Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Enzyme-Mediated Biological Control of Bactrocera Zonata Using Beauveria Bassiana Cuticle-Degrading Enzymes

Mohib Ullah
Department of Entomology, University of Agriculture Faisalabad, Pakistan

Muhammad Faheem
Department of Botany, Emerson University Multan, Pakistan

Shah Rukh Hamza
Department of Botany, Emerson University Multan, Pakistan

Sanaullah*
Institute of Plant Protection, MNS University of Agriculture Multan, Pakistan
Email: sanaullahjatoi74@gmail.com

Received: 15 February 2026 **Accepted:** 01 March 2026 **Published:** 06 March 2026

ABSTRACT

Background: This study focused on managing Bactrocera zonata, an invasive Tephritid fruit fly that attacks a variety of fruits, by applying cuticle degrading enzymes (CDE) extracted from Beauveria bassiana, a phytopathogenic agent for the biological control of insect pests, as confirmed by SDS-PAGE analysis.
Methodology: Proteases, lipases, and chitinases were among the cuticle-degrading enzymes with varying molecular weights of 19–47 kDa, 32 kDa, and 49.47 kDa, respectively. The crude enzymes that were extracted and employed at doses of 1.5, 3, 5, 7, and 10µL were applied to larvae, pupae, and adults.
Results: At 10µL/mL, the death rates for larvae and adults were found to be 78.50±2.10% and 80±2.15%, respectively. Lower amounts (1.5µL/mL) resulted in a mortality rate of 13.33±1.92%, 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.
Conclusion: The outcome demonstrated that concentration affected both adult emergence from pupae and mortality. Consequently, the pathogenicity of B. bassiana mycelium against B. zonata was increased by the addition of CDE.

Keywords: Adult Emergence, b. Zonata, b. Bassiana, Cuticle-Degrading Enzymes, Mortality, Biological Control

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

INTRODUCTION

The most prevalent polyphagous pest in the world, Bactrocera zonata, causes large financial losses in tropical and subtropical regions (Khan and Akram, 2018). Peach, mango, guava, citrus, apple, fig, and apricot are the host fruits of B. zonata. It infects not only fruits, but also crops including eggplant, tomato, and pepper, which are termed secondary hosts (Ansari et al., 2019). It has been shown that using EPF as biological control agents, such as B. bassiana, is an effective way to manage B. zonata (Chergui et al., 2020). Insect survival and environmental adaptability depend on the cuticle, a part of the integument that serves as a barrier against biotic and abiotic stimuli. The thickness and hardness of the cuticle, which are primarily formed by the cross-linking that occurs during sclerotization, serve as an efficient barrier against pathological infection (Zibaee and Ramzi, 2018). The well-known microbial agent B. bassiana, a global anamorphic fungus, infects a range of insect hosts. It is seen as a possible biological control agent in temperate agricultural settings (Motholo et al., 2019).

Protease secretion is thought to be a key pathogenic element for the fungal attack on the cuticle, and the EPF employs a number of enzymatic activities to tear down the insect cuticle. Because of its high pathogenic efficacy, B. bassiana is employed to combat significant agricultural pests (Dhawan and Joshi, 2017). One distinctive feature of B. bassiana that increases its pathogenicity is the production of cuticle-degrading enzymes. Insect cuticle, which is made up of protein and chitin fibers encased in a matrix of proteins, lipids, and N-acyl catecholamines, serves as the body's first line of protection against infection (Muniz et al., 2021).

After adhering to the hydrophobic cuticle, the fungal spore of B. bassiana produces a variety of cuticle-solubilizing enzymes and grows into an infectious structure known as an appressorium. A range of extracellular cuticle-degrading enzymes are also produced by B. bassiana (Chethana et al., 2021). Since the fungus infects the host via penetrating the cuticle, fungal proteases are essential for cuticle penetration (Shin et al., 2020). Through hyphal penetration, B. bassiana releases extracellular enzymes such lipase, chitinase, and protease to degrade the primary constituents of the insect cuticle. Disease development and other physiological processes depend on these enzymes. The insect's integument is broken down by the enzymatic machinery of B. bassiana, which includes lipases, proteases, and chitinases (Mondal et al., 2016).

