Asian Journal of Research in Crop Science

Volume 9, Issue 4, Page 1-6, 2024; Article no.AJRCS.117476
ISSN: 2581-7167

Biological Management of *Bactrocera dorsalis* (Oriental fruit fly) through Enzymatic Activity of *Beauveria bassiana*

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Authors' contributions

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

Article Information

DOI: https://doi.org/10.9734/ajrcs/2024/v9i4293

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/117476

Original Research Article

Received: 28/03/2024
Accepted: 30/05/2024
Published: 12/09/2024

ABSTRACT

Beauveria bassiana is a viable option for the biocontrol of numerous significant pest insects. Fungal spores adhere to the cuticle after invading the host body. One of the groups of enzymes that entomopathogenic fungi have that guarantees good penetration is cuticle destroying enzyme. A recent study found that it is possible to extract the crude cuticle-degrading enzymes from *B. bassiana* and combine them with mycelium to make the organism more harmful for its host. With a pH of 8.6, the molecular weights of the samples (enzymes) were calculated in kDa for both the

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Cite as: *Sumbal, Ghulam Ahmad Khan, Hafiz Muhammad Umer, Muhammad Wali Khan Sumbal, and Sanaullah. 2024. "Biological Management of Bactrocera Dorsalis (Oriental Fruit Fly) through Enzymatic Activity of Beauveria Bassiana". Asian Journal of Research in Crop Science 9 (4):1-6. https://doi.org/10.9734/ajrcs/2024/v9i4293.*

resolving gel (12%), and the stacking gel (4%). The result showed that, unlike the traditional key, discrete bands with different sizes appeared after the gel was stained and distaining. The proteases, lipases, and chitinase were confirmed by the bands detected at 19, 50, 25, 32, and 34.25 kDa, respectively. The raw enzymes that were isolated were applied in quantities of 5, 10, 15, 20, and 25µL to larvae, pupae, and adults. At 25µL/mL, the death rates for larvae and adults were found to be 78.50±2.10% and 80±2.15%, respectively. The mortality was 13.33±1.92% at lower dosages (5µL/mL), with control coming in second. The treated insects showed a low proportion of adult emergence (10±2.63%) from pupae, while the untreated group of insects showed a greater percentage (65.0±5.77%) of adult emergence. The outcome demonstrated that concentration affected both adult emergence from pupae and mortality. Consequently, the addition of CDE to *B. bassiana* mycelium increased its pathogenicity against various phases of *Bactrocera dorsalis* life cycle.

Keywords: B. bassiana; B. dorsalis; characterization; CDE; SDS-PAGE.

1. INTRODUCTION

Beauveria bassiana is an entomopathogenic fungus (EPF) that is a member of the Hypocreales order. It is commonly known that *B. bassiana* works as a biopesticide to control a variety of agricultural insect pests [1]. A common biocontrol agent for a variety of insect pests is *B. bassiana*, sometimes known as Balsamo [2]. An enzymatic complex found in *B. bassiana* facilitates spore adhesion and penetration [3]. The insect cuticle is hydrolyzed by an enzyme complex, allowing the infection cycle to penetrate and advance [4,5]. Different cuticle polymers are related to different cuticle degrading enzymes (CDE) produced by the EPF. Certain fungi generate enzymes that convert the tissues of insects into nutrients for growth. Sclerotized insect cuticles are rarely used by fungi, but the EPF has created powerful enzymes to dissolve this barrier [6].

During the infection process, the synthesis of cuticle-degrading enzymes such as lipases, chitinases, and proteases is essential. Chitinase break down the chitin network, a crucial structural element of the walls' exoskeleton that permits penetration by lessening the cuticle's stiffness. Extracellular enzymes from *B. bassiana* are necessary for the breakdown of cuticles [7]. An extracellular enzyme called chitinase has occasionally been extracted and investigated. In fungus, chitinases are involved in hyphal growth and morphogenesis. These chemicals have been observed to be produced by EPF during host infection [8,9]. The objective of study was to extract *B. bassiana's* CDE and characterize them using sodium dodecyl sulphate polyacrylamide gel electrophoresis. The extracted CDE were assayed to manage the population dynamics of *Bactrocera dorsalis* under controlled conditions.

1.1 Objectives

To extract, characterize the CDE of *B. bassiana* for enhanced pathogenicity against *B. dorsalis.*

2. METHODOLOGY

2.1 Insect Culture

The populations of *B. dorsalis* were captured from fruit orchard in district Rawalpindi and Islamabad. The insect culture were maintained at Insect rearing laboratory in the department of Entomology, PMAS, Arid Agricultural University Rawalpindi. The standard diameter of pheromone traps were used to collect and attract the adults of *B. dorsalis* using attraction pheromones for both male and female.

2.2 Fungal Liquid Medium

The main culture of the fungal isolate was made using 100 ml of mycelium medium and 4 ml of conidial suspension (1.0×10^8 conidia/ml), according to the Adamek's (Quesada-Moraga et al., 2013).

