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SELECTION OF ENVIRONMENTALLY SAFE COMPOUNDS FOR CONTROLLING THE COTTON LEAFWORM, *Spodoptera littoralis* (BOISDUVAL) IN BELL PEPPERS AT MENOFIA GOVERNORATE UNDER SEMI-FIELD CONDITIONS

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Capsicum annum L., more often known as the bell pepper, is a staple fruit vegetable crop grown worldwide due to its high nutritional value. *Spodoptera littoralis* (Boisduval), often known as the cotton leafworm, causes substantial economic losses and detrimental effects on crop quality of its voracious appetite. Biopesticides as an alternative to traditional insecticides for *S. littoralis* management have recently received more focus. We examined three commercial bioinsecticides at the recommended concentrations against *S. littoralis* 2nd instar larvae during two growing seasons in semi-field circumstances. Larval mortality was observed three days post-treatment for all drugs. Although all tested compounds were effective, emamectin benzoate consistently produced the greatest death rate over both planting periods. More importantly, in both growing seasons, the virulence of the investigated compounds was maintained for up to 10 days after treatment. Results showed that Bio-Power® exhibited the highest LC_{50} value (1.156 gm/ml), followed by Biotect® (0.1238 gm/ml) and Benzo® (0.0084 gm/ml). Furthermore, treatment with sublethal concentrations of the tested compounds lowered the total proteins, carbohydrates and lipids compared to the control. On the other hand, certain carbohydrate hydrolyzing enzyme levels were significantly reduced due to the treatment of the 2nd instar larvae with the LC_{50} of the investigated substances. While treatment with the sublethal concentration of tested compounds increased the GST level compared to the control, the chitinase activity was reduced. The results of this study show that bioinsecticides are effective replacements for synthetic insecticides. They are safe to use and have a pathogenic effect on insects, so you may use them without worrying about harming anyone.

Keywords: *Capsicum annum*; cotton leafworm; *Spodoptera littoralis*; bio-based insecticides; carbohydrate hydrolyzing enzymes.

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ABBREVIATIONS

ANOVA : Analysis of Variance
GST : Glutathione-S-Transferase
LC_{50} : Lethal Concentration of 50%
R. H. : Relative Humidity
r.p.m. : Revolution per Minute
TCA : Tricarboxylic Acid Cycle
WG : Wettable Granules
WP : Wettable Powder

1. INTRODUCTION

Bell pepper, *Capsicum annum* L., is a crucial fruit vegetable crop of the Solanaceae family cultivated worldwide for its pleasant flavor, exquisite taste and various colors. Bell pepper cultivation is increased substantially over the year in many countries worldwide [1]. Egypt ranked 6th among the producing countries in 2019 [2]. Like many field crops and vegetables, pepper is attacked by many insect pests at all its growing stages; of these pests is the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *S. littoralis* is economically significant due to its ability to attack various plant structures, unfavorably impacting crop quality and causing economic losses [3]. Many management strategies were employed to control this pest. Chemical control means were primarily and extensively used to control *S. littoralis* in Egypt [4,5]. In Egypt, *S. littoralis* was controlled by methyl-parathion, organophosphorus, synthetic pyrethroids, insect growth regulators (IGRs) and other non-conventional insecticides. However, many reports of resistance and cross-resistance development, resurgence and chemical pesticide residues have limited the employment of those pesticides [6,7]. Nowadays, more attention has been paid to using biopesticides, such as compounds based on entomopathogens. These compounds have unique modes of action [8,9], and their properties may differ considerably from the conventional agents with which growers are familiar. The entomopathogenic bacterium *Bacillus thuringiensis* (Berliner) is one of the most used biological pesticides worldwide [10,11]. It is a gram-positive bacterium that exhibits insecticidal activity against many agricultural pests [12]. *B. thuringiensis* produces an intracellular crystal composed of one or more δ-endotoxins [13,14]. It is widely used for its safety against vertebrates, non-target organisms and other ecosystems [15,16]. Moreover, utilizing entomopathogenic fungi against insects is an effective control means [17]. They infect insects through contact through their ability to secret insecticidal compounds that make them in the vanguard of the global development of alternative control strategies [17–19]. *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) has a universal distribution [20,21]. It is commonly isolated from insect cadavers and soil on selective artificial media or by insect baits [20–22]. For fungal infection, it may require several days to cause insect mortality. After application, the conidia adhering to the insect exoskeleton may also be transferred to other insects of the same or different species via physical contact [21,23,24]. The conidia on the insect's corpse are more tolerant to solar radiation under field conditions [21,25,26]. Furthermore, Emamectin benzoate is a second-generation avermectin analog with exceptional activity against lepidopterans [27]. Emamectin benzoate functions as a chloride channel activator, reducing the excitability of neurons. Within 3–4 days of exposure, insect larvae cease eating, become permanently paralyzed and die [28]. We evaluated three bioinsecticides' lethal and sublethal effects on *S. littoralis* 2nd instar larvae in semi-field circumstances throughout two consecutive growing seasons. In addition, the biochemical effects of the studied substances on soluble biomolecules, such as proteins, carbohydrates and lipids, were examined. In addition, the effect of the tested substances on enzyme activity was analyzed.