The subtilisin-like serine protease (Pr1, Pr2), which contributes to host invasion, provides the basis for the pathogenicity of the EPF. Because they break down the protein in the insect cuticle, these enzymes are essential during the early penetration stages (Petrisor and Stoian, 2017). The fungus B. bassiana produces a lot of proteases, which break down proteinaceous material and solubilize proteins, to meet its nutritional needs. Amino peptidases and exopeptidases subsequently hydrolyze these proteins to yield amino acids. Because of extracellular proteases, B. bassiana's pathogenicity toward its hosts is mostly determined by proteases (Dhawan et al., 2020). Lipases, the first enzymes that B. bassiana produces, hydrolyze the ester bonds of lipids, waxes, and lipoproteins within the insect's integument. Lipases are important for early nutrient release and cuticle penetration (Dhawan and Joshi, 2017). In order to investigate mortality and adult emergence from females under laboratory settings, a recent study examined the pathogenicity of B. bassiana cuticle-degrading

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

enzymes at varying mycelium concentrations against the three life stages of B. zonata (adult, larvae, and pupae).

Materials and methods

Insect culture

By setting up pheromone traps (15×5 cm), adults of B. zonata were gathered from guava and mango plantations in the districts of Multan and Layyah. Protein hydrolysate and methyl eugenol were used in the traps to attract females and males, respectively. Flies were collected from the field and taken to the Institute of Plant Protection's Insect Pathology lab at Muhammad Nawaz Shareef University of Agriculture Multan to be raised. Both individuals' populations were housed in 45 x 30 cm acrylic rearing cages. B. zonata was fed fresh fruits (apples, mangos, and bananas) and baking yeast. The humidity and temperature were maintained at 65.5% and 25.2°C, respectively. Regular feed changes and cage cleaning were performed.

Preparation of Fungal culture

The Insect Pathology Laboratory at Muhammad Nawaz Shareef University of Agriculture Multan's Institute of Plant Protection provided the pre-maintained cultures of B. bassiana. For fungal growth, 39 g/l of commercially prepared potato dextrose agar (PDA) (Merck KGaA, Darmstadt, Germany) was autoclaved and kept at 25±2°C and 65±5%. B. bassiana conidia were inoculated using a sterile inoculation pin following the solidification of the media. For fungal culture, the plates were incubated at 25°C for five to seven days after being wrapped with parafilm tape. After 14 days, B. bassiana conidia were removed using a 0.01% aqueous solution of Tween-80 to prepare the necessary spore concentrations.

Cuticle degrading enzymes

According to Ulker et al. (2012), the cuticle-degrading enzymes were isolated from B. bassiana conidia after they were harvested. The three life stages of B. zonata (larvae, pupae, and adults) were tested using the isolated cuticle-degrading enzymes. As indicated in Table 1, the components used to produce B. bassiana's CDE were calcium chloride (0.01%), potassium dihydrogen phosphate (0.02%), magnesium sulfate (0.01%), zinc chloride (0.01%), and olive oil.

Table 1. Chemicals and their quantity used in media for cuticle degrading enzyme extraction

Sr#	Ingredients	Quantity (Grams) per 200 ml
01	Tris HCL	3.15 g
02	NaCl	1.75 g
03	Calcium chloride	4.13 g
04	Sodium phosphate	3.45 g
05	Magnesium sulfate	6.12 g
06	Olive oil	5.00 ml

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Procedure for measuring size of CDE from B. bassiana

Using sodium dodecyl polyacrylamide gel electrophoresis (Demir et al., 2018), a 4% (w/v) stacking gel and a 10% (w/v) separating gel were used to determine the molecular mass and purify the enzymes. TAE buffer solution and 12% Acrylamide gel (AppliChem Biochemical Chemical Synthesis Services, Germany) were used to purify the enzymes. After being put into the tray, the gel was allowed to dry. To examine the bands in enzyme samples, Coomassie brilliant blue (Biochem Chemopharma, France) was used to stain the gel. The enzymes were evaluated according to their molecular weight (kDa) using several bands of dyed colors. In order to run enzymes for purification or quantitative analysis to ascertain the weight of the enzyme sample, gels were polymerized and buffer solution was added to the chambers.

Table 2. Recipe used for resolving gel (12%) and stacking gel (4%)

Sr. No.	Chemical name	Resolving gel (12%)	Stacking gel (4%)
01	Acrylamide	2.6 ml	0.75 ml
02	Tris HCl	1.5 ml	0.5 ml
03	TEMED	6µl	6µl
05	SDS 10%	150µl	200µl
06	Distill water	3.4ml	3.2ml
07	Ammonium per sulphate	3g/ 30 ml Distill water	2.5g/30ml distill water

Table 3. Chemicals used for running buffer

Sr. No.	Chemical	Weight
01	Glycine	14.4g
02	Tris base	3.02g
03	SDS	1g
04	Coomassie blue dye	25µl/sample

