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Original article

Biochemical and toxicological effects of certain bioinsecticides on the Egyptian cotton leafworm, *Spodoptera littoralis*

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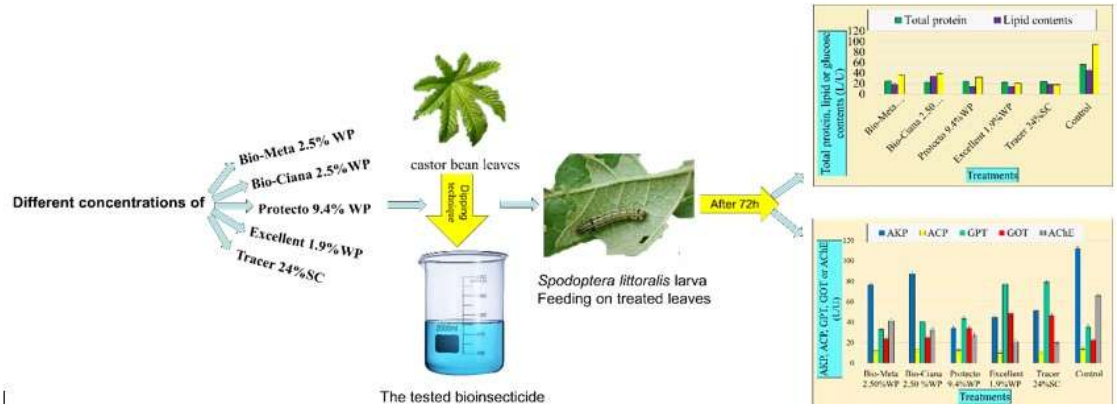
ABSTRACT

The toxicity of five bioinsecticides on the Egyptian cotton leafworm (*Spodoptera littoralis*) were evaluated under laboratory conditions. These bioinsecticides were *Metarhizium anisopliae* (Bio- Meta 2.5% WP), *Beauveria bassiana* (Bio-Ciana 2.5%WP), *Bacillus thuringiensis* (Protecto 9.4% WP), emamectin benzoate (Excellent 1.9% EC) and Spinosad (Tracer 24%SC). All experiments were evaluated on 4th larval instar to determine the LC values of the tested compounds. The biochemical parameters were also recorded on the 4th larval instar after 72h. The LC_{50} of these compounds were 30.45, 38.61, 21.15, 7,98 and 9.22 ppm after 72h for the above-mentioned compounds, respectively. The results clearly indicated that Excellent was the most effective compound, while Bio-Ciana was the least effective compound. Also, the results indicated that, all treatments reduced total protein, total lipids, glucose contents, alkaline phosphatase (AKP), and acetylcholinesterase (AChE) significantly ($P < 0.05$) in comparison with untreated check (control), while acid phosphatase (ACP) activity was decreased only after treatment with Bio-Meta, Excellent and Tracer. On the other hand, glutamic pyruvic transaminase (GPT) activity showed a significant increase ($P< 0.05$) with all treatments except Bio-Meta. Similarly, glutamic oxaloacetic transaminase (GOT) activity was increased with all treatments except Bio-Meta and Bio-Ciana. The results concluded that Excellent and Tracer were the most effective and can be successfully developed for controlling the Egyptian cotton leafworm.

Graphical abstract



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1. Introduction:

Phytophagous insects are a major constraint to crop production and often cause huge yield losses [1]. The Egyptian cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is considered one of the most destructive agriculture pests not only in Egypt but also in most countries in the world [2, 3, 4, 5]. This insect is known as a major polyphagous pest in Egypt by attacking a wide range of hosts covering over 112 species belonging to 44 different families of plant crops without any hibernation period along the year [6, 7, 8, 9]. The larvae of this insect prefer to feed on young leaves, young shoots, stake, bolls bunds, fruits and reducing the photosynthetic area and the marketability of vegetables and ornamentals [10]. Chemical control of this insect is widely used by conventional insecticides. Recently, the intensive use of many registered insecticides has led to the development of insect resistance [11, 12, 13]. Moreover, chemical insecticides may be harmful to human and the natural enemies of insect pests [14]. The environmental hazards of conventional insecticides necessitate the introduction of other new insecticides such as bioinsecticides that are effective and safe for human [15, 16]. Insects, like other animals, have enhanced defense responses to protect against insecticides, including enhanced metabolism, nerve insensitivity, and target site insensitivity; it is caused by defensive enzymes [17]. Among the defensive enzymes are acetylcholinesterase, phosphatases, and transaminases. Therefore, this study was carried out to evaluate the efficacy of five bioinsecticides and their biochemical effects on *S. littoralis* under laboratory conditions.