2. MATERIALS AND METHODS

2.1 Insects' Colony and Rearing Technique

Collections of freshly produced egg batches were transported in perforated paper bags from the field to the Cotton leafworm Research Department at the Plant Protection Research Institute at the Agricultural Research Center in Dokki, Giza, Egypt. These eggs were incubated in a controlled laboratory setting at 27±2°C and 65±5% R.H. in plastic cups lined with gauze. The freshly emerged second-instar larvae were put to use in the research process. Castor bean (*Ricinus communis* L.) leaves were provided to the freshly born larvae, and, if necessary, more leaves were added regularly [29,30].

2.2 Tested Compounds

Three commercial biopesticides were tested against the 2nd instar larvae of *S. littoralis*. A *B. thuringiensis* var. *kurstaki* under the trade name Biotect® (WP 9.4%) with a recommended application rate of 300 gm/acre obtained from the Organic BIO Technology (S. A. E.). A *Beauveria bassiana* bio-based insecticide under the trade name Bio-Power® (WP 1.15%) with a recommended application rate of 1.5 Kg/acre was supplied from Gaara Establishment (Import and Export). An emamectin benzoate compound under the trade name Benzo® (WG 5.7%) with a recommended application rate is 60 gm/acre. It

was obtained from Egypt's Land for Agricultural Development and Commercial Agencies (Agricultural Pesticides Committee, http://www.apc.gov.eg/en/default.aspx).

2.3 Semi-field Experiment Design

A semi-filed experiment was conducted to evaluate the tested compounds' effectiveness against the 2nd instar larvae of *S. littoralis*. The study was performed throughout the 2020 and 2021 bell pepper growing seasons at Astanha village (30°27'21.7"N 31°06'39.3"E), El-Bagour District, Menofia Governorate. The field area was cultivated with the Omega bell pepper variety on May 18th, 2020 and May 19th, 2021. Standard agricultural practices were applied. The tested pesticides were sprayed at the recommended concentrations with a back spray motor, taking into account the full coverage of all plant leaves in an area of 1/100 of the feddan (42 m^2) for each treatment. The tested compounds were sprayed on July 2nd, 2020, and 2021. After the leaves of the plants were completely dry, random samples were collected from the treated and untreated plants (control) and placed in perforated paper bags for one-time use. In the laboratory, clean and sterilized jars of 1-liter capacity are prepared with 25 newly hatched 2nd instar larvae per jar in four replications with 100 larvae per treatment. The larvae are fed on the treated leaves daily after spraying until the tenth day. Tested compounds evaluation was carried out during the 2020 and 2021 growing seasons. Mortalities were recorded for 2 days, 3 days, 5 days, 7 days, and 10 days post-feeding on treated leaves. The efficiency of tested compounds against the 2nd instar larvae was calculated according to Schneider-Orelli's formula [31].