Larval bioassay

B. zonata larval instars (L2) were subjected to several doses of extracted enzymes (1.5, 3, 5, 7.5, and 10µL/mL). Using the larval immersion approach, 20 larvae of comparable age were exposed to enzymes (Ugwu and Nwaokolo, 2020). Larvae of B. zonata were dipped into 9 cm sterile Petri plates with the necessary amount of CDE. A similar batch of 20 B. zonata treated with B. bassiana mycelium medium served as the untreated control. Dead larvae were removed following the count, and data were taken 24, 48, 72, and 96 hours later. After being quickly surface sterilized with 70% alcohol, the dead larvae were rinsed for three minutes with 1% sodium hypochlorite, then they were placed on a filter in sterile water.

Pupal bioassay

The 20 pupae were taken from the culture in the lab. Six treatments with varying

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

doses were used: 1.5, 3, 5, 7.5, and 10 µL/mL, as well as a control. After being submerged in the suspension bioassay procedure, roughly 20 pupae (4 days old) were maintained in sterile Petri dishes (9 cm). To help treated and untreated pupae emerge as adults, sterile sand was added to petri plates. Following 3, 6, 9, and 12 days of treatment, the adult emergence data were obtained under controlled conditions, with a temperature of 26±2°C and a relative humidity of 65±5% at 16:8 (L:D). Three replications of the experiment were conducted using a completely randomized design.

Adult bioassay

An immersion approach was used in a bioassay to assess the pathogenicity (Yousef et al., 2018). Five different concentrations of crude enzymes (1.5, 3, 5, 7.5, and 10µL per ml of mycelium liquid medium) were added to plastic rearing cages containing ten pairs of adult B. zonata three-day-olds (♂ and ♀) for testing, along with a control. Adults in the control group were only given sugar and yeast hydrolysate (Ortiz-Urquiza et al., 2013). Under CRD, three replications of six treatments involving ten pairs of B. zonata were conducted. At 16:8 (L:D), the experimental conditions were kept at 25±2°C and 60±5% relative humidity.

Statistical analysis

ANOVA was performed using a completely randomized design (CRD) to determine the percentage mortality and efficacy of five concentrations of the cuticle-degrading enzymes (1.5, 3, 5, 7.5, and 10µL per ml of mycelium liquid media). Minitab 10.0 and 8.1 were used to analyze the data. The means and standard errors were separated using Tukey's test as a post-ANOVA technique with a 5% probability (Beris, 2021).

RESULTS

Activity of CDE against second instar larvae of B. zonata

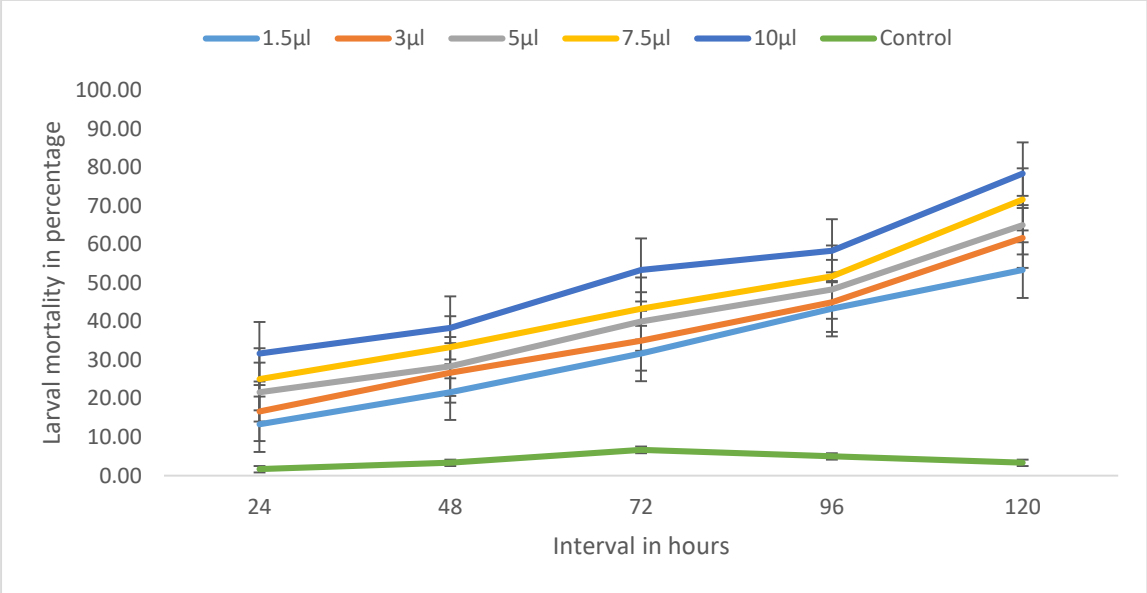
The pathogenicity of B. bassiana CDE against B. zonata larvae in their second instar was observed after a 24-hour application period. Larvae showed significant mortality ($F_{5, 12} = 29.1$, $P=0.0039$, $\alpha=0.05$) 24 hours after treatment. Concentration-dependent mortality was observed, with the batch treated with 1.5µL/mL cuticle-degrading enzymes and the untreated control group showing the lowest mortality of 13.33±1.92% and the highest mortality of 31.67±1.92% at 10µL/mL. Larval mortality rates were 31.67±1.92%, 25±1.92%, 21.68±2.72%, 16.66±1.92%, and 13.33±1.90%, respectively, for treatments 10≥7.5 ≥5 ≥3.5, and 1.5 (Fig. 3).