2. Materials and methods:

2.1. Insects:

The Egyptian cotton leafworm *Spodoptera littoralis* was obtained from The Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Dokki, Giza, Egypt which had no history of pesticides. The larvae were reared in laboratories of the Department of Plant Protection in the Faculty of Agriculture (Cairo), Al-Azhar University, Egypt.

The larvae were reared on clean fresh caster bean leaves (*Ricinus communis* L.) in a controlled environmental chamber at 25 ± 2°C and 65 ±5 R.H. and a photoperiod of 12:12 h (L:D) as described by El-Defrawi [18].

2.2. Bioinsecticides compounds:

Five bioinsecticides compounds were evaluated, two from entomopathogenic fungi, *Metarhizium anisopliae* (Biometa) and *Beauveria bassiana* (Biocinana) one from entomopathogenic bacterial, *Bacillus thuringiens*is (Protecto), one from Avermectin, emamectin benzoate (Excellent) and one from spinosyn, spinosad (Tracer). Some information on the used compounds is listed in Table (1). Different concentrations of each compound were tested on the fourth larval instar of *S. littoralis* by leaf dipping technique. For each concentration, leaves of castor bean leaves were washed, dried at room temperature and immersed in it for 2 sec, allowed to dry, and placed in a glass jar (two liters) with toilet paper on the bottom. One hundred larvae (fourth instar) were used in ten replicates (10 larvae/replicate) and covered with muslin. The larvae starved for 3 hrs. before they were placed in the jars. The larvae were fed on treated leaves for 48h, then on fresh untreated leaves until the termination of the experiment. The larvae in the control treatment were fed on leaves treated with distilled water only. After 72 h, the larval mortality was determined in each concentration to calculate the LC_{25}, LC_{50} $LC_{90,}$ and slope values according to Finney [19] using the probit analysis statistical method. The corrected mortality was used when needed by Abbott's formula [20], and the toxicity index (T.I.) was determined by Sun [21] method as follows: T.I = LC_{50} of the most effective compound, LC_{50} of the tested compound x 100.

2.3. Biochemical studies

The biochemical studies were calculated after 72h from the tested compounds treatment on the 4th larval instar of *S. littoralis* by using a diagnostic kit produced by El-Nasr Pharmaceutical Chemical Company and measured by Geneway 6105 spectrophotometer.

- **Determination of total protein**

Total protein was determined according to the method described by Doumas (1975) [22].20 µl of the protein sample was mixed with 1 ml of potassium sodium tartrate (90 g/L) in the test tube containing 20 µl of the bovine albumin (5 g/L). The blank tubes containing 20 µl of the distilled water mixed with 1 µl of potassium sodium tartrate. The tubes were incubated for 10 minutes at room temperature until a colored complex was formed. The absorbance was measured at wavelength 550 nm.

- **Determination of total Lipids**

Total lipid was determined according to the method described by Drevon and Schmitt (1964) [23]. Aliquots of 100 µl of the lipids sample were mixed with 3 ml of sulphuric acid then boiled in the water bath for 10 minutes. For colorways, 50 µl of hydrolysates was mixed with 1.5 ml of phosphoric acid (13.9 mol/L) and vanillin (8 mmol/L). The mixture was incubated at 37°C for 10 minutes. The sample absorbance was measured at 525 nm.

- **Determination of total Carbohydrates**

The total carbohydrates were determined calorimetrically by the phenol-sulphuric acid method as described by Trinder (1969) [24]. 100 ml of the supernatant solution was added to one ml of 5% aqueous phenol solution followed by 5 ml of concentrated sulphuric acid. Measurement of the

intensity of the yellow-orange color was carried out at 480 nm.

- **Determination of the acid or alkaline phosphatase activity**

Acid and alkaline phosphatase activities were measured in the larvae according to the method of Young *et al.* (1975) [25]. For the kinetic technique, 10 ml of 2-amino-2-methyl-l-propanol buffer pH 10.5 (0.9 mol/L) and magnesium sulphate (1.0 mmol/L) were mixed with 1ml of P-nitrophenyl-phosphate (5.5 mmol/L). 2ml of this mixture was mixed with 50µl of the sample. The distilled water was used as blank. The change in absorbance was measured against blank after 1, 2,3 minutes at 405 nm.

