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Cuticular Breakdown: Histopathological Manifestations of Beauveria Bassiana Infection in *Spodoptera littoralis* Larvae

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Abstract: **Objective:** The overreliance on chemical insecticides for controlling the devastating cotton leafworm, *Spodoptera littoralis*, has led to resistance and environmental concerns. This study investigated the biocontrol potential of the entomopathogenic fungus *Beauveria bassiana* by determining its median lethal concentration (LC_{50}) against fourth instar larvae and characterizing the ensuing histopathological damage to the larval cuticle, the primary barrier to infection. **Methods:** The pathogenicity of an indigenous *B. bassiana* isolate was assessed through a leaf-dipping bioassay using five conidial concentrations (1×10^5 to 1×10^9 conidia/mL). The LC_{50} was calculated via probit analysis. For histopathological examination, larvae treated with the LC_{50} (2.81×10^6 conidia/mL) were dissected at 72 hours post-treatment. Cuticle samples were processed, embedded in paraffin, sectioned, and stained with Hematoxylin and Eosin (H&E) and Periodic Acid-Schiff (PAS) for detailed microscopic analysis. **Results:** Bioassay results revealed a significant dose-dependent mortality, with the highest concentration (1×10^9 conidia/mL) causing 85.3% mortality. The calculated LC_{50} was 2.81×10^6 conidia/mL. Histopathological analysis demonstrated severe structural degradation in the cuticle of treated larvae. Key findings included the disintegration of the epicuticle and the lamellar structure of the procuticle, extensive invasion and proliferation of fungal hyphae and blastospores within the cuticular matrix, and the formation of lytic cavities. Fungal penetration reached the epidermal layer, which displayed significant disruption and vacuolization, confirming a complete breach of the host's primary physical defense. **Conclusion:** *Beauveria bassiana* proves to be a highly virulent pathogen against *S. littoralis* larvae. The histopathological evidence provides a mechanistic understanding of its efficacy, directly linking larval mortality to comprehensive cuticular disintegration and systemic fungal invasion. These findings strongly support the integration of *B. bassiana* as a sustainable and effective biocontrol agent within IPM programs to manage this economically important pest.

Key words: *Spodoptera littoralis*; *Beauveria bassiana*; Biocontrol; Histopathology; Entomopathogenic Fungus.

1. Introduction

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), is a highly destructive polyphagous pest, inflicting significant economic losses on awide range of important crops such as cotton, corn, and vegetables in Egypt and other regions **(Abdou *et al*., 2022 and Khadim and Alhilif, 2023)**. The control of this pest has historically relied on synthetic chemical insecticides. However, the overuse of these chemicals has led to the development of insecticide resistance in *S. littoralis* populations, alongside serious concerns regarding environmental contamination, human health risks, and harm to nontarget organisms **(Abdou *et al*., 2022)**. Consequently, there is anurgent need to develop effective and environmentally safe alternative control strategies.

Among the most promising alternatives are entomopathogenic fungi, particularly *Beauveria bassiana*, which act as biological control agents. These fungi are inherently pathogenic to a broad spectrum of insect pests and are considered a key component of integrated pest management programs due to their target specificity and low environmental impact **(Raja and Vinolia, 2021)**. The infection process of *B. bassiana* begins when conidia adhere to the insect's cuticle, germinate, and directly penetrate the host integument using a combination of mechanical pressure and cuticle-degrading **enzymes (Baek *et al*., 2022 and Raja and Vinolia, 2021)**. Following cuticular penetration, the fungus invades the hemocoel, proliferates, and ultimately causes host mortality.

It is important in understanding the efficacy and mode of action of *B. bassiana* to conduct a thorough investigation of the histopathological alterations it induces in the host cuticle, which serves as the primary barrier to infection. Detailed histological studies can reveal the timeline and mechanism of fungal penetration and the ensuing structural disintegration of the cuticle. Therefore, this study aims to determine the median lethal concentration (LC_{50}) of *B. bassiana* against the fourth instar larvae of *S. littoralis* and to characterize the histopathological changes in the larval cuticle following treatment with this concentration.