2.4 Determination of the Median Lethal Concentration (LC_{50}) Values of Tested Compounds

The LC_{50} values of the compounds were calculated when applied to larvae in their second instar using a leaf-dipping method [32]. Castor bean leaves were washed, dried, dipped for 10 seconds in one of six concentrations of the compounds, allowed to air dry at room temperature, and then delivered to groups of 25 second-instar larvae in clean jars. Every treatment and concentration combination was tested with three independent samples. Leaves that were soaked in water served as the control group's experiment.

2.5 Biochemical Assay

Insect samples preparation: Insect specimens were processed as reported before by Amin [33]. The second instar larvae were treated for 48 hours with the LC_{50} of the investigated drugs. One gram of surviving sixth-instar larvae was weighed and homogenized in distilled water (50 mg/1 ml). In a cooling centrifuge, homogenates were centrifuged at 8000 rpm for 15 minutes at 4° C. The deposits were removed and the supernatant, also known as enzyme extract, may be kept at 50° C for at least one week without substantial activity loss.

Determination of total proteins, total carbohydrates, and total lipids: According to Bradford [34], DuBois et al. [35], and Knight et al. [36], the effect of the LC_{50} of investigated substances on the total proteins, total carbohydrates and total lipids of the larvae that survived treatment was evaluated.

Determination of enzyme activity: The enzymes invertase, amylase, and trehalase were tested according to the standards established by Ishaaya, Ishaaya and Swirski [37,38]. Chitinase activity was measured using Bade and Stinson's methods [39]. The activity of Glutathione S-transferase (GST) was measured following the method described by Habig et al. [40].

2.6 Statistical Assessment

SPSS 22.0 (Statistical Package for Social Sciences, USA) version 22.0.0 software was used for the statistical analysis and analysis of variance (ANOVA) was performed on the data collected from each experiment independently. Four replicates were used to analyze all toxicological and biochemical parameters. The results are presented as the mean and standard deviation. Significant differences between means were identified at the $P < 0.05$ level [41]. Duncan's Multiple Range Test ($P \leq 0.05$) was used to find significant differences between the treatments [42]. Using the "LdPLine®" program, the LC_{50} values were calculated using the regression lines described in [43].

3. RESULTS AND DISCUSSION

3.1 Semi-field Application

The effectiveness of tested compounds against the 2nd instar larvae of *S. littoralis* during two successive growing seasons, 2020 and 2021, in the bell pepper field is listed in Tables 1 and 2 and Figs. 1 and 2. During the 2020 and 2021 growing seasons, the larval mortality was obtained on the 3rd day post-treatment for all tested compounds. The larval mortality rate increased gradually from the 5th-day post-treatment till the 10th day. Results also showed that Biotect® and Benzo® were more toxic than Bio-Power®.

These findings were consistent with those of Abd El-Kareem [44], who discovered steadily elevated mortality in *S. littoralis* larvae in their second instar when exposed to *B. thuringiensis* var. *kurstaki* in semi-field settings. In addition, the findings supported other research [45–47] that used bioinsecticides to treat younger larval instars of *S. littoralis*.

Determination of LC_{50} values of tested compounds: Results listed in Table 3 show the LC_{50} values of tested compounds against the 2nd instar larvae of *S. littoralis* under laboratory conditions. Obtained results showed that Bio-Power® exhibited the highest LC_{50} value (1.156 gm/ml), followed by Biotect® (0.1238 gm/ml) and Benzo® (0.0084 gm/ml). These results revealed the high toxicity of Benzo® compared to Biotect® and Bio-Power®, according to its low LC_{50} values.

3.2 Biochemical Impacts of Tested Compounds

Effect of sublethal concentrations of tested compounds on total proteins, total carbohydrates, and total lipids: The impact of sublethal concentrations of the tested compounds on total proteins, total carbohydrates and total lipids of the 6th instar larvae that survived treatment as 2nd instar larvae are shown in Table 4 and Fig. 3. Results revealed a significant reduction in total proteins, carbohydrates and lipids. Biotect® displayed the most effective compound as the reduction in total proteins, carbohydrates and lipids were more noticeable.