- **Determination of GOT and GPT activities.**

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) are determined in the larvae according to the method described by Reitman and Frankel (1957) [26].

Procedure for GOT determination. 2,4-dinitrophenyl-hydrazine aspartate was provided for the reaction and L-aspartate and 2-oxoglutarate were provided as the substrates. After incubation for 30 minutes at 37 °C, 0.5 ml of 2, 4-dinitrophenyl-hydrazine (0.1 M) was added. The mixture was stood at 20-25 °C for 20 minutes, then 5ml of sodium hydroxide 0.4 N was added and the mixture was settled for 5 minutes. The absorbance was measured against a blank at wavelength 546 nm.

Procedure for GPT determination. 0.25 ml of α-oxoglutarate (0.002 M) was mixed with 0.25 ml of di-alanine (0.2 M) and incubated for 5 minutes at 37 °C. The rest of the procedures are the same as for GOT.

- **Determination of Acetylcholinesterase activity**

Acetylcholinesterase (AChE) activity was measured using acetylthiocholine iodide as a substrate according to Agarwal *et al.* (1975) [27]. Thiocholine, the product of the hydrolysis of the substrate, reacts with 5,5-dithiobis (2-nitrobenzoic acid) to produce a yellow anion 5-thio-2-nitrobenzoic acid with absorbed light at 412 nm. The insect sample was prepared in phosphate buffer (pH 8.0, 0.1 M) (10µl sample into 100 µl buffer). 25 µl of 0.01 M of the reagent dithiobis-2-nitrobenzoic acid were added (39.6 mg of dithiobis-2-nitrobenzoic acid were dissolved in 10 ml phosphate buffer pH 7.0 (0.1M) and 15 mg of sodium bicarbonate. Finally, 20 µl of the substrate acetylcholine bromide (3mM) were added.

2.4. Statistical analysis

Mortality slopes and LC values for each bioassay were estimated via Probit analysis using LDP-Line software [*28*]. All the biochemical analyses in this study were performed by using IBM SPSS V.20 statistics (IBM®) software [*29*]. The results were analyzed by one-way ANOVA, and then means were separated by Tukey's test at ($P < 0.05$) and charted produced by using Microsoft Excel (Microsoft Office, 365).

Table (1): The formulations of the tested bioinsecticide.

Group	Common names	Trade name	Concentration and formulation	Source
Entomopathogenic fungi	*Metarhizium anisopliae*	Bio-Meta®	2.50%WP	Biopesticides production unit, PPRI
Entomopathogenic fungi	*Beauveria bassiana*	Bio-Ciana®	2.50% WP	Biopesticides production unit, PPRI
Entomopathogenic bacteria	*Bacillus thuringiensis* (Kurstaki)	Protecto®	9.4%WP	Biopesticides production unit, PPRI
Avermectin	Emamectin benzoate	Excellent®	1.9% EC	Kafr El-zyat For pesticides and chemicals company
Spinosyn	Spinosad spinosa	Tracer®	24%SC	Dow Agro Science Company Egypt

- **PPRI= Plant Protection Research Institute, Agricultural Research Center, Egypt.**

3. Results:

3.1. Bioinsecticides efficiency against *S. littoralis*:

The results in Table (2) showed the toxicity of five bioinsecticides on the fourth larval instar of the cotton leafworm under laboratory conditions after 72 hrs.

These compounds were varied in toxicity according to the LC_{50} values. The LC_{50} values clearly indicated that Excellent was the most effective with LC_{50} values 7.98 ppm, while Bio-Ciana was the least effective (LC_{50} value 38.61 ppm). Other compounds such as Tracer, Protecto, and Bio-Meta give LC_{50} values 9.22, 21.15, and 30.45 ppm, respectively.

3.2. The biochemical assay:

The results in Table (3) showed the efficiency of tested bioinsecticides compounds on total protein, total lipids, and glucose contents. These results clearly indicated that

all compounds significantly reduced the total protein, total lipids, and glucose contents in comparison with untreated check (control). No significant difference was found between these compounds in the case of total protein. In the case of total lipids, the Protecto and Excellent were reduced significantly. While both fungi-bioinsecticides were used, Bio-Meta and Bio-Ciana were the least effective in reducing the total lipids. In the case of glucose content, the results indicated that all treatments were reduced significantly. The Tracer was the most effective than other compounds followed by Excellent, Protecto, Bio-Ciana, and Bio-Meta, respectively.