Figure 3. Larval mortality of B. zonata at various intervals after application of CDE of the B. bassiana

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about



After 48 hours, the substantial mortality was observed (F5, 12 = 38.33±2.63, P=0.000, α=0.05). The B. zonata larvae treated with 10µL/mL cuticle-degrading enzymes had the highest mortality rate (38.33±2.63), while the untreated group had the lowest mortality rate (21.66±2.50) at 1.5µL/mL. When substantial mortality (F5, 12 = 15.81, P=0.0001, α=0.05) was recorded at 58.33±3.96% and 78.33±2.80% in a batch of insects treated with 10µL/60mL, respectively, the cuticle-degrading enzymes from B. bassiana were found to be fatal after 72 and 96 hours of treatment. Following 72 and 96 hours of treatment with 1.5 µL/mL of cuticle-degrading enzymes, the lowest death rate of 43.32± 2.96% was observed, followed by an untreated group of 3.00±2.80% (Fig. 3).

The activity of CDE of B. bassiana against 4 days old pupa to observe adult emergence

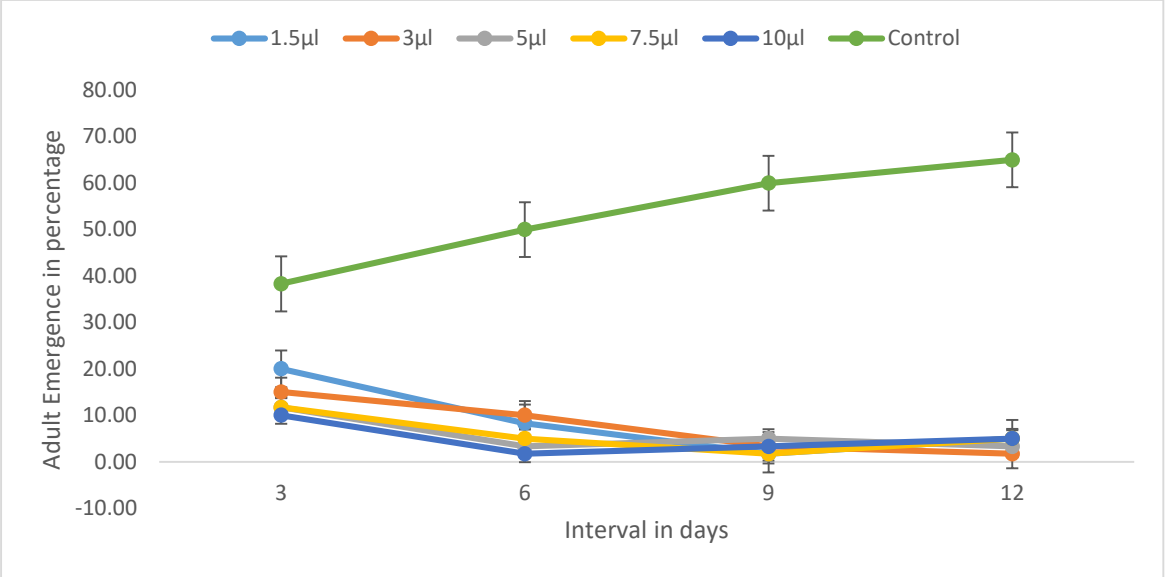
Adult emergence was 8.33±3.11% at 10µL of cuticle-degrading enzymes after 6 days of treatment, compared to 50±3.12% in pupae that were not treated. After 9 days of treatment with 10µL of B. bassiana's cuticle-degrading enzymes, poor adult emergence was observed at 3.33±2.72%, while in an untreated group, adult emergence was observed at 60±2.99%. Adult emergence was poor at 8.33±2.22% at 10µl of cuticle-degrading enzymes after 12 days of treatment (F5, 12 = 23.93, P=0.0002, α=0.05), compared to 65.00±5.77% in pupae that were not treated. Following a review of all the data, the control group showed the highest adult emergence, followed by the less concentrated ones, and the 10µL concentrated checks showed the lowest adult emergence after three