Table 1. Ingredients used for enzymes extraction and assay

Sr. no.	Ingredients	Quantity (Grams) per 200 ml
01	NaCl	1.25g
02	Tris HCL	1.50g
03	Sodium phosphate	3.10g
04	Calcium chloride	2.45g
05	Magnesium sulfate	5.7g
06	Olive oil	6.00ml

2.3 Bioassays of Larval, Pupal, and Adult Stage of *B. dorsalis*

Concentrations of extracted enzymes were applied to *B. dorsalis* larvae, pupae and adults at 5, 10, 15, 20, and 25µL/mL. About 25 individuals of a similar age were exposed to enzymes using the immersion method (Ugwu and Nwaokolo, 2020).

2.4 Statistical Analysis

The ANOVA were used to find the mean value. MS excel were used for graphical representation of data. The data were examined using Minitab 8.1 (Beris, 2021).

3. RESULTS

3.1 Enzymatic Activity of *B. bassiana* against Larval Stage of *B. dorsalis*

B. bassiana CDE was found to be pathogenic against *B. dorsalis* larvae in their second instar. There was a significant mortality of larvae after one treatment day ($F_{5, 12} = 29.1$, $P = 0.0039$, $\alpha = 0.05$). The batch that received 5µL/mL cuticle-degrading enzyme treatment had the lowest mortality, at 13.33±1.92%; the group that did not receive any treatment had the lowest concentration-dependent mortality, at 31.67±1.92% at 25µL/mL. The treated 25 ≥20 ≥15 ≥10 group exhibited no change in mortality patterns, with larval mortality rates of 31.67±1.92%, 25±1.92%, 21.68±2.72%, 16.66±1.92%, and 13.33±1.90%, respectively.

3.2 Enzymatic Activity of *B. bassiana* against Adult Emergence of *B. dorsalis*

After 25µL of cuticle-degrading enzymes for two days, the adult emergence rate was 8.33±3.11%, whereas the adult emergence rate in pupae that were not treated was 50±3.12%. After three days of treatment with 25µL of *B. bassiana* cuticle-degrading enzymes, poor adult emergence was found at 3.33±2.72%, while in the untreated group, adult emergence was observed at 60±2.99%. Poor adult emergence was seen at 8.33±2.22% at 25µl of enzymes ($F_{5, 12} = 23.93$, $P = 0.0002$, $\alpha = 0.05$) after a 5-day treatment with cuticle-degrading enzymes, compared to 65.00±5.77% adult emergence in pupae that were not treated.

3.3 Pathogenic Activity of CDE against Adult Stage

After three and four days of treatment, the death rate of adult *B. dorsalis* was 50±2.88% and 80±2.15% at 25µl, respectively, whereas the lowest mortality rate was 30±2.88% and 36±2.33% at 5µl. In experimental settings, the dose and duration of exposure to trends based on insects were observed to have the largest and most notable mortality.

