0.0921 ppm, respectively. The results showed that, as compared to control, the duration of the larvae increased by 4.76% and 20.67% for Bio-power and lufenuron, respectively. Pupation rates decreased by 49.5% and 47.5%, respectively, for Bio-power and lufenuron, when compared to controls. Additionally, pupal duration was shorter for each of Bio-power and lufenuron when compared to controls, by 23.9 % and 22.2 %, respectively. When compared to the enzymatic activity in control insects, the enzyme activities for Bio-power and lufenuron show an increase in protease by 82.450 and 77.993% and a decrease in chitinase by 116.702 and 86.801%, respectively. These enzymes are a variety of hydrolases that are crucial for the physiological operation of the targeted insect and, as a result, for its metabolic pathway.The total level of protein decreased by 32.413% and 13.702%, lipid by 12.795% and 12.934%, and carbohydrate by 23.872% and 38.846%, according to the results of both treatments.

This study found that using B. bassiana (Bio-Bower), an entomopathogenic fungus (EPF), as a very safe alternative to chemical pesticides because this study found that it was a very effective treatment for the black cutworm Agrotis ipsilon. Chemical pesticides also cause significant harm to soil and water quality.

Keywords: Beauveria bassiana, Entomopathogenic fungi, chitin synthesis inhibitor, Agrotis ipsilon, Insect growth regulators.

1. Introduction

The black cutworm *A. ipsilon* (H.), is a significant lepidopterous pest of several ecologically important crops, including cowpeas, sorghum, bermudagrass, soybeans, and corn. Black cutworm control has only been possible using insecticides. This insect has thus developed resistance to the main classes of pesticides in many different locations. The use of synthetic chemical insecticides with a high control efficacy of *A. ipsilon* has a negative influence on human health, agriculture, and natural enemies because of chemical residue, environmental pollution, and the development of insect resistance to these chemical insecticides (Batta, 2016; Fernandes et al., 2010). It is now commonly recognised that the biodiversity of agroecosystems contributes significant ecosystem services to agricultural productivity, such as biological pest management (Entomopathogenic fungus, *Beauveria bassiana*), (Meyling and Eilenberg, 2007).

Insect growth regulators (IGRs), which have recently entered the market, have an impact on insects by regulating or inhibiting specific biochemical pathways or processes necessary for insect growth and development (Abdel-Aal, et al., 2009). These IGRs are primarily responsible for insect death due to abnormal hormone-mediated cell or organ regulation (El-Akad et al., 2016). Wormatin (Lufenuron), one of these IGRs known as chitin synthesis inhibitors (CSIs), prevents the formation of chitin (Maqsood, et al., 2016).

The goal of the current study was to investigate the biological and biochemical effects of the CSI's lufenuron on the homogenate of the sixth larval instar of the black cutworm A. ipsilon, as well as assess the sensitivity of the fourth instar larvae to the fungus *B. bassiana*.

2. Materials and Methods

Rearing of *Agrotis ipsilon*

Twenty newly emerged *A. ipsilon* moths were acquired from the cutworm department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, moved to the laboratory of the Vegetables Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, and maintained in glass jars covered with tissue paper and held in place by rubber bands. Adults consumed a 20% concentration of honey solution as sustenance. Females were permitted to deposit their eggs on muslin strips fixed to the jars' tops. After egg laying, these strips were put into Petri dishes and maintained in an incubator at a constant temperature of 25 °C 1 °C and 70–80 RH% until the eggs hatched (Zhang et al. 2019). Castor leaves were served daily as a source of food for the newly hatched larvae kept in small jars. The 4th-instar were placed in glass plates either singly or in small group to prevent cannibalism.

Bio-Bower is the commercial formulation of the entomopathogenic fungus, *Beauveria bassiana* provided by T. Stanes and Company Limited, India. The international unit was 32 x 10^6 viable spores/mg, or 32,000 viable spores per milligram. The recommended application rate was 200 gm/100 liters of water or feddan, and the active ingredient was 10% W.P.

Wormatin 5% EC (Lufenuron): is a widely used commercial insect growth regulator. The suggested application rate was 160 cm3 per feddan, and the active ingredient had a concentration of 5% EC.

Toxicological studies:

The fourth instar larvae of *A. ipsilon* were fed for three seconds on castor bean leaves treated with different concentrations of the tested biopesticides, Bio-Bower and lufenuron. Each tested biopesticide had five concentrations prepared: Bio-Bower had 6, 4, 2, 1, and 0.5 spores/mg, while lufenuron had 0.75, 0.375, 0.1875, 0.0938, and 0.0469 ppm. For each concentration, 45 larvae of the 4th instar were divided into three replicates and fed on the treated leaves for 48 hours.

Additionally, 30 fourth-instar larvae were employed as a control treatment that fed on castor bean leaves soaked in water. The larvae who survived were then transferred to other clean jars and given fresh castor bean leaves daily for seven days after treatment. Every day, the mortality % was calculated and adjusted using the (Abbott, 1925) algorithm. Statistics were used to statistically calculate percentages of correcting mortalities in accordance with Finney (1971), and the LC50 value was established.