Figure 4. Adult emergence percentage of B. zonata at different intervals after application of CDE

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about



The activity of CDE of B. bassiana against 3 days old adult of B. zonata

Significant mortality was detected at 36.66±1.92% at 10µL after 2 days of treatment ($F_{5, 12} = 22.31$, $P=0.012$, $\alpha=0.05$). The lowest mortality was recorded at 11±1.50% at 1.5µL, followed by untreated adults at 5±1.22%. Likewise, following three days of therapy, the highest fatality rate was 41.66±2.43% at 10uL, while the lowest mortality rate was 20±2.2% at 1.5µL, with untreated people coming in last. After 4 and 5 days of therapy, the adult B. zonata mortality rate was 50±2.88% and 80±2.15% at 10 µl, respectively, while the adult mortality rate was 30±2.88% and 36±2.33% at 1.5 µl. Under laboratory conditions, the largest and most substantial mortality was seen in relation to the concentration and exposure time of insect-based trends (Fig. 5).

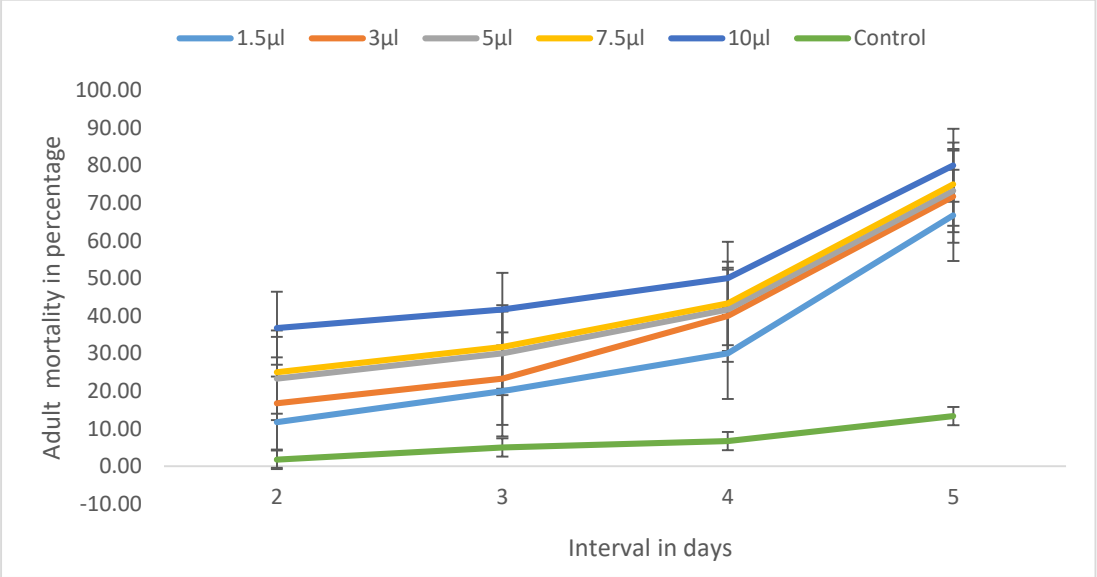


Figure 5. Adult mortality of B. zonata at different intervals after application of CDE

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Physical deformities resulted in different life stages of B. zonata after application of CDE

Physiological malformation in arthropods can be caused by chemicals, mutagens, diseases, and physical disruptions. This might happen naturally or as a result of any other condition. Despite the fact that coleopterans are known to be sensitive to physical disruption, little research has been done on how this impacts juvenile development and results in malformations (Bong et al., 2018). Numerous arthropods have been discovered to have morphological abnormalities. It can occur spontaneously (idiopathic) or be brought on by physical disturbances, illnesses, and substance mutagens (Barbosa et al., 2015).

The effects of several B. bassiana cuticle-degrading enzymes, such as proteases, chitinases, lipases, and proteinases, significantly altered the structure of the insect cuticles in the current study. Certain enzymes cause the development of insects from pupae to adults to be delayed, which results in aberrant development of B. zonata from pupae to adults. After dipping in the different concentrations of B. bassiana's CDE, numerous physical abnormalities were seen in the larvae of B. zonata. The creation of melanin, which is triggered by immunological reactions after dipping in enzymes, causes the cuticle of insects to turn black, which lowers the likelihood that pupae will form from two to three old day larvae. Melanization was also observed in pupae, which decreased the proportion of adult emergence.