Table 1. Corrected mortality percentage of 2nd instar larvae of *Spodoptera littoralis* during the 2020 growing season

Tested compounds	% Corrected mortality after indicated days					% General mean
	2 days	3 days	5 days	7 days	10 days	
Biotect®	0	51.46	81.46	95.44	95.44	64.76
Bio-Power®	0	50.96	79.96	93.44	93.44	63.56
Benzo®	0	60.96	80.72	96.72	96.72	67.02
Control	0	0	0	1	1	0.6

Table 2. Corrected mortality percentage of 2nd instar larvae of *Spodoptera littoralis* during the 2021 growing season

Tested compounds	% Corrected mortality after indicated days					% General mean
	2 days	3 days	5 days	7 days	10 days	
Biotect®	0	52.33	88.89	94.44	95.44	66.22
Bio-Power®	0	48.72	87.44	94.44	94.44	65.08
Benzo®	0	65.72	96.30	96.30	98.96	71.46
Control	0	0	0	1	2	0.9

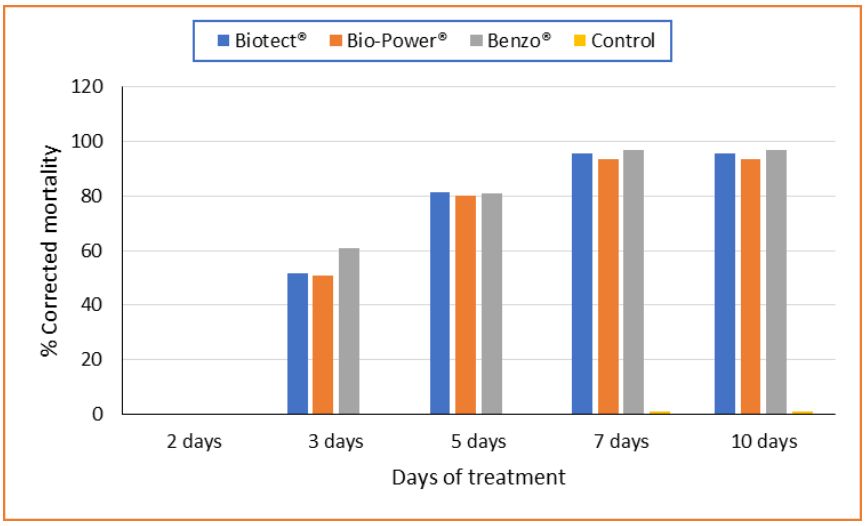

Fig. 1. Corrected mortality percentage of 2nd instar larvae of *Spodoptera littoralis* during the 2020 growing season

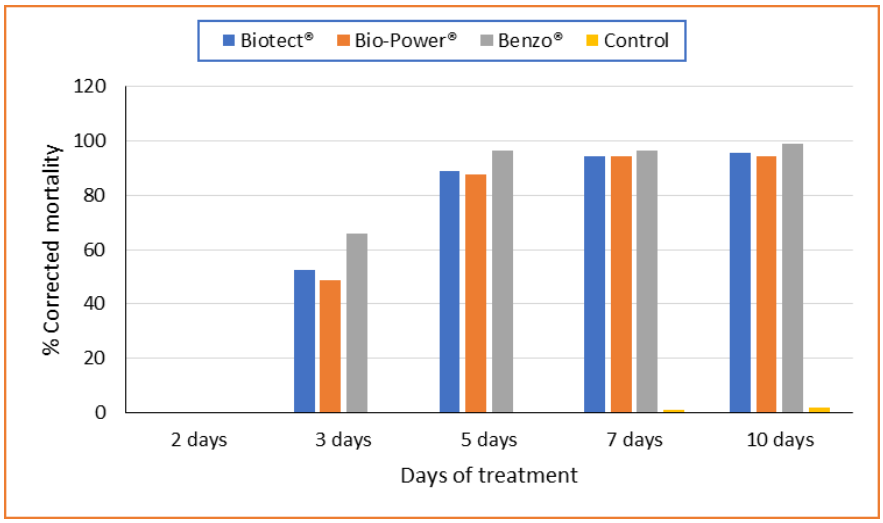

Fig. 2. Corrected mortality percentage of 2nd instar larvae of *Spodoptera littoralis* during the 2021 growing season