Biological studies:

Newly ecdysed fourth instar larvae (30 larvae for each treatment and 30 for controls) were taken from the maintained insect culture. For larvae treated by biopesticides, larvae fed on castor bean leaves treated with the attained LC50 values of Bio-Bower and lufenuron. For the control treatment, the larvae were fed on castor bean leaves treated merely with water. Daily examinations of the larvae allowed for the study of the following parameters: pupation percentage, pupal duration, and pupal deformity percentage.

Biochemical studies:

Sample preparation for biochemical studies:

Treated and control larvae were collected six days after the fourth instar, put in ice containers, and homogenized for three minutes in the proper buffer using a Teflon homogenizer encased by a jacket of crushed ice. Using a Biofuge 28RS Heraeus centrifuge from Sepatech, homogenates were spun at 8000 rpm for 10 minutes at 4°C. The resulting supernatants were immediately used to measure the amount of enzyme activity according to Ishaaya et al(1971).

Determination of chitinase activity:

In accordance with the procedure outlined by Ishaaya and Casida (1974), chitinase was tested using the 3, 5-dinitrosalicylic acid reagent to detect the free aldehydic groups of hexosamine liberated on chitin digestion. Making the DNSA (3, 5-dinitrosalicylic acid reagent) With the use of a magnetic stirrer, one gramme of 3, 5-dinitrosalicylic acid was dissolved in 20 ml of 2N NaOH solution and 50 ml of distilled water to create the dinitrosalicylic acid reagent. The addition of 30 g of potassium sodium tartrate was followed by prolonged magnetic stirring until a clear solution was attained. The final volume was then increased to 1000 ml by adding distilled water.

Assay of chitinase activity:

The reaction mixture contained 0.18 ml of sample homogenate, 0.12 ml of 0.2 M phosphate buffer (pH 6.6), and 0.3 ml of 0.5% colloidal chitin. By adding 1.2 ml of the 3,5-dinitrosalycilic acid reagent (DNSA), the enzyme activity was stopped after 60 min of incubation at 37°C. The reaction mixture was first heated for 5 minutes at 100 oC, cooled in an ice bath, and then diluted with 1.2 ml of distilled water. The absorbance of the supernatant was measured spectrophotometrically at 550 nm after undigested chitin was centrifuged for 15 min. at 6000 rpm. When N-acetylglucosamine (NAGA) is directly reacted with DNSA

reagent under circumstances resembling those of an enzyme reaction, a linear plot of absorbance value versus amount of NAGA is produced, with 1 mg of NAGA producing an absorbance value of 0.78.

Determination of protease activity:

The casein digestion method established by Ishaaya et al. (1971) was used to measure the proteolytic enzyme activity. The reaction mixture contained 0.2 ml (0.2 M) glycine buffer (pH 10), 0.4 ml 1.5% casein solution, and 0.2 ml homogenate sample. After 60 minutes of incubation at 37°C and the addition of 1.2 ml of a 5% trichloroacetic acid solution, the enzyme activity was stopped. After centrifuging the reaction mixture at 6000 rpm for 15 min, the supernatant was removed to test the enzyme activity. At 280 nm, the proteolytic activity was calculated as O.D. unit x 10^3.

Determination of the main components:

The homogenated larval bodies were analysed for total proteins, total lipids, and total carbohydrates using the methods of Bradford (1976), Singh and Sinha (1977), and Knight et al. (1972), respectively.

Procedure for statistical analysis:

Student t-tests and Duncan's multiple range tests (p = 0.05) were used to determine the significance of various treatments. The software "Costat" from Berkley, California-based cohort software Ine was used to conduct all prior analyses (Duncan, 1955).

3. Results

1. Toxicity effects of Bio-Bower and lufenuron on the 4th instar larvae of *Agrotis ipsilon*:

The effectiveness of Bio-Bower and lufenuron on *A. ipsilon* larvae in their fourth instar is shown in Table (1). Regression lines and the LC_{50} were calculated. For Bio-Bower and lufenuron had LC_{50} values of 1.0677 g/l and 0.0921 ppm, respectively. Additionally, the slope values for Bio-Bower and Lufenuron, respectively, were 4.949 and 1.5387, demonstrating the homogeneity of the larvae.

Table (1): Toxicity values of Bio-Bower and Lufenuron on the 4th instar larvae of *Agrotis ipsilon*

Parameters	Bio-Bower gm/ 1litter	Lufenuron (ppm)
LC_{50}	1.0677	0.0921
Lower Confidential limit (95%) LC_{50}	0.7194	0.0561
Upper Confidential limit (95%) LC_{50}	1.4285	0.1293
Slope+S.E	4.9489+ 0.3094	1.5387+ 0.2188
Accumulative mortality% (At the end of larval stage)	51.2	50.5

2. Biological studies:

Table (2) showed that the *A. ipsilon* treated in the 4th instar larvae with LC_{50} of Bio-Bower lasted 11.0 days, which was 0.5 (12 hours) days longer than the control treatment, with an increase of 4.76%. For lufenuron the treated larval lifespan achieved 12.67 days as opposed to 10.5 days in the control, where lufenuron treatment resulted in increased larval longevity by 2.17 days compared to the control, with a 20.67% increase.