DISCUSSION

Fungi create mycelium to penetrate the epicuticle and penetrate epithelial tissue, causing infection of the epidermis, which is one of the most common ways of infection. This study aimed to demonstrate the beneficial effects of B. bassiana cuticle-degrading enzymes on larval mortality and deformities in various stages of B. zonata. B. bassiana ultimately enters insects through a combination of enzymatic and mechanical processes, primarily through intact cuticles rather than wounds or natural openings (Kaur, 2022).

Instead of hyphal growth, blastospores are the primary method of reproduction for the anamorphic fungi B. bassiana (Chandler, 2017). Proteases produced by B. bassiana aid in the hydrolysis of cuticuler proteins found in the insect's epidermis and render antifungal proteins inactive. The breakdown of lipids and lipoproteins found in insect cuticles is significantly aided by B. bassiana lipases. It facilitates the germination and adhesion of spores on the insect cuticle by improving hydrophobic interactions between the fungal surface and the cuticle and breaking triacylglycerol ester bonds to release free fatty acids. Additionally, B. bassiana produces phospholipase C, which aids in the hydrolysis of phosphodiester bonds and breaks down phospholipids in insect cell membranes.

It enables the fungus to infect other tissues by piercing the insect's hemocoel. Tissue penetration, extensive growth in the hemolymph, and the subsequent manufacture of poisons all contribute to the insect host's demise. By spreading throughout the insect's body cavity via hemolymph, these blastospores infiltrate vital organs, obstructing the circulatory system and ultimately resulting in the insect's death. Following the death of the host, the fungus enters a facultative feeding stage, during which it begins hyphal development outside the epicuticle and generates a large number of spores

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

(Altinok et al., 2019).

Germ tubes on the cuticle can be impacted by environmental stressors such high temperatures and UV radiation, as well as insect defensive reactions in the cuticle. The virulence of the fungus may be positively impacted by accelerated cuticle breaching since it may reduce the duration of exposure to these conditions and, thus, increase the chance of successful infection. Concurrent enzymatic cuticle breakdown is beneficial in addition to the mechanical pressure required to break through the cuticle. A chitin-based framework, along with auxiliary proteins and lipids, make up the majority of the cuticle (Zhang et al., 2010).

Hydrolytic enzymes including lipases, chitinases, and proteases are created and released when a fungus breaks through the cuticle of its host. The initiation of the infection process, which leads to cuticle transposition, depends on these enzymes. When B. bassiana is cultivated in the cuticle of an arthropod or chitin as the sole source of carbon and nitrogen, it produces a number of extracellular enzymes that break down the components of the host cuticle (Cheong et al., 2020).

One important factor in B. bassiana's pathogenicity was thought to be its extracellular enzyme activity. B. Bassiana had the highest extracellular chitinase activity and was the most harmful to its hosts. The pathogenicity of B. bassiana chitinase has been linked to the enzyme's ability to break down the chitin polymer of an insect's cuticle. According to Dhawan and Joshi (2017), protease was considered to be an essential enzyme during the infection stage of EPF.

Second only to chitin, protein is one of the most important components of an insect's cuticle. Chitinase is one of the most crucial and early enzymes engaged in the invasion, after the proteolytic breakdown of the target proteins and the ultimate exposure of the chitin in the host cuticle. There were extracellular proteases in insect hemolymph as well. The B. bassiana isolates' protease activity varied significantly from one isolate to another, and their peak protease production time varied as well (Cheong et al., 2020). The timing of maximum protease synthesis is not always determined by the media components, as seen by the high levels of protease release observed in media with varying nitrogen sources three days after inoculation.

Proteolytic activity declined as the cultures of B. bassiana isolates grew older; this was probably due to either culture autolysis or nutrient restriction. In almost every isolate of B. bassiana, two trypsin-like proteases with molecular weights of 30-kDa and 27-kDa, together with a 66-kDa protease, were found. B. bassiana conidia were shown to have a metalloprotease with a molecular weight of 103 and 12 kDa (Dhar and Kaur, 2010). Among the promising biocontrol agents is B. bassiana. It has been used in agroecosystems for a long time and has proven to be quite successful in controlling insect outbreaks. Due to their unusual ability to enter their host insects through the cuticle, these microbes heavily depend on a class of hydrolyzing enzymes.