Table 3. The LC_{50} values of tested compounds against the 2nd instar larvae of *Spodoptera littoralis* under laboratory conditions

Tested compounds	Median lethal concentration (LC_{50}) (gm/m)	Fiducial limits (C.I. 95%) (gm/ml)		Slope
		Lower	Upper	
Biotect®	0.1238	0.0832	0.1747	1.3412 ± 0.2132
Bio-Power®	0.1567	0.1056	0.2282	1.2448 ± 0.2071
Benzo®	0.0084	0.0058	0.0114	1.6199 ± 0.2390

Table 4. Impact of median lethal concentrations of tested compounds on total proteins, carbohydrates, and lipids in the 6th instar larvae of *Spodoptera littoralis* that survived treatment as 2nd instar larvae

Tested compounds	Total proteins (µg/g b.w.) (Mean ± S.E.)	Total carbohydrates (µg/g b.w.) (Mean ± S.E.)	Total lipids (µg/g b.w.) (Mean ± S.E.)
Biotect®	34.6 ± 0.9^{c}	44.3 ± 1.4^{c}	35.0 ± 1.2^{c}
Bio-Power®	43.0 ± 1.0^{b}	50.6 ± 1.2^{b}	42.0 ± 1.1^{b}
Benzo®	42.0 ± 1.1^{b}	50.6 ± 0.7^{b}	37.6 ± 0.7^{c}
Control	46.3 ± 0.3^{a}	71.3 ± 0.9^{a}	46.3 ± 0.9^{a}
Df	3	3	3
F-value	70.0	420.75	74.0
***P*-value**	0.0000***	0.0000***	0.0000***

*Means followed by the same small letter in a column are not significantly different at the 5% probability level (Duncan's Multiple Range Test) [42] b.w., body weight; DF: degree of freedom *** Highly significant effect*

Insect susceptibility to tested pesticides and changes in their function may be linked to any shift in energy stores such as carbohydrates, lipids, proteins and glycogen [48]. Proteins are crucial building blocks influencing body size, growth rate and fertility. They have been connected to life cycles, population dynamics and even biological diversity at higher levels of the organization [49]. The harmful effects of the studied bioinsecticides might be responsible for reducing protein content. Moreover, the reduction in protein contents could be allocated to the breakdown of protein into amino acids, so with the entrance of these amino acids to the tricarboxylic acid cycle (TCA) as a keto acid, they will help supply energy for the insect. So, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress to provide intermediates to the TCA cycle by retaining free amino acid content in the hemolymph [50]. For insects, carbohydrates are a crucial source of energy. Carbohydrates can be transformed into lipids and help produce amino acids. Numerous

carbohydrates, including sugars, are effective appetite enhancers [51]. The enhanced metabolism during toxicant stress may be the reason for the decreased carbohydrate intake. The decrease in carbohydrates raises the prospect that under stressful circumstances, vigorous glycogenolysis and the glycolytic pathway might provide more energy than needed [52–55].