Meanwhile, the pupal duration for Bio-power treatment was 8.67 days as opposed to 11.0 days in the control, or by 2.33 days, **or a reduction of -23.9%.**

Pupation percentages in treated larvae with Bio-Bower and lufenuron, decreased to 49.0 and 47.5%, respectively, which were nearly half that of untreated insects. On the other hand, the proportion of deformed pupae increased reaching 4.04 and 33.33% in Bio-Bower and lufenuron treatments, respectively.

Table (2): Biological parameters of the 4th instar larvae of *Agrotis ipsilon* treated with Bio-Bower and Lufenuron at the LC50 levels.

Treated compound	Bio-Bower	Lufenuron	Control	F. Value	L.S.D.
Mean larval duration (days± S.E.)	11.00^{b} ± 1.04	12.67^{a} ± 0.88 (20.67)	10.5^{b} ± 0.57	21.973**	0.8389
Pupation %	49.5	47.5	97.0		
Mean pupal stage (days± S.E.)	8.67^{b}± 0.88 **(23.9)**	7.67^{c} ± 0.2 **(22.2)**	11.0^{a} ± 0.59	85.369***	0.6399
Pupal malformation %	4.04	33.33	0.0		

The percentage reduction from the control is indicated by the numbers in brackets
Numbers of the same letters have no significant difference.

3. Biochemical studies:

3.1 Effect of Bio-Bower and lufenuron on certain activities of several *Agrotis ipsilon* enzyme systems:

The activities of the selected enzymatic systems were assessed on the sixth day of treating the 4th instar *A. ipsilon* larvae with the established **LC_{50}** values of Bio-Bower and lufenuron. The activity of chitinase and protease in Bio-Bower treated larvae were calculated to be 27.38 µg NAGA/min/g and 354.6 µg casein/min, respectively, higher than those in control untreated insects. These values were calculated to be significantly lower by 82.450 and 77.993%, respectively, than the values of the control (Table 3 and Figure 1). The **LC50** values for chitinase and protease, respectively, after treatment of 4th instar *A. ipsilon* larvae with lufenuron were 49.95 µg NAGA/min/g and 245.2 µg casein/min, respectively (Table 3 and Figure 1). In untreated insects these values were 42.80µg NAGA/min/g and 282.5 µg casein/min to the respective mentioned enzymes which represent a significant decrease than the enzymatic activities in control insects by 116.702 and 86.801%.

Table (3) : Agrotis ipsilon 4th instar larvae's enzyme activity after six days of treatment with Lufenuron and Bio-Bower biopesticides

Compound	Control	Bio-Bower	Control	Lufenuron
Chitinase µg NAGA/min/g	33.21 ± 0.108	27.38 ± 0.294	42.80 ± 1.233	49.95 ± 1.345
Activity % according to control	82.450		116.702	
Protease µg casein/min	454.3 ± 10.15	354.6 ± 2.288	282.5 ±6.593	245.2 ± 5.199
Activity % according to control	77.993		86.801	

% Activity in the control= 100% less than 100% or more than 100% (decrease or increase)

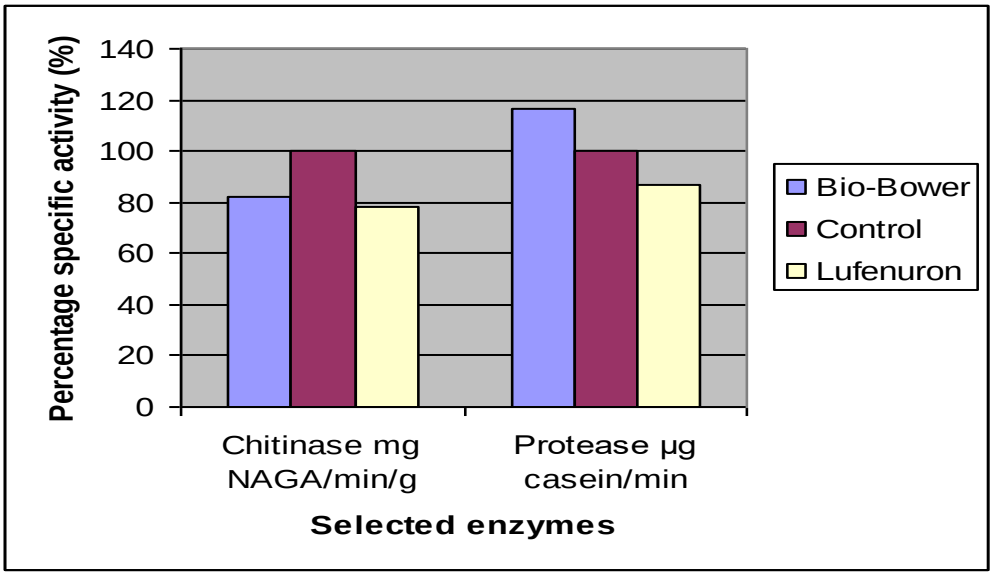

Figure (1): Effects of Bio-Bower and Lufenuron on the particular activities of chitinase and protease *Agrotis ipsilon* larvae.