2. Materials and Methods

2.1. Insect Rearing

A laboratory colony of the cotton leafworm, *Spodoptera littoralis* (Boisduval), was maintained under controlled conditions of 27 ± 2°C, a photoperiod of 14:10 (L:D) hours, and 65 ± 5% relative humidity. Larvae were reared collectively on fresh castoroil leaves (*Ricinus communis*), which were replaced daily. Adults were provided with a 10% sucrose solution for nourishment **(El-Sawaf, 1971)**. The newly molted fourth instar larvae, selected based on head capsule width and uniform size, were used for all bioassays and histopathological investigations.

2.2. Fungal Inoculum Preparation

The study utilized an indigenous isolate of the entomopathogenic fungus, *Beauveria bassiana*, which was isolated in the biopesticide production unit and formally accessioned in the gene bank under the identification number PQ550640". A stock suspension was prepared by suspending the fungal spores in an aqueous solution of 0.05% Tween 80 to facilitate even dispersion of hydrophobic conidia **(Abd El-Kareem and Ibrahim)**. The concentration of viable conidia was determined using an improved Neubauer hemocytometer and confirmed by plating serial dilutions on Potato Dextrose Agar (PDA) to assess colony-forming units (CFUs). A series of five spore concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8, and 1×10^9 conidia/mL) was prepared from the stock for the bioassay.

2.3. Bioassay and LC_{50} Determination

The pathogenicity of *B. bassiana* was evaluated using a leaf-dipping bioassay method. Discs of castor leaves (4 cm diameter) were dipped in the respective conidial suspensions for 30 seconds, with control leaves dipped in 0.05% Tween-80 solution only **(Abdou *et al*., 2022)**. After air-drying, the

treated leaf discs were placed in plastic containers (14 x 8 cm). Groups of twenty 4th instar larvae were introduced into each container, with each concentration replicated five times. Larval mortality was recorded daily for 7 days post-treatment. Larvae were considered dead if they showed no movement upon prodding with a fine brush or displayed visible mycosis. Mortality data were corrected using Abbott's formula **(Abbott, 1925)**. The median lethal concentration (LC_{50}) and its 95% fiducial limits were calculated using probit analysis **(Finney, 1971)**.

2.4. Histopathological Examination

Fourth instar larvae were treated with the established LC_{50} concentration of *B. bassiana* using the leaf-dipping method described above. Control larvae were fed on untreated leaves. At 72 hours post-treatment, cuticle samples from both treated and control moribund larvae were dissected. The tissues were immediately fixed in Formalin Acetic Alcohol (FAA) for 48 hours **(Raja and Vinolia, 2021)**. Following fixation, tissues were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin wax. Serial sections of 5–7 µm thickness were cut using a rotary microtome. The sections were mounted on glass slides and stained with Hematoxylin and Eosin (H&E) for general tissue morphology and with Periodic Acid-Schiff (PAS) to visualize fungal structures **(Raja and Vinolia, 2021 and Baek *et al*., 2022)**. The stained sections were examined and photographed under a light microscope for detailed histopathological analysis.

2.5. Statistical Analysis

Mortality data from the bioassay were subjected to a one-way analysis of variance (ANOVA). Means were separated using the Least Significant Difference (LSD) test at a 0.05 probability level. All statistical analyses were performed using SPSS software (Version 19).

3. Results

3.1. Bioassay and Virulence of *Beauveria bassiana*

The bioassay results demonstrated a clear dose-dependent relationship between the concentration of *Beauveria bassiana* conidia and the mortality of *Spodoptera littoralis* fourth instar larvae. Mortality rates increased progressively with increasing conidial concentration over the 7-day observation period (Table 1). The highest concentration of 1×10^9 conidia/mL resulted in a final corrected mortality of 85.3%, while the lowest concentration of 1×10^5 conidia/mL caused 30.3% mortality. Statistical analysis confirmed that the effect of concentration was highly significant (F-value = 14.36; P-value = 0.0052). Probit analysis of the concentration-mortality data estimated the median lethal concentration (LC_{50}) to be 2.81×10^6 conidia/mL after 7 days of treatment.

