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EXTRACTION AND CHARACTERIZATION OF CUTICLE DEGRADING ENZYMES OF *BEAUVERIA BASSIANA* FOR ENHANCED PATHOGENICITY AGAINST *BACTROCERA DORSALIS*

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ABSTRACT

Beauveria bassiana is a promising candidate for the biocontrol of many important insect pests. The cuticle is the site of attachment of fungal spores after invading the host body. Cuticle-degrading enzymes (CDE) are a set of enzymes possessed by entomopathogenic fungi that ensure successful penetration. The current study indicated the extraction of crude cuticle-degrading enzymes from *B. bassiana*, as a mixture of mycelium and CDE, offers enhanced pathogenicity to its host. Tris-HCL, calcium chloride, potassium hydrogen phosphate, sodium phosphate, magnesium sulfate, zinc chloride, and olive oil were mixed in a 50 ml Erlenmeyer flask to extract CDE from the lab strain of *B. bassiana*. After centrifugation of the mixture, the supernatant was separated as extracellular enzymes. The media was subjected to SDS-PAGE analysis for enzyme characterization. Stacking gel (4%) and resolving gel (12%) were used with a pH of 8.6 to determine the molecular masses of the samples (enzymes) in kDa. The results showed that after staining and de-staining the gel, different bands of various sizes appeared. When compared with a standard key, the bands were found at 19, 50, 25, 32, and 34.25 kDa, confirming the presence of proteases, lipases, and chitinase, respectively. The extracted CDE can be used with different combinations of mycelial medium of *B. bassiana* or other entomopathogenic fungi to enhance their pathogenicity against insect pests. The extracted crude enzymes were used against larvae, pupae, and adults at concentrations of 5, 10, 15, 20, and 25µl. The mortality rate in larvae and adults was recorded as 78.50±2.10% and 80±2.15% at 25µl/ml, respectively. At lower concentrations (5µl/ml), the mortality was 13.33±1.92%, followed by the control group. A low percentage of adult emergence (10±2.63%) from pupae was observed in treated insects, and a higher adult emergence (65.0±5.77%) was observed in the untreated group of insects. The results showed that mortality and adult emergence from pupae were concentration-dependent. Therefore, adding CDE to the mycelium of *B. bassiana* enhanced its pathogenicity against different life stages of *Bactrocera dorsalis*.

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INTRODUCTION

The fungus *Beauveria bassiana* belongs to the order Hypocreales and is entomopathogenic in nature. *B. bassiana* is well recognized as a biopesticide for the management of many agricultural insect pests (Javed et al., 2019; Bara et al., 2020; Shehzad et al., 2021, 2022). *B. bassiana* (Balsamo) is a good biocontrol agent. This fungus is frequently used to control a range of insect pests (Malan et al., 2018). *B. bassiana* has an enzymatic complex that supports spore attachment and penetration, producing extracellular enzymes on substrates (Fernandes et al., 2012). The enzyme complex hydrolyzes the insect cuticle for penetration and progression of the infection cycle. The entomopathogenic fungus makes a variety of cuticle-degrading enzymes (CDE) that relate to distinct cuticle polymers. Some fungi transform insect tissues into nutrients for growth and produce enzymes. Although fungi seldom use sclerotized insect cuticles, the entomopathogenic fungi have developed effective enzymes to break down this protective layer (Stevenson et al., 2020). The production of cuticle-degrading enzymes like proteases, chitinases, and lipases is crucial in the infection process. These enzymes hydrolyze polymer proteins and chitin complexes, which are essential components of the insect's cuticle. An important structural component of the exoskeleton, the chitin network, is degraded by chitinases, allowing penetration by reducing the rigidity of the cuticle. Extracellular enzymes of *B. bassiana* are essential for cuticle degradation (Svedese et al., 2013). Chitinases are extracellular enzymes that have been isolated and studied in some cases. Chitinases play a role in hyphal development and morphogenesis in fungi. Entomopathogenic fungi (EPF) have been found to produce these compounds during host infection. The chitinases produced by *B. bassiana* have six isoforms (30, 33, 43.5, 45, 60, and 110 kDa). Chitinases hydrolyze the chitin polymer's bonds to produce N-acetyl b-D-glucosamine monomers. This enzymatic process is crucial in breaking down the chitin polymer in the insect cuticle (Rameshthangam et al., 2018).