3.2. Lufenuron and Bio-Bower 's combined effects on the total soluble protein, total carbohydrate, and total lipid composition of *Agrotis ipsilon* larvae:

The total soluble protein level was significantly lower in larvae treated with Bio-Bower and lufenuron 235.0 mg/g and 396.8 mg/g, respectively, than it was in untreated insects. These values were calculated to be much lower (32.413 and 32.413%, respectively) than the values of the control (Table 4 and Figure 2). In a similar way, the activity of the total carbohydrates was significantly reduced, with 273.3 mg of glucose and 318.4 mg of glucose per larva, respectively. This is a considerable reduction of 12.795 and 12.934% compared to the total carbohydrates in control insects, which were 313.4 and 365.7 mg glucose/larva to the comparable component in untreated insects (Table 4 and Figure 3). The data in Table

demonstrated a significantly significant difference in the total lipid levels between the control and the tested substance, Bio-Bower and lufenuron. They were calculated to be 254.8 and 261.8 mg oleic/larva, corresponding to a significant reduction in the total lipid contents of the corresponding compound untreated insects to 23.872 and 38.846% (Table 4 and Figure 4).

Table (4): Weights of total protein, total carbohydrate, and total lipid in *Agrotis ipsilon* larvae following six days of treatment as 4th instar larvae with Bio-Bower and Lufenuron.

The biochemical components	**Treatments compound**			
	Control	**Bio-Bower**	**Control**	**Lufenuron**
Total protein mg/larva	347.7 ± 20.19	235.0*** ± 5.863 (32.413)	459.8 ± 6.347	396.8*** ± 3.602 (13.702)
Total carbohydrate mg glucose/larva	313.4 ± 7.818	273.3*** ± 4.573 (12.795)	365.7± 4.53	318.4*** ± 4.126 (12.934)
Total lipid mg oleic/larva	334.7 ± 6.332	254.8*** ± 5.82 (23.872)	428.1 ± 6.851	261.8*** ± 6.572 (38.846)

Numbers between brackets presented % decrease or increase in case the biochemical component ***: highly significant ($p < 0.001$), (student-t test).

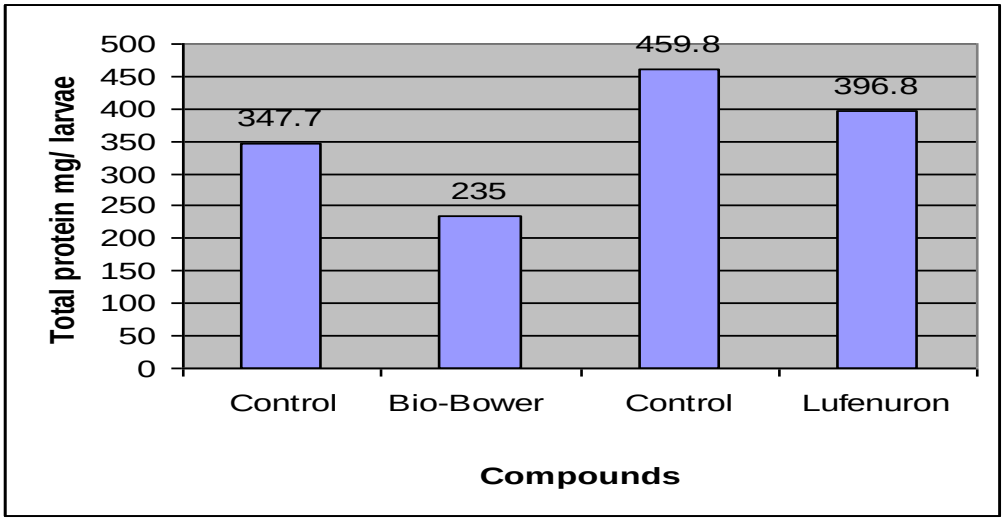

Figure (2) : Total protein content in *Agrotis ipsilon* larvae after six days of treatment as 4th instar larvae with Bio-Bower and Lufenuron.