Table (1). Mean percent mortality (± Standard Error) of *Spodoptera littoralis* 4th instar larvae after treatment with different concentrations of *Beauveria bassiana*

Concentration (conidia/mL)	Mortality (%) ± S.E.
1×10^5	30.3 ± 0.3
1×10^6	38.6 ± 0.7
1×10^7	43.3 ± 0.3
1×10^8	70.6 ± 0.9
1×10^9	85.3 ± 0.9
LC_{50} (95% FL)	**2.81×10^6**

3.2. Histopathological Alterations in the Larval Cuticle

Microscopic examination of histological sections revealed severe structural degradation inthe cuticle of larvae treated with the LC_{50} of *B. bassiana*, in stark contrast to the intact architecture observed in control larvae.

3.2.1. Control Larvae

The integument of untreated *S. littoralis* larvae exhibited a normal, well-organized, and multi-layered structure (Figure 1A, 1B). The epicuticle (Ep) appeared as a thin, continuous, and electron-dense outer layer. Beneath it, the procuticle, comprising the exocuticle (Ex) and the thicker endocuticle (En), displayed a uniform, tightly packed, and lamellar arrangement of chitin-protein fibers. The epidermal cell layer (Ed) underlying the cuticle was intact and mononucleated. No fungal elements or signs of degradation were observed.

3.2.2. Treated Larvae

In larvae treated with the LC_{50} of *B. bassiana*, the cuticle underwent profound histopathological changes (Figure 2A, 2B). The most salient feature was the extensive invasion by fungal elements. Germinated conidia were observed on the cuticle surface, giving rise to penetration pegs and hyphae (Hy) that breached the epicuticle.

The structural integrity of the procuticle was severely compromised. The well-defined lamellar structure of the endocuticle and exocuticle was disintegrated, appearing fragmented and amorphous. Dense networks of fungal hyphae (Hy) and blastospores (FS) were visible, proliferating within the cuticular matrix, leading to widespread lysis and the formation of cavities.

The invasion was not superficial; hyphae penetrated the entire cuticle, reaching the epidermal layer (Ed), which showed signs of disruption and vacuolization. In advanced stages of infection, hyphae were observed emerging from the inner surface of the cuticle into the hemocoel. The Periodic Acid-Schiff (PAS) stain confirmed the presence of fungal cell walls throughout the degraded cuticular tissues, highlighting the extensive colonization.