The results in Table (4) showed the efficiency of the tested compounds on alkaline phosphatase, acid phosphatase, glutamic pyruvic transaminase, and acetylcholinesterase, respectively. The results clearly indicated that all treatments were significantly reduced alkaline phosphatase in comparison with the control treatment. The Tracer was the most effective followed by Protecto.

Table (2): Efficiency of the tested bioinsecticides on 4th larval instar of *S. littoralis*at 72 h post-exposure under laboratory conditions.

Treatments		LC_{25} (ppm)	LC_{50} (ppm) (Confidence limits)	LC_{90} (ppm)	Slope± SE	Toxicity index
Common names	**Trade names**					
M. anisopliae	Bio-Meta 2.50% WP	16.14	30.45 (28.11-32.25)	54.81	1.61±0.26	26.21
B. bassiana	Bio-Ciana 2.50 %WP	18.63	38.61 (36.19-40.33)	69.50	1.77±0.21	20.67
B. thuringiensis	Protecto 9.4%WP	10.58	21.15 (19.31-22.19)	38.07	1.56±0.18	37.73
Emamectin benzoate	Excellent 1.9% EC	3.44	7.98 (6.50-8.21)	14.36	1.85±0.22	100
Spinosad	Tracer 24%SC	4.61	9.22 (8.11-11.27)	16.60	1.89±0.13	86.56

Toxicity index (T.I.) =LC_{50} of the most effective compound / LC_{50} of the tested compound×100 (Sun,1950).

Table (3): Total protein, lipid and glucose contents of 4th instar *S. littoralis* after 72h post-treatment to LC_{50} of the tested bioinsecticides.

Treatments	Total protein (mg/ml)	Lipid content (mg/ml)	Glucose content (mg/ml)
Bio-Meta	$24.89±1^{b}$	$18.83±0.76^{c}$	$36.82±0.54^{c}$
Bio-Ciana	$22.92±1.8^{b}$	$34.13±2.4^{b}$	$39.7±0.51^{b}$
Protecto	$24.2±0.25^{b}$	$13.96±1.6^{d}$	$32.54±0.41^{d}$
Excellent	$22.48±0.19^{b}$	$13.99±1.1^{d}$	$20.87±0.61^{e}$
Tracer	$23.97±1.9^{b}$	$18.39±1.4^{c}$	$18.26±0.99^{f}$
Untreated check (control)	$56.47±1.1^{a}$	$45.87±0.72^{a}$	$94.87±0.84^{a}$

Data are given as (mean ± S.E.). Values followed by different letter(s) within each column are significantly different when analyzed by Tukey's test (P < 0.05).

The results also showed that Excellent was the most effective in reducing acid phosphatase. Also, Bio-Meta and Tracer without significant difference between the two compounds. Bio-Ciana and Protecto were the least effective and no significant difference was found with untreated check (control). The effect of the tested compounds on glutamic pyruvic transaminase are shown in Table (4) from these data, the results indicated that all treatments increased the glutamic pyruvic transaminase contents. Excellent and Tracer were the most effective in increasing the glutamic pyruvic transaminase followed by Protecto and Bio- Ciana, Bio-Meta was the least effective. Also, these treatments increased the glutamic oxaloacetic transaminase. The Excellent and Tracer are significantly the most effective followed by Protecto. The Bio-Ciana and Bio-Meta were the least effective without significant difference between them and untreated check (control) treatment. Also, the data in Table (4) clearly indicated that all treatments significantly reduced the acetylcholinesterase. The most effective compounds were Excellent and Tracer while Bio-Meta was the least effective. Also, Protecto and Bio-Ciana were significantly reduced the acetylcholinesterase.

Table (4): Enzymes activities of 4th instar *S. littoralis* after 72h post-treatment to LC_{50} of the tested bioinsecticides.