Proteases are the most prevalent family of industrial enzymes, having a variety of uses. The epicuticle, the outermost cuticle layer of insects, is mostly composed of proteins and lipids that are maintained by a variety of biopolymers (Lemoine et al., 2020). Proteases work on the cuticle before chitinolytic enzymes target the weaker microfibers of the insect (Grizanova et al., 2019). The lipase enzymes released by *B. bassiana* facilitate the degradation of proteinaceous substances in insect cuticles. Indigenous isolates of *B. bassiana* contain cuticle-degrading enzymes such as chitinase, proteinase, and lipase, and the type of isolates used for the synthesis of enhanced myco-pesticides in an integrated pest control program determines how to isolate variability is characterized (Zibaee et al., 2018).

The most prevalent polyphagous pest, *Bactrocera dorsalis*, is responsible for enormous economic losses in tropical and subtropical regions of the world. *B. dorsalis* hosts fruits including peaches, mangos, guavas, citrus fruits, apples, figs, and apricots. It affects secondary hosts, such as crops like eggplant, tomato, and pepper, in addition to fruits (Ansari et al., 2019). The use of EPF as biological control agents, such as *B. bassiana*, has been shown to be the most effective strategy for the management of *B. dorsalis* (Chergui et al., 2020). The cuticle is a part of an insect's integument that serves as a barrier against biotic and abiotic stimuli and is crucial to an insect's ability to survive and adapt to its environment. The cuticle's thickness and hardness, both of which are formed by the cross-linking that occurs during sclerotization, serve as an excellent barrier against pathological infection (Zibaee and Ramzi, 2018). Many insect hosts are infected by the well-known microbiological agent *B. bassiana*, a global anamorphic fungus. In temperate agricultural contexts, it is considered a potential biological control agent (Motholo et al., 2019). The recent study aimed to extract cuticle-degrading enzymes of *B. bassiana* and characterize the enzymes using Sodium dodecyl sulfate polyacrylamide gel electrophoresis. The extracted crude enzymes were assayed against different life stages of *B. dorsalis* under laboratory conditions.

The objectives of the study are to extract cuticle-degrading enzymes of *B. bassiana* from different isolates, to characterize these enzymes using the SDS-PAGE technique, and to assess their activity against different life stages of *B. dorsalis*.

MATERIALS AND METHODS

Insect culture

Pheromone traps (155 cm) were used to capture adult *B. dorsalis* flies in the mango and guava orchards of the districts of Multan and Layyah. Methyl eugenol and protein hydrolysate were used in the traps for male and female attraction, respectively. After being collected

from the field, the flies were taken to the Insect Pathology laboratory at the Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan for rearing. The populations of the flies were housed in acrylic rearing cages measuring 45 × 30 cm. *B. dorsalis* flies were fed with baking yeast and fresh fruit (apples, bananas, and mangoes). The temperature and humidity were maintained at 25.2°C and 65.5%, respectively. Regular cage cleaning and feeding adjustments were performed.

Preparation of fungal culture

The pre-maintained cultures of *B. bassiana* were obtained from the Insect Pathology Laboratory, Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan. Commercially formulated potato dextrose agar (PDA) (Merck KGaA, Darmstadt, Germany) (39 g/l) was used for fungal growth, autoclaved, and maintained at 25±2°C and 65±5% relative humidity. After the media had solidified, a sterile inoculation pin was used to inoculate *B. bassiana* conidia. The plates were wrapped with parafilm tape and incubated at 25°C for 5-7 days for fungal culture (Zibaee and Bandhani, 2009). Conidia of *B. bassiana* were washed off after 14 days with a 0.01% aqueous solution of Tween-80 to prepare the required concentrations of spores.