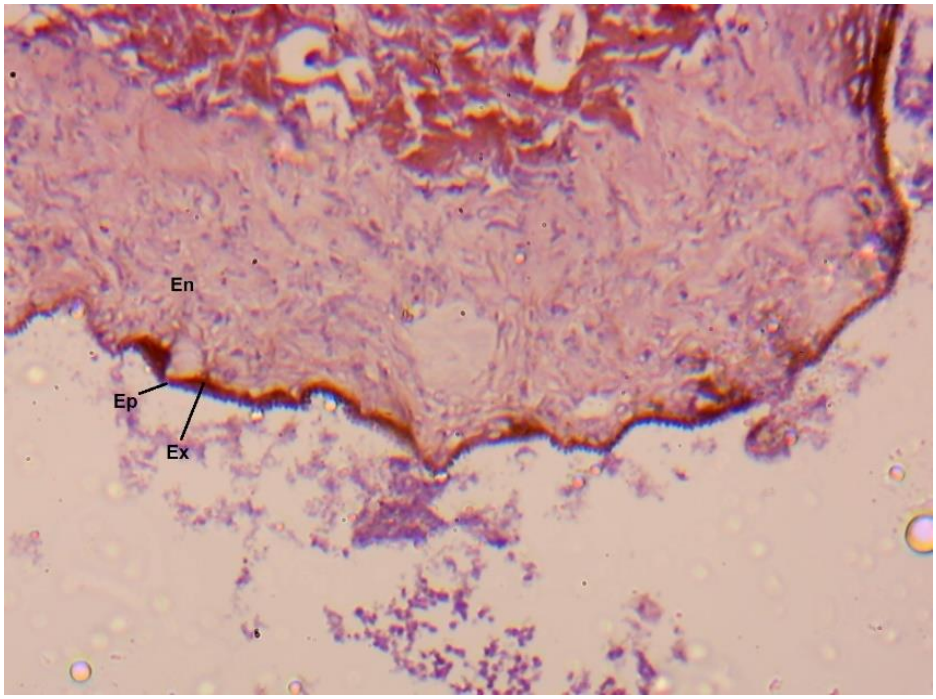

Figure (1). Photomicrograph of the integument from an untreated *S. littoralis* larva showing the normal, layered structure of the epicuticle (Ep), exocuticle (Ex), and endocuticle (En). The epidermal layer (Ed) is intact. (Stain: H&E)

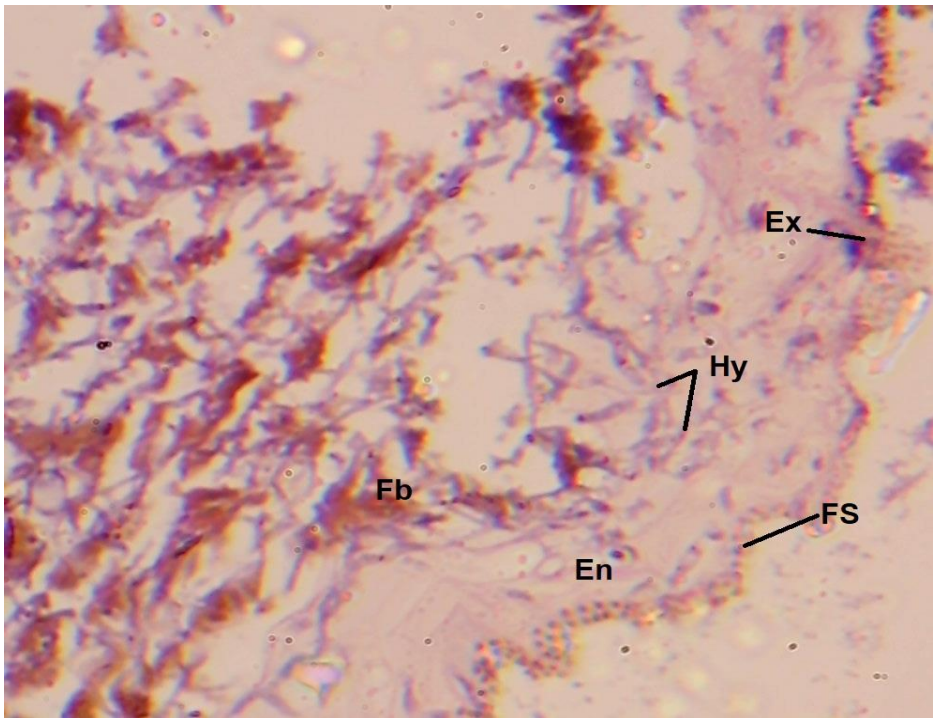

Figure (2). (A) Photomicrograph of the integument from a larva treated with the LC_{50} of *B. bassiana*, showing severe degradation. Note the extensive fungal hyphae (Hy) within the cuticle and the disintegration of the procuticular layers (Ex, En). The epicuticle (Ep) is disrupted. (Stain: PAS & H&E)

Discussion

The results of this study demonstrate the significant pathogenic potential of *Beauveria bassiana* against the fourth instar larvae of *Spodoptera littoralis*. The observed dose-dependent mortality and the calculated LC_{50} of 2.81×10^6 conidia/mL are consistent with previous research, confirming the virulence of this entomopathogenic fungus against lepidopteran pests **(Vivekanandhan *et al*., 2022 and Abou-Elkassem *et al*., 2024)**. The histopathological findings provide a clear cytological explanation for this mortality, revealing a sequence of cuticular degradation and fungal proliferation.