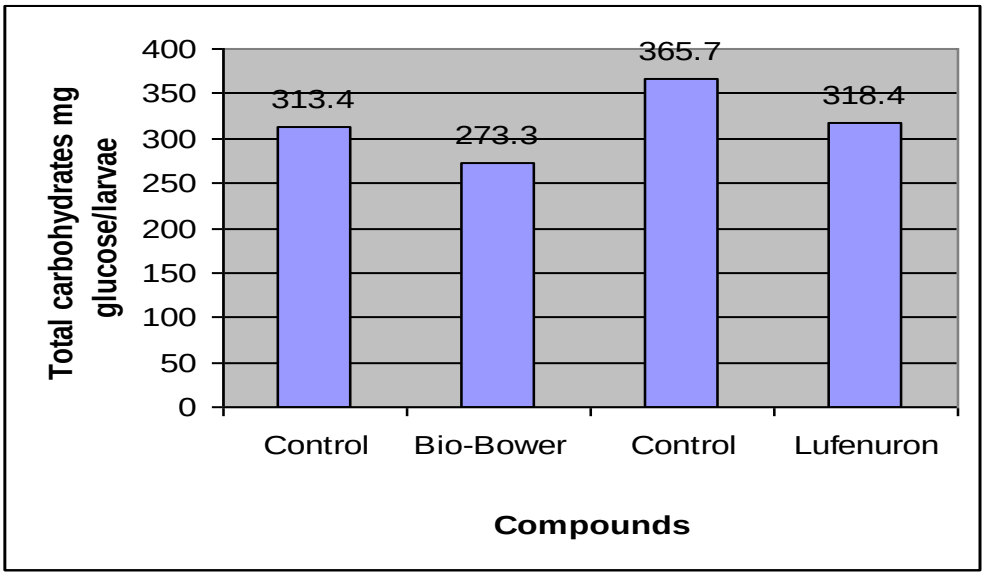

Figure (3) : Total carbohydrate contents of *Agrotis ipsilon* larvae after six days of treatment as 4th instar larvae with Bio-Bower and Lufenuron.

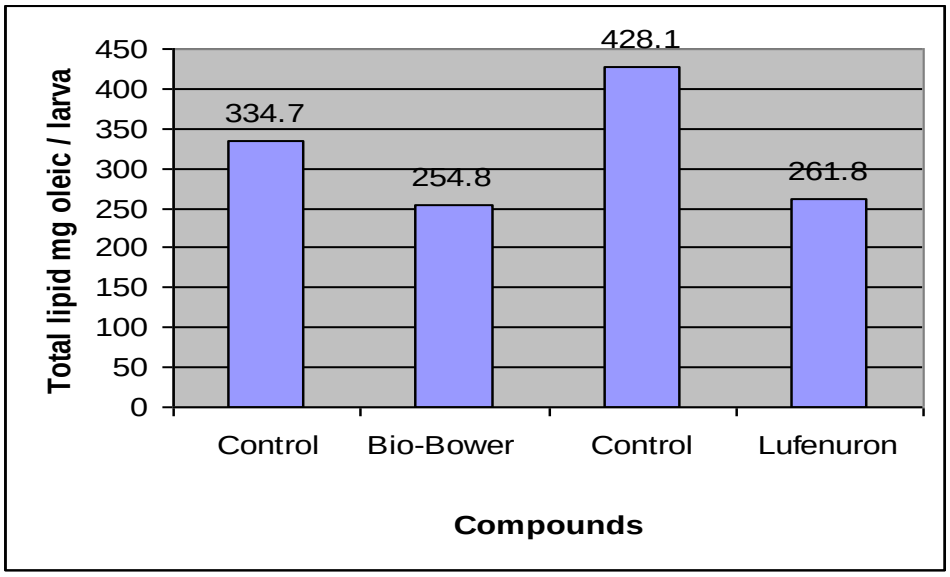

Figure (4) : Total lipid contents of *Agrotis ipsilon* larvae after six days of treatment as 4th instar larvae with Bio-Bower and Lufenuron.

4. Discussion

The unspecific adherence of the fungus spores to the insect cuticle and penetration into the insect hemolymph, where substantial development and the synthesis of toxins occur, before spreading to the insect tissue and causing death, is the mode of action for *B. bassiana* infection in the insect host.

A. ipsilon's chitin syntheses are inhibited when it is treated with *B. bassiana* supportrd by El Husseini's (2019) hypothesis that *M. anisopliae*, an entomopathogenic fungus, is effective at repelling *S. littoralis* when used at various concentrations. Additionally, Ramos et al. (2020) noted that while regulating *S. frugiperda* with *B. bassiana* and *M. anisopliae*, *M. anisopliae* induced the highest sporulation rates. While the direct inhibition of chitin synthesis within the integument rather than any indirect extracuticular effects on hormone levels may be responsible for the lufenuron-induced larval mortality, the ingestion of chitin synthesis inhibitor compounds by insect larva also disrupted endocuticular deposition during moulting process and abortive moulting because they block chitin synthesis. In general, treated larvae were shown to move less actively and with clear muscle contractions. Before dying, the treated larvae also showed significant tremors followed by paralysis. These results are consistent with those of Wahba and Shaker (2020), who investigated the toxicity of lufenuron to *S. littoralis* larvae in their fourth instar. These results concur with those from Abdel-Aal and El-Shikh (2012) and Maqsood et al. (2016), who found that lufenuron was the most efficient insecticide for controlling *S. littoralis*.

