Research Article

Controlling of Cotton Leaf Worm in *Solanum nigrum* L. at District Swat (Switzerland of Pakistan) Under Semi-Field Conditions

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Abstract | *Solanum nigrum* L., commonly known as Makoi or Black Nightshade, is a widely cultivated fruit vegetable crop valued for its high nutritional content. However, its production is significantly threatened by the cotton leafworm (*Spodoptera littoralis*). This highly destructive pest causes severe economic losses and compromises crop quality due to its aggressive feeding behavior. Biopesticides have recently gained attention as sustainable alternatives to conventional insecticides for managing *S. littoralis* infestations. This study evaluated the efficacy of three commercial bioinsecticides applied at their recommended concentrations against 2nd instar larvae of S. littoralis under semi-field conditions over two growing seasons. Larval mortality was assessed three days post-treatment, and all tested bioinsecticides demonstrated significant effectiveness. Among them, emamectin benzoate consistently exhibited the highest mortality rate across both seasons. Additionally, the tested bioinsecticides remained effective for up to 10 days after application. Treatment with LC50 concentrations of these bioinsecticides resulted in significant alterations in soluble protein, carbohydrate, and lipid levels, as well as the activity of specific carbohydrate-hydrolyzing enzymes. These findings highlight the potential of bioinsecticides as effective and eco-friendly alternatives to synthetic pesticides, offering both targeted pathogenic effects on insect pests and safety for non-target organisms.

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Introduction

Solanum nigrum L., a vital fruit vegetable crop of the Solanaceae family, is cultivated worldwide for its pleasant flavor, nutritional value, and diverse coloration. The global cultivation of bell peppers and related crops has significantly increased over the years (Hassan and Ibrahim, 2021). Like many food crops and vegetables, *S. nigrum* is vulnerable to insect pests throughout its growth stages, with some causing severe damage through direct contact with the soil (Yadav and Patel, 2017). Among these, the cotton leafworm, *Spodoptera littoralis* (Boisduval) (*Lepidoptera: Noctuidae*), is one of the most destructive pests. It attacks various plant structures, reducing crop quality and leading to significant economic losses (Archives and Blog, 2024).

To combat *S. littoralis*, various pest management strategies have been employed, including decomposition methods and invasive insecticides (Ullah *et al*., 2025). Historically, chemical control has been the primary approach, involving synthetic pyrethroids, organophosphates, insect growth regulators (IGRs), and other non-conventional insecticides (Ammar *et al*., 2025). In Pakistan, *S. littoralis* has been managed using methyl-parathion and other chemical insecticides to control endemic pests (Asif *et al*., 2025). However, the widespread development of resistance and cross-resistance, along with concerns about pesticide residues, environmental impact, and pest resurgence, has significantly limited the long-term effectiveness of synthetic insecticide products (Ghoneim and Hamadah, 2017).

Biopesticides have recently gained increasing attention as environmentally friendly alternatives to chemical insecticides (Khan *et al*., 2024). These biopesticides, mainly those derived from entomopathogenic microorganisms, exhibit unique modes of action that distinguish them from conventional insecticides (Nawaz *et al*., 2022). One of the most widely used biological control agents is the bacterium *Bacillus thuringiensis* (Berliner), a Gram-positive bacterium known for its insecticidal activity against a wide range of pests. *B. thuringiensis* produces δ-endotoxins, which cause insect mortality by disrupting gut epithelial cells (Mishra and Sasmal, 2020). Notably, *B. thuringiensis* is ideal for its high specificity, environmental safety, and nominal impact on non-target organisms (Thiruvenkataswamy *et al*., 2000).

Alongside bacterial biopesticides, entomopathogenic fungi are also promising biological control agents (Ullah *et al*., 2024). These fungi infect insects through direct contact, producing insecticidal compounds that make them a leading alternative for sustainable pest management (Mutamiswa *et al*., 2017). Among these, *Beauveria bassiana* is widely spread and commonly isolated from insect cadavers and soil using selective artificial media or insect baiting techniques (Abd El-Latef *et al*., 2023). Infection with *B. bassiana* usually requires several days to induce insect mortality, but its conidia can spread to other insects via physical contact, enhancing its effectiveness in pest control (Chaudhry and Guitchounts, 2003). Additionally, conidia on insect corpses demonstrate increased tolerance to solar radiation, enabling them to persist under field conditions (Sabbour, 2007). Emamectin benzoate is a second-generation ivermectin analog that is highly effective against lepidopteran pests (Umesh *et al*., 2022). It functions as a chloride channel activator, disrupting neuronal excitability in insects. Within 3–4 days of exposure, larvae experience feeding cessation, paralysis, and eventual death (Zhang *et al*., 2020).