Treatments	AKP	ACP	GPT	GOT	AChE
Bio-Meta 2.50% WP	76.64±1.7^{c}	11.91±0.4^{ab}	33.03±0.57^{c}	24.05±0.67^{c}	41.59±0.47^{b}
Bio-Ciana 2.50 %WP	87.45±0.9^{b}	13.21±1^{a}	40.34±1^{b}	24.89±0.76^{c}	32.69±0.83^{c}
Protecto 9.4%WP	34.14±0.8^{d}	12.78±1.1^{a}	43.45±0.9^{b}	34.12±1.4^{b}	27.66±1.15^{d}
Excellent 1.9%EC	45.15±1.9^{f}	9.98±0.6^{b}	77.5±1.6^{a}	48.84±1.6^{a}	21±1^{e}
Tracer 24%SC	51.23±1.4^{e}	11.76±0.6^{ab}	79.14±1.8^{a}	46.54±1.3^{a}	19.93±1.38^{e}
Control	112.28±1.2^{a}	13.52±1^{a}	35.75±1.3^{c}	22.69±0.78^{c}	66.19±0.96^{a}

Data are given as (mean ± S.E.). Values followed by different letter(s) within each column are significantly different when analyzed by Tukey's test (P < 0.05).
***AKP: Alkaline phosphatase (phenol/ml/minute)**
***ACP: acid phosphatase (phenol/ml/minute)**
***GPT: Glutamic pyruvic transaminase (µg pyruvate/ml/min.)**
***GOT: Glutamic oxaloacetic transaminase (µg oxaloacetate/ml/min)**
***AChE: Acetylcholinesterase (µg/min/g.b.wt)**

4. Discussion

The LC_{50} values recorded at 72h post-treatment on the 4th larval instar of the cotton leafworm indicated variation in toxicity of these compounds. The results clearly showed that emamectin benzoate was the most effective compound, while the fungus *B. bassiana* -based bioinsecticide was the least effective compound. The current results are similar to those reported by Temerak who found that emamectin benzoate was the most effective insecticide against the 4th instar larvae of the cotton leaf worm, followed by Spinosad [30]. Emamectin benzoate was the most potent compound among the tested bioinsecticides against the cotton leafworm larvae [3, 31]. Emamectin benzoate causes a high reduction in larval populations of *S. littoralis*, ranging between 78.53 to 83.96% [32].

To understand the responses of *S. littoralis* 4th larval instar to the tested bioinsecticides (*M. anisopliae*, *B. bassiana*, *B. thuringiensis*, emamectin benzoate, and Spinosad), the biochemical changes in protein, lipid, glucose, alkaline phosphatase, acid phosphatase, GPT, GOT, and AChE were detected at 72h post-treatment. Total proteins, carbohydrates as glucose, lipids, alkaline phosphatase, and acetylcholinesterase are vital components for insect development and perform its vital activities [33, 34]. In this study total protein, carbohydrates as glucose, lipids, alkaline phosphatase, and acetylcholinesterase decreased significantly after bio-insecticides applications. Similar results were observed when *S. littoralis* was treated with the LC_{50} values of Radiant, Protecto, and Agerin [35]. Also, when *S. littoralis* larvae were treated by emamectin and Spinosad as bioinsecticides or after hexaflumuron and teflubenzuron treatment as insect growth regulators (IGR's) [36]. A decrease in total protein contents in *Tribolium castaneum* treated with Spinosad was reported by other authors [37]. Total protein was significantly decreased by using spinetoram on *S. littoralis* [38]. Some bioinsecticides decrease the acetylcholinesterase activity of the 4th instar larvae of *S. littoralis* [39]. However, the sublethal effects of some bioinsecticides can be integrated into *S. littoralis* control to reduce the overuse of insecticides, this effect appears as the reduction in the acetylcholinesterase and acid phosphatase activities which result from blocking of the action potential of the nervous system by inhibition of neuronal cholinesterase activity or resulted from cell damage as indicated by acid phosphatase[5]. Changes in alkaline phosphatase activities after treatment with bioinsecticides indicated an alternation in the physiological balance of the midgut which might affect these enzymes [40].

On the other hand, the present study showed a significant increase in GOT and GPT activities. These transaminases are key enzymes that help in the production of energy by the metabolism of the nitrogen compound and serve as a strategic link between carbohydrates and protein metabolism [41, 42]. This interprets the relationship between increases in these enzymes and inhibition of glucose and protein content [43]. The current results are in agreement with some results for *S. littoralis* after several treatments of bioinsecticides and IGRs [44, 45, 46, 41].

Conclusion: In the present study, Excellent and Tracer can control *S. littoralis* successfully and the LC_{50} of the tested bioinsecticides can disturb the activities of some vital components and enzymes effectively. This change in certain physiological parameters can disturb the growth and development of the insect and finally leads to death.

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