The findings demonstrated that B. bassiana (Bio-Bower**)** and lufenuron significantly extended the larval duration of *A. ipsilon* larvae, which was directly related to the larvae's slower metabolic rate and caused by the insecticide treatment. The reduced ability of treated larvae to feed could result in the extension of larval instars and a consequent decrease in the percentage of pupation. El-Akad et al. (2016) identified alterations in the many biological characteristics of the newly

hatched larvae of *P. gossypiella* treated with both *B. bassiana* and *M. anisopliae*, supporting these findings. Additionally, Jennifer et al. (2014) and Kimberly and Seow (2017) found that *M. anisopliae* had a greater impact on *Diatraea flavipennella* larval phase. El-Sayed et al. (2017) studied the toxicity of lufenuron and flufenoxuron against the second instar larvae *of S. littoralis*, treated with the sublethal concentrations of it exhibited also dramatically reduced larval duration and pupation percentage. Changes in larval and pupal durations may indicate metamorphic disturbance, which is consistent with the proportion of deformed pupae found in our results for lufenuron.

Cells in the epidermis, stomach, salivary glands, or fat body of insects produce chitinolytic enzymes (Kramer and Koga, 1986). In the process of moulting, the enzymes chitinase and protease both play a role in the digestion of old endocuticle. Therefore, any alteration in this enzyme's activity may be caused by insecticides (Hussain et al., 2010). Production of cuticle-degrading enzymes, including chitinases, lipases, and proteases, affected and facilitated penetration of certain fungus while also supplying food for future growth. Additionally, St Leger et al. (1991) found that low nutrition levels stimulated the development of appressoriums, hydrophobins, and the expression of cuticle-degrading proteases in *M. anisopliae*. As a result of using several insect growth regulators and bioinsecticides against *S. littoralis*, Assar et al. (2016) observed an increase in the activity of the enzymes that hydrolyze carbohydrates, including chitinase, phenoloxidase, and others. Assar et al. (2016) observed an increase in the activity of the enzymes that hydrolyze carbohydrates, including chitinase, phenoloxidase, and others recorded that chitinase and protease are essential for digestion of old endocuticle in the moulting process as a result of using several insect growth regulators and bioinsecticides against *S. littoralis*.

The most significant molecules found in the cells of all living things are proteins, which contain a variety of substances like enzymes and hormones that are essential to the survival of the living things (Fagan et al., 2002). The energy needed for cellular development is provided by carbohydrates (Lee et al., 2002). An important source of energy is lipids (Ali, 2011). According to Ahmed et al. (1985), the considerable decrease in total protein may be the result of binding with exogenous chemicals such as pesticides. According to Remia et al. (2008), the effect of anti-feedent and enhanced metabolism during toxicant stress may be to blame for the decrease in carbs. The current findings are consistent with those of El-Badawy et al. (2018), who discovered that *P. lilacinum* isolation significantly increased total carbs and total lipids while lowering *S. littoralis's* total protein content. Additionally, Nirupama (2015) found that the fungus infection gradually decreased the total protein of the silkworm, *Bombyx mori*. Additionally, Vidhya et al. (2016), who exhibited a substantial decrease in total protein content after infecting the army worm *S. litura* (Fabricius) with *B. bassiana* and *M. anisopliae*, supported our findings. In addition, Sobhi et al. (2020) confirmed our findings by recording suppression of total lipid and total protein of *S. littoralis* treated with essential camphor oil, while reporting stimulation of total carbs following treatment with essential *C. cyminum* oil, contrary to their findings. The findings of Kungreiliu Panmei et al. (2021) are in favour of the hypothesis that lufenuron infection reduced the body nutrition of *Ae. aegypti* larvae, with the metabolic abnormalities affecting the larvae's growth and development. The effects of sublethal concentrations of lufenuron have been seen in *Glyphodes pyloalis*, according to

Aliabadi et al. (2016). Due to starvation or injury to the alimentary canal caused by the tested IGRs, the hemolymph and body homogenate total carbohydrate contents of the treated larvae of *S. littoralis* (2nd and 4th instar) decreased (Saleh and Abdel-Gawad, 2018).

Conclusions

The current research aimed to evaluate the biological and biochemical impacts of the CSI's lufenuron on the homogenate on the sixth larval instar of the fall worm *A. ipsilon* and on the fourth instar larvae assess their susceptibility to fungi, *B. bassiana* (Bio-Bower). The outcome showed an increase in larval duration and a decrease in pupation percentage and pupal duration. The enzyme activities demonstrate an increase in protease and a decrease in chitinase. Additionally, both treatments' results show a decrease in the total content (lipid, protein, and carbohydrates. The present results suggest that *Beauveria bassiana* (Bio-Bower), an entomopathogenic fungus (EPF), was found to be a very effective treatment for the black cutworm *A. ipsilon*, making it a very safe alternative to chemical pesticides.

Abbreviations

EPF: The entomopathogenic fungi; IGRs: Insect growth regulators; CSIs: Chitin synthesis inhibitors

Declarations

Ethics approval and consent to participate.

Not applicable.

Competing interests

I, the author do not have competing interests.

Funding

No funding from any source

Availability of data and materials

Access to all of the datasets used is simple.