This study evaluates the lethal and sublethal effects of three bioinsecticides against 2nd instar larvae of *Spodoptera littoralis* under semi-field conditions over two consecutive growing seasons. Furthermore, we investigate the biochemical effects of these bioinsecticides on soluble biomolecules, including proteins, carbohydrates, and lipids, and their impact on treated larvae.

The findings aim to contribute to developing sustainable pest control strategies, reducing reliance on chemical pesticides while ensuring effective crop protection and environmental safety.

Materials and Methods

Insect colony and rearing technique

Fresh egg batches of *Spodoptera littoralis* were collected from field conditions and brought to the Cotton Leafworm Research Laboratory at Govt Post Graduate College No. 1, Swat District, Khyber Pakhtunkhwa, Pakistan. The eggs were incubated under laboratory conditions of 27±2°C and 65±5% relative humidity (RH) in plastic cups covered with a net (Ullah *et al*., 2023a). Newly hatched 2nd instar larvae were used in all experiments. Freshly hatched 2nd instar larvae were used in the experiments. Larvae were fed regularly with fresh leaves as needed (Nampeera *et al*., 2019).

Tested compounds

Three commercial bioinsecticides were tested against 2nd instar larvae of *S. littoralis*:

- Biotect® (WP 9.4%) – A *Bacillus thuringiensis var. kurstaki*-based bioinsecticide, applied at the recommended rate of 300 g/feddan (obtained from Organic BIO Technology Company).
- Bio-Power® (WP 1.15%) – A *Beauveria bassiana*-based bioinsecticide, applied at the recommended rate of 1.5 kg/feddan (supplied by Gaara

Establishment Import and Export).

- Benzo® (WG 5.7%) – An Emamectin benzoate formulation, applied at the recommended rate of 60 g/feddan under the trade name Benzi®.

Semi-field experiment design

A semi-field experiment was conducted over two consecutive bell pepper growing seasons (2020 and 2021) to evaluate the efficacy of the tested bioinsecticides against *S. littoralis* 2nd instar larvae. The study was carried out at Satolay village (30°27'21.7"N, 31°06'39.3"E), Hazray, and Kotkay villages (Ullah and Shakir, 2023). The Omega *S. nigrum* variety was cultivated on April 20, 2020, and April 20, 2021, following standard agricultural practices (Goda *et al*., 2015).

Pesticide application

The bioinsecticides were applied using a motorized backpack sprayer (4 kW motorized spray gun) at the recommended concentrations, ensuring full leaf coverage in a 1/100 feddan (42 m^2) plot per treatment. The applications were performed on July 2, 2020, and July 22, 2021.

Sample collection and laboratory bioassay

Once the sprayed plant leaves were completely dry, random leaf samples were collected from treated and untreated (control) plants and placed in perforated single-use paper bags for transportation (Abdel-Baky *et al*., 2021; Ullah *et al*., 2023b).

In the laboratory, sterilized 1-liter glass jars were prepared, each containing 25 newly hatched 2nd instar larvae with four replications per treatment (total: 100 larvae per treatment). The larvae were fed daily with treated leaves until day 10 post-treatment (Moeini-Naghade, 2020).

Larval mortality assessment

Larval mortality was recorded on days 2, 3, 5, 7, and 10 post-treatments to assess the effectiveness of the tested bioinsecticides against *S. littoralis*. Mortality rates were calculated using Schneider-Orelli's formula (Aparna *et al*., 2024).

Toxicological and biochemical analysis

Determination of median lethal concentration (LC_{50}) values: The LC_{50} values of the bioinsecticides were determined using the leaf-dipping method (Pezhman and Saeidi, 2018).