Free and bound fatty acids, short- and long-chain alcohols, steroids, esters, phospholipids and other substances make up the lipids found in living things. Insects can convert carbohydrates into lipids; many can synthesize lipids and store them as body fat. Cell walls are made up of fatty acids, phospholipids and sterols, each of which serves other distinct purposes [48]. The detoxification process in larvae, which necessitates the conversion of a significant amount of eaten food into energy following treatment with pesticides, may be to blame for the decrease in total lipid concentrations [56]. The findings demonstrated that the LC_{50} of the investigated compounds lowered the amylase, invertase and trehalase activities in the late 6th instar larvae after treatment of the 2nd instar larvae. These enzymes carry out the hydrolyses of carbohydrates. Furthermore, the reduction in all bodily components brought on by therapy may be linked to the depletion in enzyme levels. The lower levels of these enzymes demonstrate a lower rate of metabolism, a lower rate of phosphorous release for energy metabolism and a lower rate of transport of metabolites [57].

Effect of sublethal concentrations of tested compounds on Amylase, Invertase, and Trehalase activities: The latent impact of tested compounds' LC_{50} on the activity of some carbohydrate hydrolyzing enzymes is shown in Table 5 and Fig. 4. Results revealed that treatment with the median lethal concentrations of the tested compounds significantly decreased the activity of amylase and invertase compared to the control. However, there was an insignificant decrease in trehalase activity when treating larvae with Bio-Power® and Benzo®. On the other hand, treating larvae with Biotect® has significantly reduced the trehalase activity compared to the control. The inhibition of carbohydrate hydrolyzing enzymes may impact molting [58], explaining the observed larval mortality [59].

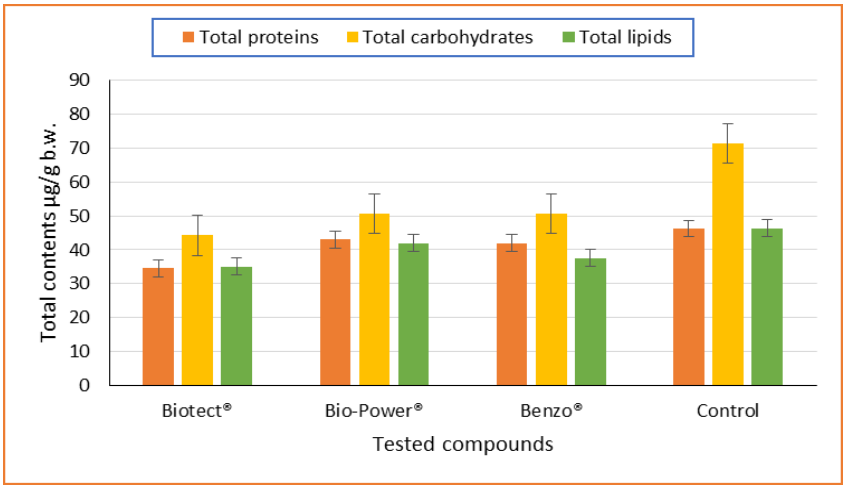

Fig. 3. Effect of LC_{50} of tested compounds on the total body contents in the 2nd instar larvae of *S. littoralis*

Table 5. Impact of median lethal concentrations of tested compounds on amylase, invertase, and trehalase activities in the 6th instar larvae of *Spodoptera littoralis* that survived treatment as 2nd instar larvae

Tested compounds	Mean ± S. E. (µg glucose/min./gm b.w.)		
	Amylase	Invertase	Trehalase
Biotect®	200.6 ± 0.7^{c}	563.0 ± 2.1^{d}	383.6 ± 2.7^{b}
Bio-Power®	197.0 ± 1.2^{d}	572.6 ± 2.2^{c}	409.0 ± 0.6^{a}
Benzo®	206.0 ± 0.6^{b}	583.0 ± 3.0^{b}	405.0 ± 2.9^{a}
Control	213.6 ± 0.9^{a}	652.6 ± 1.4^{a}	409.6 ± 2.6^{a}
Df	3	3	3
F-value	150.0	4921.0	456.0
P-value	0.0000***	0.0000***	0.0000***

*Means followed by the same small letter in a column are not significantly different at the 5% probability level (Duncan's Multiple Range Test) [42] b.w., body weight; DF: degree of freedom *** Highly significant effect*