Fungal liquid medium

According to the Adamke's, 4 ml of conidial suspension (1.0×10^8 conidia/ml) and 100 ml of mycelium medium were combined to create the primary culture of the fungal isolate (Silva et al., 2022). After mixing 2.5 ml of the primary culture with 250 ml of liquid medium and shaking the culture at 26°C and 200 rpm in a rotary shaker, a secondary culture (1%) was produced after six days of incubation at 26°C and 150 rpm. The mycelia were separated by centrifugation at 10,000 rpm for 30 minutes at 4°C, and the filtrate was then extracted from the supernatant using a 2 mm pore-size filter.

Enzymes extraction and assay

The required amount of fungal concentration was inoculated and incubated on a rotary shaker at 9000 rpm for 15 minutes in an Erlenmeyer flask with a volume of 40 ml (Dhawan et al., 2017). The supernatant was extracted from the culture by centrifugation at 10,000 rpm for different time intervals ranging from 2 to 10 minutes, and this was used as the crude enzyme (Coutinho et al., 2016). The obtained mycelium was rinsed with ice-cold 25 mM Tris-HCl at pH 8 and then centrifuged at 8000 rpm for 15 minutes. The recovered mycelium was crushed into tiny particles and placed in a solution containing 1 g/mL of lysis buffer (25 mM Tris-HCl at pH 8). The extracellular and intracellular soluble fractions were obtained from the extracted supernatant and culture filtrates, respectively. Subsequently, protease activity was determined using both fractions (Dias et al., 2008) (Table 1).

Table 1. Chemicals and their quantity used in media for CDE extraction.

Sr. No.	Ingredients	Quantity (g) 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

Characterization of cuticle degrading enzymes using SDS-PAGE

Sodium dodecyl polyacrylamide gel electrophoresis, employing a 4% (w/v) stacking gel and a 10% (w/v) separating gel, was used to determine the molecular mass and purify enzymes (Demir et al., 2018). The German company AppliChem Biochemical Chemical Synthesis Services' 12 percent Acrylamide gel and TAE buffer solution were used for purifying the enzymes. The gel was placed into the tray and allowed to dry. To examine the bands in the enzyme samples, the gel was stained with Coomassie brilliant blue (Biochem Chemopharma, France). The enzymes were evaluated using several bands of dyed colors, which indicated their molecular weight (kDa). For running the enzymes to purify or quantitatively analyze and calculate the weight of the enzyme sample, gels were polymerized, and buffer solution was added to the chambers. Subsequently, the samples were loaded to calculate the molecular mass of the enzymes (Table 2, 3).

Bioassay

Larval bioassay

The second larval instar (L2) of *B. dorsalis* was exposed to extracted enzyme concentrations of 5, 10, 15, 20, and 25µl/ml. The larval immersion method was used to expose about 25 larvae of similar age to the enzymes (Mutamiswa et al., 2021). Sterile Petri dishes (9 cm) with the necessary quantity of CDE were used to dip *B. dorsalis* larvae. A similar batch of 25 *B. dorsalis* larvae treated with *B. bassiana* mycelium medium served as the untreated control. Dead larvae were removed following the count, and the data were recorded after 1, 2, 3, and 4 days. The dead larvae were placed on a filter in sterilized Petri dishes after being quickly surface sterilized with 70% alcohol and washed with 1% sodium hypochlorite for 3 minutes.

Table 2. 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

Table 3. 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
04	APS 10%	80µl	50µl
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

Pupal bioassay

The 25 pupae were collected from a lab culture. Six different treatments, including a control and concentrations of 5, 10, 15, 20, and 25µl/ml, were applied. Approximately 25 pupae (4-day-old) were placed in sterile Petri dishes (9 cm) using the suspension bioassay technique. To enhance the emergence of adults from both treated and untreated pupae, Petri plates were filled with sterile sand. After treatment, data on adult emergence were collected 2, 3, 5, and 7 days later under carefully monitored conditions (26°C temperature, 65±5% relative humidity at a 16:8 h L: D period).

Adult bioassay

The pathogenicity was assessed using a bioassay with an immersion technique (Wang, et al., 2021). A total of five crude enzyme concentrations (5, 10, 15, 20, and 25 µl per ml of mycelium liquid medium) were evaluated, along with a control, on approximately 12 pairs of 3-day-old adult *B. dorsalis*. Adults in the control group were fed only sugar and yeast hydrolysate (Ortiz-Urquiza et al., 2013). Three replications were performed for each of the six treatments, with 12 pairs of *B. dorsalis* under a completely randomized design. The experimental environment was maintained at 25°C with a relative humidity of 60% and a 16:8 (L: D) photoperiod.