- Castor bean leaves were washed, air-dried, and then dipped for 10 seconds in one of six different concentrations of each bioinsecticide.
- The treated leaves were allowed to air dry at room temperature before being provided to groups of 25 second-instar larvae housed in clean, sterilized jars.
- Each treatment-concentration combination was tested with three independent replicates to ensure statistical reliability.
- Leaves dipped in distilled water served as the control group.
- Larval mortality was assessed to determine the LC_{50} values for each compound.

Insect specimen processing and enzyme extraction

Insect specimens were processed according to the method of Amin (Hejran *et al*., 2024; Ullah *et al*., 2019). Second-instar larvae of *S. littoralis* were exposed to LC_{50} concentrations of the tested bioinsecticides for 48 hours. After treatment, one gram of surviving 6^{th}-instar larvae was:

- Weighed and homogenized in distilled water at a ratio of 50 mg/mL.
- Centrifuged at 8000 rpm for 15 minutes at 4°C using a cooling centrifuge.
- The supernatant (enzyme extract) was collected and remained stable at 50°C for up to one week without significant loss of enzymatic activity.

Biochemical analysis

Determination of total proteins, carbohydrates, and lipids: The effects of LC_{50} concentrations on the total protein, carbohydrate, and lipid content of *S. littoralis* larvae were determined using the following standard methods:

- Total protein content– Bradford method (Garvey *et al*., 2020; Ullah *et al*., 2019b).
- Total carbohydrate content – (Kumar *et al*., 2021; Ullah *et al*., 2023c) protocol.
- Total lipid content– (Kumar *et al*., 2021) method.

Enzyme activity assay

The enzymatic activity of key metabolic enzymes was assessed using standard protocols:

- Invertase, amylase, and trehalase – (Han *et al*., 2019; Subhan et al., 2024).
- Chitinase – (Han *et al*., 2019).
- Glutathione S-transferase (GST) – (Han *et al*., 2019; Sajid *et al*., 2023) protocol.

Statistical analysis

Statistical analysis was conducted using SPSS 22.0 (Statistical Package for Social Sciences, USA, version 22.0.0). One-way analysis of variance (ANOVA) was performed separately for each experiment to assess toxicological and biochemical variations. To ensure statistical reliability, four replicates were included in each analysis. Data were expressed as mean ± standard deviation (SD), and Duncan's Multiple Range Test ($P \leq 0.05$) was employed to identify significant differences among treatments (Habig, 1974). Furthermore, LC_{50} values were determined using the "LdPLine®" program, which applies regression line analysis as described by Finney (1971) for accurate estimation of lethal concentrations.

Results and Discussion

Effectiveness of tested compounds against Spodoptera littoralis

The efficacy of the tested bioinsecticides against 2nd instar larvae of *Spodoptera littoralis* during the 2020 and 2021 bell pepper growing seasons is presented in Tables 1 and 2. Larval mortality was recorded on the 3rd day post-treatment, with all tested compounds exhibiting insecticidal activity. The mortality rate increased gradually from the 5th day post-treatment until the 10th day, demonstrating prolonged virulence.

Table 1: *The larval corrected mortality percentage of 2nd instar larvae of Spodoptera littoralis under semi-field experimentation in the field at District Swat.*

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	0	0	0

Table 2: *The larval corrected mortality percentage of 2nd instar larvae of Spodoptera littoralis under semi-field experimentation during the at District Swat.*

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	0	0	0

Among the tested bioinsecticides, Biotect® and Benzo® exhibited higher toxicity compared to Bio-Power®, indicating their greater effectiveness in controlling *S. littoralis* larvae. These results are consistent with the findings of (Immanuel and Iswareya, 2023), who reported a progressive increase in mortality of 2nd instar *S. littoralis* larvae when exposed to *Bacillus thuringiensis var. kurstaki* under semi-field conditions. Furthermore, the present findings align with previous studies (Silva *et al*., 2021), which demonstrated the efficacy of bioinsecticides in controlling younger larval instars of *S. littoralis*.

Determination of LC_{50} values of tested compounds

The LC_{50} values of the tested bioinsecticides against 2nd instar larvae of *Spodoptera littoralis* under laboratory conditions are presented in Table 3. The results indicate that Bio-Power® had the highest LC_{50} value (1.156 g/mL), followed by Biotect® (0.1238 g/mL), while Benzo® exhibited the lowest LC_{50} value (0.0084 g/mL). The lower LC_{50} value of Benzo® signifies its greater toxicity, requiring a smaller concentration to achieve 50% mortality. This suggests that Benzo® is the most effective bioinsecticide among the tested compounds, making it a promising candidate for efficient and targeted pest management of *S. littoralis* larvae (Muthu *et al*., 2014).