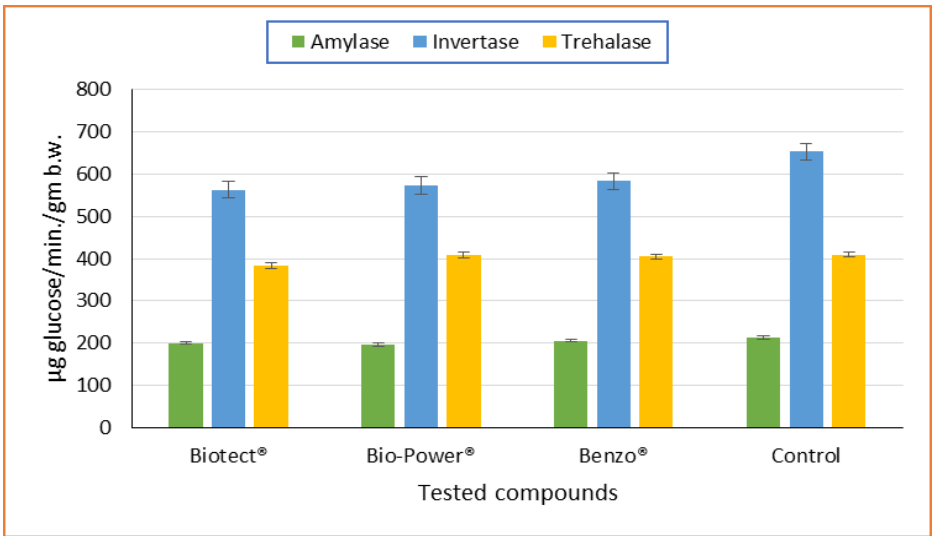

Fig. 4. Effect of sublethal concentrations of tested compounds against certain carbohydrate hydrolyzing enzymes in the 2nd instar larvae of *S. littoaralis*

Table 6. Impact of median lethal concentrations of tested compounds on chitinase and glutathione-S-transferase (GST) activities in the 6th instar larvae of *Spodoptera littoralis* that survived treatment as 2nd instar larvae

Tested compounds	Chitinase (µg NAGA/min/gm b.w.) (Mean ± S. E.)	GST (µmole/min/ml) (Mean ± S. E.)
Biotect®	236.3 ± 3.2^{ab}	217.3 ± 2.2^{a}
Bio-Power®	202.0 ± 1.5^{c}	224.0 ± 3.1^{a}
Benzo®	231.6 ± 1.2^{b}	205.0 ± 2.5^{b}
Control	241.3 ± 1.9^{a}	191.3 ± 1.8^{c}
Df	3	3
F-value	924.75	628.75
***P*-value**	0.0000^{***}	0.0000^{***}

*Means followed by the same small letter in a column are not significantly different at the 5% probability level (Duncan's Multiple Range Test) [42] b.w., body weight; DF: degree of freedom *** highly significant effect*

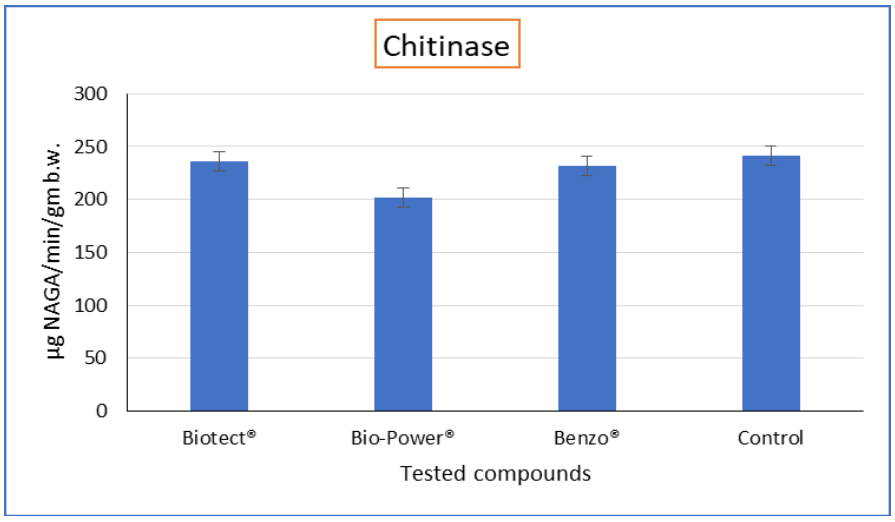

Fig. 5. Mean activity level of chitinase after treatment of the 2nd instar larvae of *S. littoralis* with LC_{50} of tested compounds