5. REFERENCES

Abbott, W. S. (1925): A method for computing the effectiveness of an insecticide. J. Econ. Entoml., 18: 265-267.

Abdel-Aal, A. E. and El-Shikh, T.A. (2012): Efficiency of Diple 2x (Bacillus thuringiensis var. kurstaki) alone and its mixture with two insecticides against the Egyptian cotton leaf worm Spodoptera littoralis (Lepidoptera: Noctuidae). Egyptian Academic Journal of Biological Sciences, 4 (1):61- 68.

Abdel-Aal, A. E.; El- Sheikh, T. A. and Farag, A. M. (2009): Effectiveness of insect growth regulators on the cotton leafworm, S. littoralis (Boisd.) population on Egyptian cotton in Menofia Governorate. Egypt. J. Agri. Res., 87 (2): 177-190.

Ahmed, M.S.; Ali, F.A. and Shakoori, A.R. (1985): Effect of dieldrin on the whole-body protein content of Periplaneta americana. Pakistan Journal of Zoology, 17(1):105-109.

Ali, R.M.S. (2011): Combined effect of gamma radiation and entomopathogenic nematoda on some store product pests. Ph. D., Thesis, Fac. of Agric., Ain Shams University.

Aliabadi, P.F.; Sahragard, A. and Ghadamyari, M. (2016): Lethal and sublethal effects of a chitin synthesis inhibitor, lufenuron, against Glyphodes pyloalis Walker (Lepidoptera: Pyralidae). J. Crop Prot., 5(2):203–214.

Assar, A. A.; Abo El-Mahasen, M. M.; Dahi, H. F. and Amin, H. S. (2016):

Biochemical effects of some insect growth regulators and bioinsecticides against cotton leafworm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae). J. Biosci. Appl. Rese., 2(8): 587- 594.

El-Akad, A. S.; El-Banna, A. A.; Abd El-Wahab, I. S.; Abd El- Aal, A. E. and Amin, N.S. (2016): Effects of Beauveria bassiana and Metarhizium anisopliae on some biological aspect of Pectinophora gossypiella (Saunders) (Lepidoptera: Gelechiidae). Egypt. Acad. J. Biolog. Sci., 9 (3): 31–39.

El-Badawy, S.S.; Ali, S. S. ; El-Hefny, A.A. and Heikal, G.A.M. (2018): Study of infection impact by entamopathogenic fungi on some biochemical contents in Haemolymph of cotton leaf worm, Spodoptera littoralis (Boisduval). Journal of Plant Protection and Pathology, Mansoura Univ., 9 (9):605-610.

El Husseini, M. M. M. (2019): Efficacy of the entomopathogenic fungus, Metarhizium anisopliae (Metsch.), against larvae of the cotton leafworm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae), under laborato0ry conditions. Egyptian Journal of Biological Pest Control, 29:50

El-Sayed, E. K.;Massoud, M. A. Z. and Attia, M. A. (2017): Biochemical and biological influences of sub-lethal concentrations of emamectin benzoate and certain IGR insecticides against Spodoptera littoralis (Lepidoptera: Noctuidae). Alexandria Science Exchange Journal, 38 (2): APRIL-JUNE.

Batta, Y. A. (2016): Recent advances in formulation and application of entomopathogenic fungi for biocontrol of stored-grain insects. Biocontrol Sci. Technol., 26: 1171-1183.

Bradford, M.M. (1976): A rapid and sensitive method for the quatitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Duncan, D.B. (1955): Multiple range and multiple F-test. Boimetrics, 11(1): 1-24.

El Husseini, M. M. M. (2019): Efficacy of the entomopathogenic fungus, Metarhizium anisopliae (Metsch.), against larvae of the cotton leafworm, Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae), under laborato0ry conditions. Egyptian Journal of Biological Pest Control, 29:50.

Fagan, W.F.; Siemann, E.R.; Mitter, C.; Denno, R.F. and Huberty, A.F. (2002): Nitrogen in insects: implication for trophic complexity and species diversification. American Naturalist, 160:784-802.

Fernandes, F.L.; Bacci, L. and Fernandes, M.E.S. (2010): Impact and selectivity of insecticides to predators and parasitoids. Entomo Brasilis, 3(1): 1-10.

Finney, D.J. (1971): Probit Analysis: a Statistical Treatment of the Sigmoid Response Curve. P. 33. Cambridge Univ. Press, London.

Hussain, A.; He Tian YR; Bland, J.M. and Gu, W.X. (2010): Behavioral and electrophysiological responses of C. formosanus towards entomopathogenic fungal volatiles, Biological Control, 55:166-173.

Ishaaya, I. and Casida, J.E. (1974): Dietary TH 6040 alters composition and enzyme activity of housefly larval

cuticle. Pestic. Biochem. Physiol., 4: 484- 490.

Ishaaya, I.; Moore, I. and Joseph, D. (1971): Protease and amylase activity in larvae on the Egyptian cotton worm, Spodoptera littoralis. J. Insect Physiol., 17: 45-53.