According to studies, proteases are essential enzymes for fungal penetration through insect cuticles because they are released early in the pathogenic process. Almost all virulent entomopathogenic fungi have elevated proteolytic activity for trypsin-like proteases (Pr2) and subtilisin-like proteases (Pr1) because these two proteases directly affect their host insects. The genetic engineering of entomopathogenic fungi has brought attention to the role of proteases in virulence and their potential use in the development of novel mycoinsecticides. The majority of research on the effects of

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

entomopathogenic fungi has focused on the possibility of inoculum formation and mortality in target insects in order to differentiate between various fungal species or strains.

It is essential to take into account other pathogenic elements, such as the ability of enzymes to secrete, and try to overexpress them in order to ensure efficacy. Proteases would be good choices as they guarantee fungal penetration and are directly poisonous to insects. Furthermore, research on the pathological management of insect pests has shifted its focus to the use of genetic engineering to increase the virulence and speed of entomopathogenic fungi's ability to kill insects. The study's findings will aid in our comprehension of the degree of protease production in various B. bassiana isolates, as well as the length of incubation and the generation of Pr1 and Pr2 by these isolates. This will lead to new research on the role of chitinases, lipases, and proteases in entomopathogenic fungus virulence.

Conclusion

Numerous insect pests can be managed by the amorphous entomopathogenic fungus Beauveria bassiana. Because B. bassiana contains enzymes that break down cuticles, it is harmful to insects. The SDS-PAGE technique was used to analyze the isolated cuticle-degrading enzymes. Based on their molecular weights, it was separated into three cuticle-degrading enzymes following investigation. These enzymes, which vary in their molecular weights in Kilo Dalton (kDa), include lipases, chitinases, and proteases.

After the gel was stained, the bands of their molecular weights were plainly evident, indicating the presence of these enzymes that break down cuticles. When combined with the mycelial body of less pathogenic strains of B. bassiana, these enzymes can increase the activity of the species. Consequently, it is possible that these enzymes contribute significantly to the pathogenicity of numerous insect pests. Protease, lipase, and chitinases are among the enzymes found in B. bassiana that break down the antifungal protein found in the B. zonata cuticle. As a result, B. bassiana is an effective biological control agent for B. zonata management.

Novelty statement

B. bassiana is a potent biological control agent that releases certain cuticle degrading enzymes. These cuticle degrading enzymes cause direct toxicity in the insect cuticle by destroying the antifungal protein present in the insect cuticle.

Conflict of Interest

All the authors participated equally and have no conflict of interest in this manuscript.

REFERENCES

Altinok, H. H., M.A Altinok and A.S. Koca. 2019. Modes of action of entomopathogenic fungi. Curr. Tre. Nat. Sci. 8(16), 117-124.

Ali, S., Huang, Z., S.X. Ren. 2009. Production and extraction of extracellular lipase from the entomopathogenic fungus Isaria fumosoroseus (Cordycipitaceae; Hypocreales). Biocontrol Sci Technol , 19(1), 81-89.

Ansari, M. S., R. Basri and S.S. Shekhawat. 2019. Insect Pests Infestation During

Online ISSN 3007-3197
Print ISSN 3007-3189

Field and Storage of Fruits and Vegetables. In Health and Safety Aspects of Food Processing Technologies Springer, Cham. (pp. 121-207).

Barbosa, W. F., H.V.V. Tomé, R.C. Bernardes, M.A.L. Siqueira, G. Smagghe and R.N.C. Guedes. 2015. Biopesticide-induced behavioral and morphological alterations in the stingless bee Melipona quadrifasciata. Environ. Toxicol. Chem., 34(9), 2149-2158.

Beris, E. (2021). Evaluation and environmental testing of entomopathogenic fungi for their effectiveness as bio-control agents of major vineyard pests. Plant Prot., 5(1), 13-23.

Bong, L. J., K.B. Neoh and T. Yoshimura. 2018. Developmental irregularity and abnormal elytra formation in the oriental wood borer induced by physical disturbance. J. Insect Sci., 18(1), 12.

Chandler, D. 2017. Basic and applied research on entomopathogenic fungi. In Microbial control of insect and mite pests, Academic Press (pp. 69-89).

Chergui, S., Boudjemaa, K., Benzehra, A., and Karaca, I. 2020. Pathogenicity of indigenous Beauveria bassiana (Balsamo) against Ceratitis capitata Wiedemann (Diptera: Tephritidae) under laboratory conditions. Egyp. J. Bio. Pes. Con. 30:128.