Statistical analysis

ANOVA was used in a completely randomized design to examine the percentage mortality and effectiveness of five concentrations of the cuticle-degrading enzymes (5, 10, 15, 20, and 25 L per ml of mycelium liquid medium). Minitab 8.1 was used to analyze the data. In a post-ANOVA analysis, Tukey's test was employed to differentiate the means and standard errors at a 5% probability level (Beris, 2021).

RESULTS

Gel electrophoresis analysis for enzymes

The molecular weight of the isolated enzyme was found to be 31 kDa using SDS-PAGE. The optimum conditions for enzyme activity were 35°C and pH 7.0. For SDS-PAGE gel electrophoresis, samples were loaded onto a 10% polyacrylamide gel with 0.1% SDS and a stacking gel.

The gels were stained with Coomassie blue following electrophoresis (Ola-Oladimeji et al., 2018).

Visualization of enzymes in the gel for their molecular masses

After de-staining the gel, the bands of the expected enzymes were characterized for their molecular weight. The protein bands were determined by comparing them to standard enzyme markers. The analysis of the gel, when compared with the standard enzyme markers, revealed that bands of 19 kDa and 50 kDa were detected in the enzyme samples from *B. bassiana* when subjected to SDS-PAGE. Several experiments revealed that the molecular mass of proteases ranges from 19 to 47 kDa. The secretion and activity of proteases vary among isolates and strains of *B. bassiana*. The pathogenicity and virulence of proteases depend on the isolates and host insects. Proteases facilitate causing direct toxicity in target insects (Zibaee et al., 2018) (Figure 1).

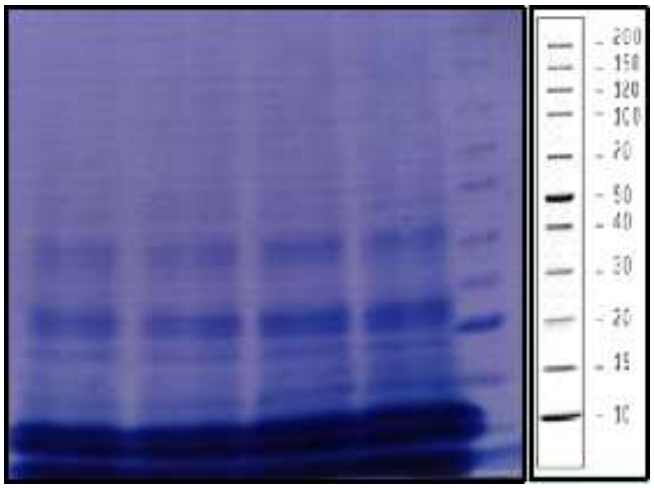

Figure 1. A visual band of 19 and 50 KDa proteases from *B. bassiana.*

After extracting cuticle-degrading enzymes from various isolates of *B. bassiana*, molecular weights ranging from 32 to 25 kDa were observed, indicating the presence of proteinase enzymes. When *B. bassiana* was cultured on different carbon and nitrogen sources, it produced two chymotrypsin-like serine proteinases. The molecular mass of the purified enzymes was found to be 32 kDa. Following the purification of enzymes, a molecular mass of 25 kDa was recorded at a pH range of 7 and a temperature of 35.8°C (Zibaee et al., 2011) (Figure 2).