The severe disintegration of the cuticular layers observed in treated larvae is a direct consequence of the fungal infection process. The penetration of the integument by *B. bassiana* is a critical first step, achieved through a combination of mechanical pressureand enzymatic degradation **(Raja and Vinolia, 2021)**. Our observations of germ tubes and penetration pegs breaching the epicuticle and procuticle align with the established mode of action, where fungal enzymes suchas proteases and chitinases break down the protein-chitin matrix of the cuticle **(Vivekanandhan *et al.*, 2022)**. The extensive lysis and fragmentation of the procuticle, leading to a loss of structural integrity, directly compromise the insect's primary defense barrier. Similar histopathological disruptions in the cuticle have been documented in other insects infected with *B. bassiana*, including *Graptostethus servus* and *Nezara viridula* **(Nada *et al*., 2022 and Soliman *et al*., 2022)**.

Furthermore, the extensive colonization of the cuticular matrix and the subsequent invasion of the epidermal layer and hemocoel by fungal hyphae and blastospores indicate a successful evasion of the host's cellular immune responses. The transformation of invasive hyphae into hyphal bodies within the hemolymph, as reported in *Spodoptera litura* **(Raja and Vinolia, 2021)**, facilitates rapid proliferation and systemic infection, ultimately leading to septicemia and host death. The histopathological damage observed in this study is not limited to the cuticle; similar profound malformations have been reported in the midgut tissues of insects treated with microbial agents, including disruption of epithelial cells and

the peritrophic membrane **(Saleh *et al*., 2021 and Aziz *et al*., 2024)**. This internal tissue damage likely contributes to mortality through nutrient malabsorption and physiological failure.

The efficacy of *B. bassiana* demonstrated here, coupled with its minimal non-target impact as shown in studies on earthworms **(Vivekanandhan *et al*., 2025)**, underscores its value as a biocontrol agent. While conventional insecticides like abamectin may initially cause higher larval mortality **(Abou-Elkassem *et al*., 2024)**, the development of resistance and environmental hazards associated with their use necessitate sustainable alternatives. The ability of *B. bassiana* to cause significant mortality and severe histopathological damage positions it as a cornerstone for integrated pest management programs aimed at controlling *S. littoralis*, offering an effective and eco-friendly strategy to mitigate crop losses.

5. Conclusion

This study conclusively demonstrates the high efficacy of the entomopathogenic fungus *Beauveria bassiana* as a potent biocontrol agent against the fourth instar larvae of *Spodoptera littoralis*. The toxicological bioassay established a clear dose-dependent relationship, with a median lethal concentration (LC_{50}) of 2.81×10^6 conidia/mL, confirming the virulence of the fungus. More significantly, the histopathological analysis provided a mechanistic understanding of this pathogenicity, revealing severe cuticular degradation characterized by disintegration of the epicuticle and procuticle, and extensive internal colonization by fungal hyphae and spores. The breach of this primary physical barrier and subsequent invasion of the body cavity are the key factors leading to larval mortality.

These findings align with the mode of action of *B. bassiana* reported in other insect pests, underscoring its reliability. The profound structural damage to the integument, coupled with its proven lethality, positions *B. bassiana* as a highly viable and sustainable alternative to synthetic chemical insecticides for managing *S. littoralis*. Its use in Integrated Pest Management (IPM) programs can help mitigate the issues of insecticide resistance and environmental pollution, thereby contributing to more sustainable and ecologically responsible agricultural practices. Future research should focuson field evaluations and formulation development to enhance the persistence and efficacy of this promising biocontrol agent under real-world conditions.

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