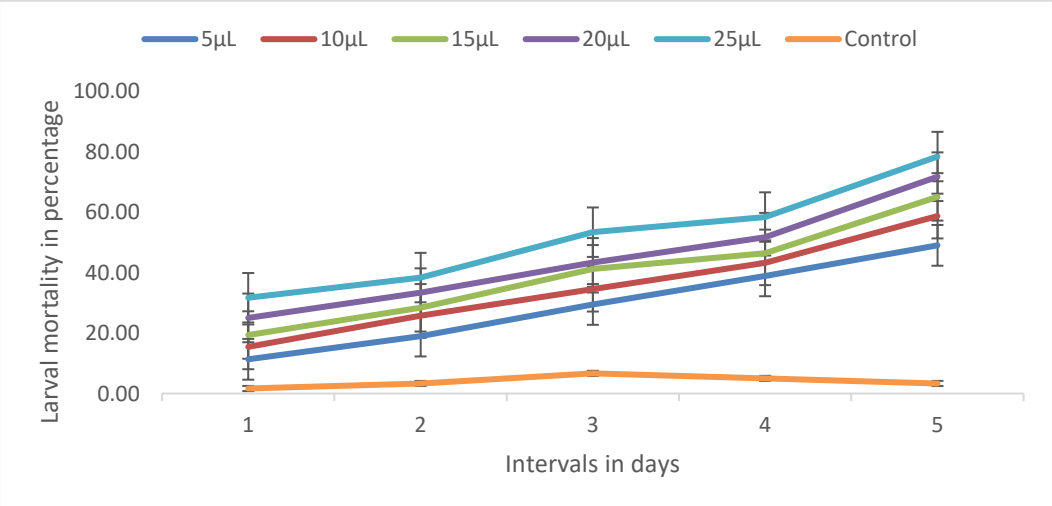

Fig. 1. Mortality percentage after different time intervals

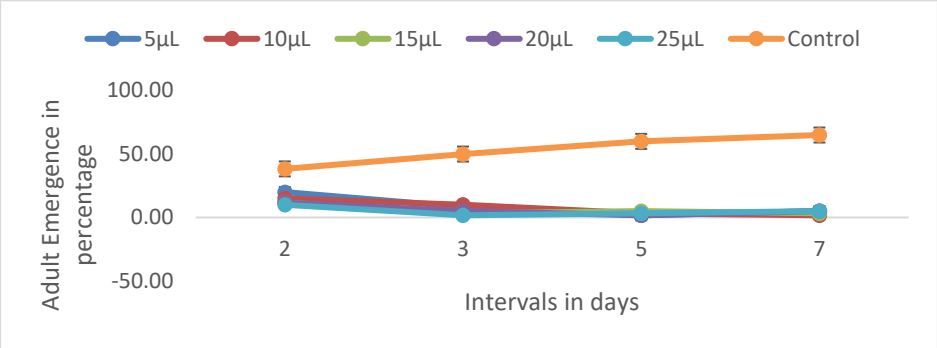

Fig. 2. Adult emergence percentage

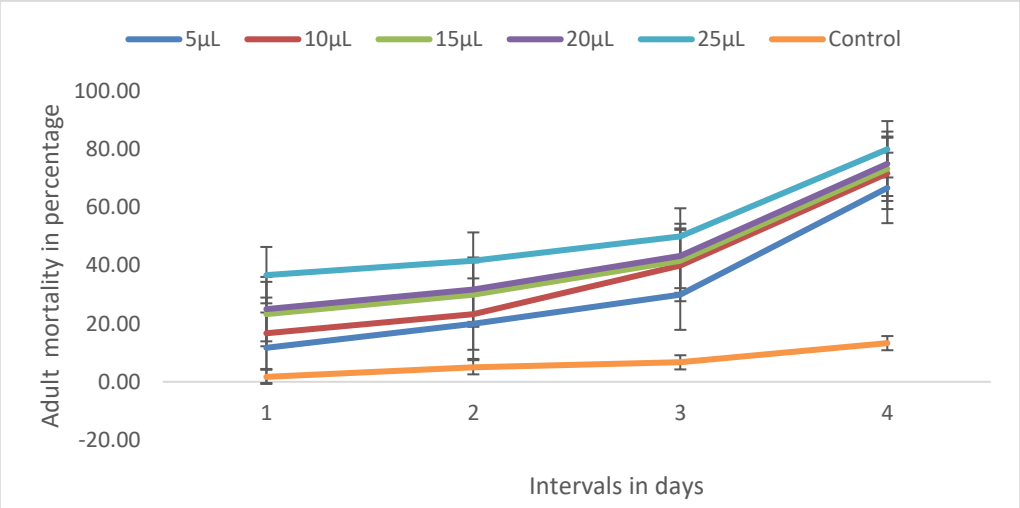

Fig. 3. Adult mortality percentage

4. DISCUSSION

In the current study, Protease enzymes were detected in the *B. bassiana* culture with band sizes of 19 and 47 kDa, indicating a pH range of 7 to 12 and a temperature range of 35 to 45°C. By deactivating the antifungal protein in the insect's epidermal layer, it is essential to the hydrolysis and breakdown of the cuticles of the insects. Using standardized enzyme keys, the molecular weight of proteases was observed in the gel.

Moreover, *B. bassiana* has been found to cause epicuticle degeneration in the early stages of infection. However, when it deteriorated, the cuticle's function was rendered unnecessary. Thus, only during the cuticle penetration phase do the entomopathogenic fungi degrade lipid substrates [10].

According to Görgün and Zengin, native-PAGE analyses were carried out devoid of SDS-PAGE. One naphthyl acetate was used to colour the gels in order to detect the esterase bands in the samples. Petrisor et al. [11] investigated the complexity of chitinase and found that the fungus released many chitinase enzymes. Two distinct chitinases found in *B. bassiana* have been found to be regulated and activated by products of chitin degradation.

During fungal invasion, extracellular acidic chitinase has also been found on the cuticle surfaces of hosts. Many entomopathogenic fungi have demonstrated chitin lytic activity, which is assumed to be important for pathogenicity; however, using rudimentary chitinase preparations, the enzymes linked to pathogenicity have not been well characterised. Different isolates of *B. bassiana* were shown to have chitinase with varying molecular weights, ranging from 43.5 kDa to 33 kDa, 45 kDa, and 110 kDa [12]. Our findings showed that *B. bassiana* have strong pathogenic and enzymatic virulent power against *B. dorsalis* by causing

direct toxicity through cuticle penetration by degrading antifungal proteins [13-15].

5. CONCLUSION

The *Beauveria bassiana* is effective at managing a variety of insect pests. Because *B. bassiana* contains enzymes that break down cuticles, it is harmful to insects. The SDS-PAGE method was used to analyse the isolated cuticle-degrading enzymes. Based on their molecular weights, it was separated into three cuticle-degrading enzymes after investigation. Proteases, lipases, and chitinase are among the enzymes with varying molecular weights measured in kilo Dalton (kDa). After the gel was separated, the bands corresponding to their molecular weights were plainly visible, indicating the presence of these enzymes that break down cuticles. As a result, these enzymes might be very harmful against a variety of insect pests. Among other enzymes, *B. bassiana* has protease, lipase, and chitinase, which break down the antifungal protein in the cuticle of *B. dorsalis*. *B. bassiana* serves as a potent biological control agent for *B. dorsalis*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://doi.org/10.9734/ARRB/2017/34005

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/117476