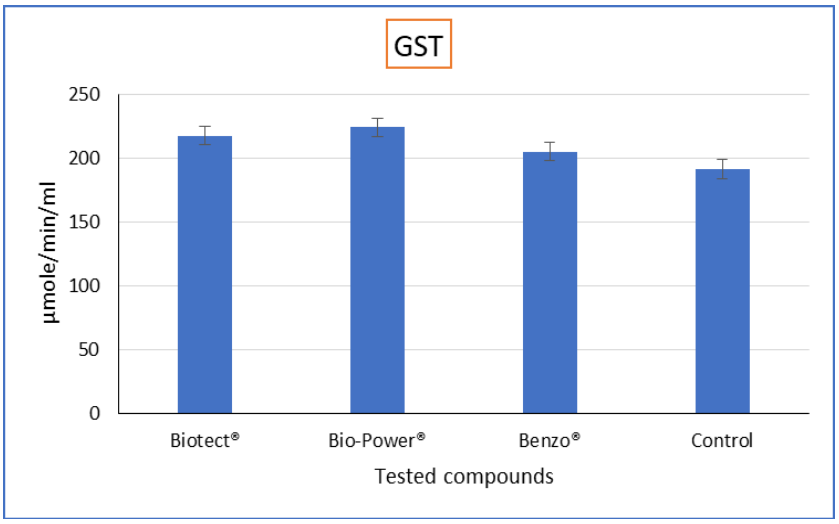

Fig. 6. Mean activity level of GST after treatment of the 2nd instar larvae of *S. littoralis* with LC_{50} of tested compounds

Effect of sublethal concentrations of tested compounds on chitinase and glutathione-S-transferase (GST) activities: Table 6 shows the latent effect of tested compounds on chitinase and GST activities in the 6th instar larvae that survived treatment as the 2nd instar larvae. Results revealed reduced chitinase activity due to treatment with the sublethal concentration of tested compounds. Results also showed that the lowest chitinase level was observed in the Bio-Power® treatment Fig. 5. Results showed that Bio-Power® demonstrated the highest GST activity, followed by Biotect® and Benzo® Fig. 6. Treatment with sublethal concentrations of tested compounds significantly lowered the chitinase activity compared to the control. In contrast, Bio-Power® showed the lowest chitinase activity, followed by Benzo® and Biotect®. Furthermore, the GST activity was significantly increased due to treatment with sublethal concentrations of the tested compounds compared to the control. An insect's exoskeleton may be a good insecticide target [60]. Chitinase participation in the peritrophic membrane's turnover could impact gut physiology during the ecdysis chitin process. Pesticide tolerance or resistance can develop due to immune response activation and induction of detoxifying enzymes such as glutathione-S-transferases in response to sublethal insecticide exposure [54]. Before they reach the target areas, these enzymes break down the deadly compounds in insects [61]. Due to its function in the degradation of hazardous and insecticide compounds, GST has acquired prominence. Eliminating metabolites, defense against free radical damage to tissues, and potential defense against pathogen and toxin exposure in insects are other functions of GST [62,63]. The overproduction caused by the treatment with the tested chemicals as a defense mechanism against those compounds may cause elevated GST [64,65].

4. CONCLUSION

The employment of bioinsecticides against the younger larval instar of *S. littoralis* can represent excellent substitutes for conventional insecticides. This group of biobased insecticides has a unique mode and site of action and a latent effect on insects' biological and physiological aspects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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