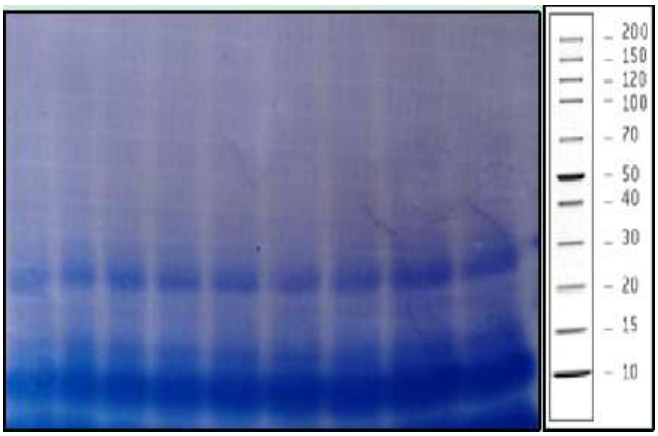

Figure 2. A visualize band of 32 and 25 KDa of chitinase from *B. bassiana.*

Microbial lipases are the most prevalent type of lipases produced for commercial purposes. Due to their enormous potential in various commercial fields, studies on lipases, particularly those of microbial origin, have increased recently. Extracellular lipases produced by *B. bassiana* have been found to successfully manage several insect pests through the release of fungal and bacterial lipases. One of these enzymes, called lipase L1, is a monomeric enzyme with a molecular weight of 24 kDa and has demonstrated the ability to cleave all the ester bonds present in trifolin (Figure 3).

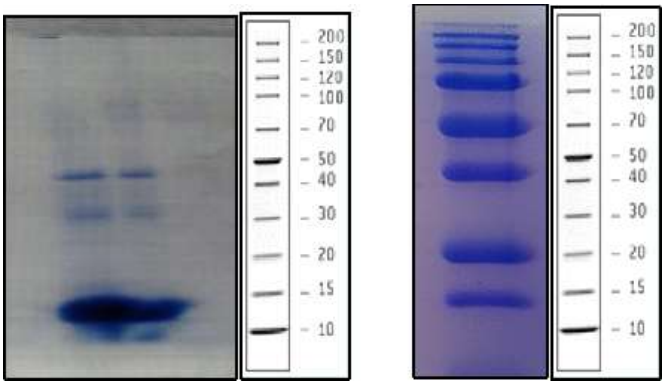

Figure 3. A visualize band of 34 and 49.7 KDa of lipases from *B. bassiana.*

Extracellular chitinase has been implicated as a virulence factor in fungal entomopathogenicity. The isoelectric points of the endochitinases could be classified as basic or acidic. SDS-PAGE allowed the separation of the acidic chitinases from *B. bassiana* into two major bands (43.5 and 45 kDa) with related N-terminal sequences. By using polyclonal antibodies to

the 45-kDa isoform and conducting ultrastructural immunocytochemistry, researchers observed the synthesis of chitinase during the penetration of the host cuticle. Only a minimal amount of chitinase was produced by infection structures on the cuticle surface and during the initial penetration of the cuticle. However, a significant accumulation of chitinase occurred in the areas of proteolytic breakdown, indicating that chitinase release is dependent on substrate accessibility (Ahmad et al., 2021).

Activity of CDE against second instar larvae of *B. dorsalis*

After 1 day of application, the pathogenicity of CDE from *B. bassiana* was recorded against second instar larvae of *B. dorsalis*. Significant mortality (F5, 12 = 29.1, P = 0.0039, α = 0.05) of larvae was observed after 1 day of treatment. Concentration-dependent mortality was recorded with the maximum mortality of 31.67±1.92% at 25µl/ml and the lowest mortality of 13.33±1.92% in a batch treated with 5µl/ml cuticle degrading enzymes, followed by the untreated one. The trend of larval mortality remained as treated 25 ≥ 20 ≥ 15 ≥ 10 ≥ 5 with mortality of 31.67±1.92%, 25±1.92%, 21.68±2.72%, 16.66±1.92%, 13.33±1.90%, respectively (Figure 4). The significant mortality (F5, 12 = 38.33±2.63, P = 0.000, α = 0.05) was noted after 2 days. The maximum mortality was recorded in *B. dorsalis* larvae at 38.33±2.63% treated with 25µl/ml cuticle degrading enzymes, and the lowest mortality was 21.66±2.50% at 5µl/ml, followed by the untreated group with mortality of 3.33±1.50%. The cuticle degrading enzymes from *B. bassiana* were found lethal after 3 and 4 days of treatment when significant mortality (F5, 12 = 15.81, P = 0.0001, α = 0.05) was recorded highest at 58.33±3.96% and 78.33±2.80% in a batch of insects that were treated with 25µl/ml, respectively. The lowest mortality, 43.32±2.96%, was recorded after 3 and 4 hours of treatment with 5µl/ml of cuticle degrading enzymes, followed by an untreated group with a mortality rate of 3.00±2.80% (Figure 4).