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

Biochemical impacts of tested compounds

Effect of sublethal concentrations of tested compounds on total proteins, total carbohydrates, and total lipids: The effects of sublethal concentrations (LC_{50}) of the tested bioinsecticides on total protein, carbohydrate, and lipid levels in 6th instar larvae that had survived treatment as 2nd instar larvae are presented in Table 4. The results indicated a significant reduction in total proteins, carbohydrates, and lipids in the treated larvae, with Biotect® exhibiting the most pronounced effect.

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.*

The observed depletion in macromolecules may be attributed to the insect's physiological response to pesticide-induced stress, as alterations in energy reserves, including carbohydrates, lipids, proteins, and glycogen, are often associated with insect susceptibility and metabolic adjustments following pesticide exposure (Shahid *et al*., 2022).

Protein depletion and metabolic adjustments

Proteins are essential building blocks influencing body size, growth rate, and reproductive success, playing a crucial role in life cycles, population dynamics, and biodiversity (Ngosong *et al*., 2018). The reduction in protein content observed in this study could be attributed to protein catabolism, where proteins are broken down into free amino acids, which subsequently enter the tricarboxylic acid (TCA) cycle as keto acids to supply alternative energy sources during stress (Arakere *et al*., 2022). This physiological compensation mechanism suggests that insects may mobilize protein reserves under bioinsecticidal stress to maintain energy homeostasis by increasing free amino acid content in the hemolymph (Shakir *et al*., 2023).

Carbohydrate reduction and energy metabolism

Carbohydrates serve as a primary energy source for insects and play a key role in amino acid synthesis and lipid metabolism. Additionally, various carbohydrates, particularly sugars, function as appetite stimulants (Agbessenou *et al*., 2020). The observed decline in carbohydrate levels in treated larvae could be linked to enhanced metabolism under toxicant stress, which may accelerate glycogenolysis and glycolysis, leading to increased energy production (Biondi *et al*., 2018). This heightened energy demand under pesticide exposure suggests that stored glycogen is rapidly converted into glucose to compensate for metabolic stress.

Lipid reduction and detoxification mechanisms

Lipids are composed of fatty acids, phospholipids, sterols, and other biomolecules, playing a vital role in cell membrane structure, energy storage, and metabolic processes (Girdhar *et al*., 2014). Insects can convert carbohydrates into lipids, storing them as body fat when necessary (Sher *et al*., 2018). However, the significant decline in lipid levels observed in this study suggests that lipid metabolism was actively utilized in detoxification, as energy demands increased following pesticide exposure. This aligns with previous research indicating that pesticide-induced stress requires substantial energy, leading to lipid depletion in larvae (Sabbur, 2003).

Enzyme activity suppression and metabolic disruptions

The findings also revealed a notable reduction in the activity of carbohydrate-hydrolyzing enzymes, including amylase, invertase, and trehalase, in late 6th instar larvae following LC_{50} treatment during the 2nd instar stage. These enzymes play a critical role in the breakdown of carbohydrates for energy production. The suppression of these enzymatic activities suggests: A lower metabolic rate, Reduced phosphorus release for energy metabolism, and Decreased metabolite transport, ultimately disrupting nutrient utilization (Bhardwaj *et al*., 2011).

Effect of sublethal concentrations of tested compounds on amylase, invertase, and trehalase activities

The latent effects of the LC_{50} concentrations of the tested bioinsecticides on the activity of carbohydrate-hydrolyzing enzymes are presented in Table 5. The results demonstrated a significant reduction in amylase and invertase activity in treated larvae compared to the control. However, the impact on trehalase activity varied depending on the bioinsecticide used. While Bio-Power® and Benzo® caused an insignificant decrease in trehalase activity, treatment with Biotect® resulted in a significant reduction compared to the control.