Chethana, K. T., Jayawardena, R. S., Chen, Y. J., Konta, S., Tibpromma, S., Phukhamsakda, C., and Hyde, K. D. 2021. Appressorial interactions with host and their evolution. Fun. Dive. 110(1), 75-107.

Cheong, P. C., Glare, T. R., Rostás, M., Haines, S., Brookes, J. J., and Ford, S. 2020. Lack of involvement of chitinase in direct toxicity of Beauveria bassiana cultures to the aphid Myzus persicae. J. Inver. Patho, 169, 107276.

Dhawan, M., and N. Joshi. 2017. Enzymatic comparison and mortality of Beauveria bassiana against cabbage caterpillar Pieris brassicae LINN. Braz. J. Microbiol., 48, 522-529.

Dhar, P. and Kaur, G. 2010. Production of cuticle-degrading proteases by Beauveria bassiana and their induction in different media. Afr. J. Biochem. Res, 4, 65-72.

Dhawan, M., Joshi, N., Ghuman, S., Sandhu, S., and Sharma, M. 2020. Deciphering the relationships among enzymatic systems and virulence of Beauveria bassiana: A Review.

Demir, Y., Dikbaş, N. and Beydemir S. 2018. Purification and biochemical characterization of phytase enzyme from Lactobacillus coryniformis (MH121153). Mole. Biotech. 60, 783-790.

Kaur, C. P. 2022. Analeptic Mushrooms. Agriculture & food e-newsletter, 2.

Mondal, S., S. Baksi, A. Koris and G. Vatai. 2016. Journey of enzymes in entomopathogenic fungi. Pacific Science Review A: Nat. Sci. Engi., 18(2), 85-99.

Motholo, L. F. 2019. Endophytic establishment of Beauveria bassiana in wheat (Triticum aestivum) and its impact on Diuraphis noxia (Doctoral dissertation, North-West University (South Africa). Potchefstroom Campus).

Muniz, E. R., C.S. Ribeiro-Silva, W. Arruda, N.O. Keyhani and E.K. Fernandes. 2021. The Msn2 transcription factor regulates acaricidal virulence in the fungal pathogen Beauveria bassiana. Front. Cell. Infect. Microbiol. 11, 604.

Ortiz-Urquiza, A., N.O. Keyhani and E. Quesada-Moraga. 2013. Culture conditions

Online ISSN 3007-3197
Print ISSN 3007-3189

affect virulence and production of insect toxic proteins in the entomopathogenic fungus Metarhizium anisopliae. Biocontrol Sci Technol., 23(10), 1199-1212.

Petrisor, C., and Stoian, G. 2017. The role of hydrolytic enzymes produced by entomopathogenic fungi in pathogenesis of insects mini review. Rom. J Plant Prot, 10, 66-72.

Sarwar, M., M. Hamed, B. Rasool, M. Yousaf and M. Hussain. 2013. Host preference and performance of fruit flies Bactrocera zonata (Saunders) and Bactrocera cucurbitae (Coquillett)(Diptera: Tephritidae) for various fruits and vegetables. Int. J. Environ., 1(8), 188.

Sanjaya, Y., Ocampo, V. R., and Caoili, B. L. 2015. Infection process of entomopathogenic fungi Beauveria bassiana in the Tetrancyhus kanzawai (Kishida)(Tetranychidae: Acarina). Arthro. 4(3), 90-97.

Shin, T. Y., M.R. Lee, S.E. Park, S.J. Lee, W.J. Kim and J.S. Kim. 2020. Pathogenesis-related genes of entomopathogenic fungi. Arch. Insect Biochem. Physiol., 105(4), e21747.

Ugwu, J. A., V.M. Nwaokolo. 2020. Biocidal activity of selected botanicals and Beauveria bassiana on oriental fruit fly, Bactrocera dorsalis (Diptera; Tephritidae). j. res. for. wildl.12 (1), 53-61.

Ülker, S., and S.A. Karaoğlu. 2012. Purification and characterization of an extracellular lipase from Mucor hiemalis f. corticola isolated from soil. JBB.114(4), 385-390.

Zhang, Y., J. Zhang, X. Jiang, G. Wang, Z. Luo, Y. Fan and Y. Pei, Y. 2010. Requirement of a mitogen-activated protein kinase for appressorium formation and penetration of insect cuticle by the entomopathogenic fungus Beauveria bassiana. Appl. Environ. Microbiol. 76(7), 2262-2270.

Zibaee, A., and Ramzi, S. 2018. Cuticle-degrading proteases of entomopathogenic fungi: from biochemistry to biological performance. Arch. Phyto. Pla. Protec. 51(13-14), 779-794.