Jennifer, G.; Eedmilson, J. M.; Valéria, W.; Auristela, C. D.; Liana M. D. P.; Cínthia, C. M. S. and Alvaro, A. C. T. (2014): Sublethal effect of concentrations of Metarhizium anisopliae (Metsch sorok) on the larval stage and immunologic characteristics of Diatraea flavipennella (Box) Lepidoptera: Crambidae. Anais da Acad. Bras. de Ciênc., 86 (4): 1973-1984.

Kimberly, M. S. A. and Seow, M. H. (2017): Mode of infection of Metarhizium spp. fungus and their potential as biological control agent's fungi. J. Fungi 3(2):30:1-20.

Knight, J.A.; Anderson, S. and Rawle, J.M. (1972): Chemical basis of the sulfophosphovanillin reaction for estimating total serum lipids. Clin. Chem., 18: 199-202.

Kramer, K. J. and Koga, D. (1986): Insect Chitin: Physical state, synthesis, degradation and metabolic regulation. Insect Biochem., 16 (6): 851- 877.

Kungreiliu Panmei; Roopa Rani Sa mal; Lanbiliu, P. and Sarita Kumar (2021): Infuence of lufenuron on the nutrient content and detoxifcation enzyme expression in Aedes aegypti L. (Diptera: Culicidae). International Journal of Tropical Insect Science.

Lee, K.P.; Behmer, S.T.; Simpson, S.J. and Raubenheimer, D. A. (2002): geometric analysis of nutrient regulation in the generalist caterpillar Spodoptera littoralis (Boisduval). Journal of Insect Physiology, 48: 655-665.

Maqsood, S.; Sabri, M. A.; Ali, A.; Abbas, M. and Aziz, A. (2016): Comparative toxicity of some insecticides against armyworm, Spodoptera litura L. (Lepidoptera: Noctuidae) under laboratory conditions. Journal of Entomology and Zoology Studies, 5(1): 770-773.

Meyling, N.V. and Eilenberg, J. (2007): Ecology of the entomopathogenic fungi Beauveria bassiana and Metarhizium anisopliae in temperate agroecosystems: Potential for conservation biological control. Biological Control, 43: 145–155.

Nirupama, R. (2015): Biochemical studies on total protein, carbohydrate and lipids content level during the infection by fungi white muscardine disease of silkworm, Bombyx mori L. Munis. Entomology and Zoology Journal, 10(2).

Ramos, Y.; Alberto Daniel Taibo; Jorge Ariel Jiménez and Orelvis Portal (2020): Endophytic establishment of Beauveria bassiana and Metarhizium anisopliae in maize plants and its effect against Spodoptera frugiperda (J. E. Smith) (Lepidoptera: Noctuidae) larvae. Egyptian Journal of Biological Pest Control, 30:20.

Remia, K. M.; Logaswamy, S. ; Logankumar, K. and Rajmohan, D. (2008): Effect of an Insecticide (Monocrotophos) on some biochemical constituents of the fish Tilapia mossambica. Pollution Res., 27.

Saleh, T.A. and Abdel-Gawad, R.M. (2018): Electrophoretic and colorimetric pattern of protein and isozyme as reflex to diflubenzuron and chromafenozide treatments of Spodoptera littoralis (Boisd.). J

Entomol Zool Stud, 6(3):1651–1660.

Singh, N.B. and Sinha, R.N. (1977): Carbohydrates, lipids and protein in the developmental stages of Sitophillus oryzea and Sitophillus grannarius. Ann. Ent. Soc. Amer., 107-111.

Sobhi, A. S. ; El Kousy, S.M. and ElSheikh, T.A.A. (2020): Some toxicological and physiological aspects induced by camphor oil, Cinnamomum Camphora on the cotton leafworm, Spodoptera littoralis (Boisduval). (Lepidoptera: Noctuidae). Egyptian Academic Journal of Biological Sciences, 12(2):63- 73. Assar, A. A.; Ab

St Leger, R.J.; Cooper, R.M. and Charnley, A.K. (1991): Characterization of chitinase and chitobiose produced by the entomopathogenic fungus Metarhizium anisopliae. Journal of Invertebrate Pathology, 58:415-426.

Vidhya, D.; Rajiv, P. and Nalini Padmanabhan (2016): Impact of entamopathogenic fungal infection on the detoxifying enzyme in cotton leaf worm, Spodoptera litura (fabricius). International Journal of Pharma and Bio Sciences, 7(4): (b) 943- 948.

Wahba, T.F. and Shaker, N. N. (2020): Interaction of agricultural drainage water cations with insecticide potency Egypt. J. Plant Prot. Res. Inst., 3 (1): 167- 178.

Zhang Z, Xu C, Ding J, Zhao Y, Lin J, Liu F, Mu W (2019). Cyantraniliprole seed treatment efficiency against Agrotis ipsilon (Lepidoptera: ctuidae) and residue concentrations in corn plants and soil. Pest Manag Sci 75:464-1472