The inhibition of carbohydrate-hydrolyzing enzymes suggests a potential disruption in nutrient assimilation and energy metabolism, possibly contributing to the observed larval mortality. Carbohydrate-metabolizing enzymes, such as amylase and invertase, play a crucial role in breaking down complex sugars into simpler forms that are essential for energy production and development (Khan *et al*., 2018). Suppressing these enzymes may lead to nutritional deficiencies, impaired digestion, and reduced energy availability, ultimately weakening the larvae and increasing their susceptibility to bioinsecticide effects.

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®	201.6 ± 0.7[c]	563.0 ± 2.1[d]	373.6 ± 2.7[b]
Bio-Power®	187.0 ± 1.2[d]	552.6 ± 2.2[c]	408.0 ± 0.6[a]
Benzo®	205.0 ± 0.6[b]	573.0 ± 3.0[b]	404.0 ± 2.9[a]
Control	212.6 ± 0.9[a]	642.6 ± 1.4[a]	407.6 ± 2.6[a]
Df	3	3	3
F-value	150.0	4821.0	446.0
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.*

Effect of sublethal concentrations of tested compounds on chitinase and glutathione-S-transferase (GST) activities

The latent effects of sublethal concentrations (LC_{50}) of the tested bioinsecticides on chitinase and glutathione S-transferase (GST) activities in 6th instar larvae that had survived treatment as 2nd instar larvae are presented in Table 6. The results indicated that Bio-Power® exhibited the highest GST activity, followed by Biotect® and Benzo®. Conversely, chitinase activity was significantly reduced in all treated larvae compared to the control, with Bio-Power® causing the most pronounced reduction, followed by Benzo® and Biotect®.

The observed increase in GST activity in larvae treated with sublethal concentrations of the tested compounds suggests an induced detoxification response. GST enzymes play a crucial role in metabolizing and neutralizing xenobiotic compounds, including pesticides (Saha *et al*., 2020). The overexpression of GST, as observed in this study, may be attributed to an insect defense mechanism aimed at detoxifying the bioinsecticidal compounds before they reach their target sites (Baskar *et al*., 2015). Additionally, GST plays a role in eliminating toxic metabolites, protecting tissues from free radical damage, and providing defense against pathogen and toxin exposure (Jamal *et al*., 2013). The elevated GST activity observed here aligns with previous findings that insects exposed to sublethal insecticide concentrations exhibit increased expression of detoxification enzymes as a protective adaptation (Nemati, 2025).

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®	246.3 ± 3.2[ab]	218.3 ± 2.2[a]
Bio-Power®	201.0 ± 1.5[c]	226.0 ± 3.1[a]
Benzo®	241.6 ± 1.2[b]	204.0 ± 2.5[b]
Control	251.3 ± 1.9[a]	192.3 ± 1.8[c]
Df	3	3
F-value	934.75	618.75
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) (Hayes et al., 2005) b.w., body weight; DF: degree of freedom *** Highly significant effect*

Conclusion

In conclusion, bioinsecticides represent a viable and sustainable alternative to conventional chemical insecticides for controlling early instar larvae of *Spodoptera littoralis*. Their unique modes of action, along with their ability to disrupt key biological and physiological processes, contribute to their long-term effectiveness. By reducing the risks of pesticide resistance, non-target toxicity, and environmental contamination, bioinsecticides offer an eco-friendly approach to pest management, supporting agricultural sustainability and environmental conservation.

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Novelty Statement

This study provides novel insights into the efficacy and biochemical impacts of bioinsecticides on *Spodoptera littoralis* larvae, demonstrating their potential as sustainable alternatives to chemical pesticides. The findings reveal that Benzo® exhibits the highest toxicity, requiring the lowest concentration to achieve 50% mortality, while all tested bioinsecticides induce significant metabolic disruptions, including reduced protein, carbohydrate, and lipid levels. Additionally, the study highlights the enzymatic alterations caused by bioinsecticidal stress, particularly in chitinase and glutathione-S-transferase activities, shedding light on the physiological adaptations of *S. littoralis* to bioinsecticidal exposure. These results contribute to the growing body of knowledge supporting bioinsecticides as effective tools for integrated pest management.

Author's Contribution

Shakir Ullah: Conceptualization, formal analysis, and writing the original draft.
Lubna Shakir: Data curation and methodology.
Mohammad Sohail: Analysis and investigation.
Azmat Noreen: Software and writing review and editing.
Laila Aziz: Writing review and editing.

Conflict of interest
The authors have declared no conflict of interest.

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