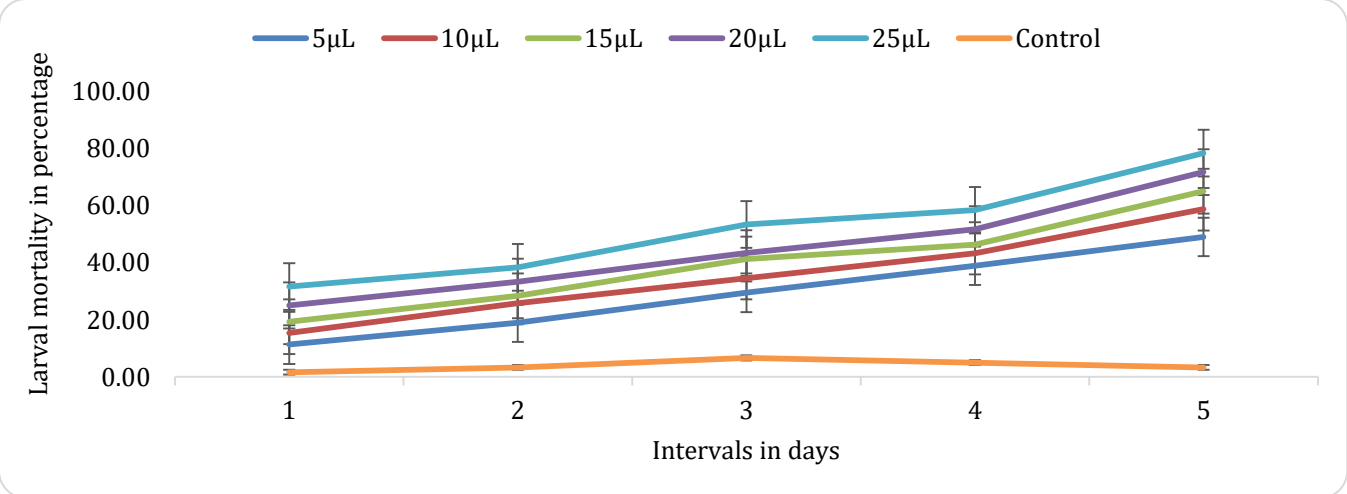

Figure 4. Percentage of larval mortality of *B. dorsalis* in different intervals.

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

After 2 days of treatment, adult emergence was recorded at 8.33±3.11% with 25µl of cuticle degrading enzymes, compared to 50±3.12% adult emergence in untreated pupae. Poor adult emergence was recorded after 3 days of treatment, at 3.33±2.72% with 25µl of cuticle After overviewing all the recorded data, the maximum adult emergence was recorded in the control group, followed by less concentrated ones, while the minimum adult emergence was noted in the 25µl concentrated degrading enzymes of *B. bassiana*, while the untreated group showed adult emergence at 60±2.99%. After 5 days of treatment with cuticle degrading enzymes, poor adult emergence was observed at 8.33±2.22% with 25µl of enzymes (F5, 12 = 23.93, P = 0.0002, α = 0.05), compared with an adult emergence of 65.00±5.77% in untreated pupae.

checks after 2, 3, 5, and 7 days of application (Figure 5).

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

After 1 day of application, significant mortality (F5, 12 =

22.31, P = 0.012, α = 0.05) was observed at 36.66±1.92% with 25µl, and the lowest mortality was recorded at 11±1.50% with 5µl, followed by untreated adults at 5±1.22%. Similarly, after 2 days of treatment, the highest mortality was observed at 41.66±2.43% with 25µl, and the lowest mortality was seen at 20±2.2% with 5µl, followed by untreated adults. The highest mortality of *B. dorsalis* adults was recorded after 3 and 4 days of treatment at 50±2.88% and 80±2.15% with 25µl, and the lowest mortality was noted after treatment at 30±2.88% and 36±2.33% with 5µl, respectively. The concentration and time of exposure showed trends with the highest and most significant mortality under laboratory conditions (Figure 6).

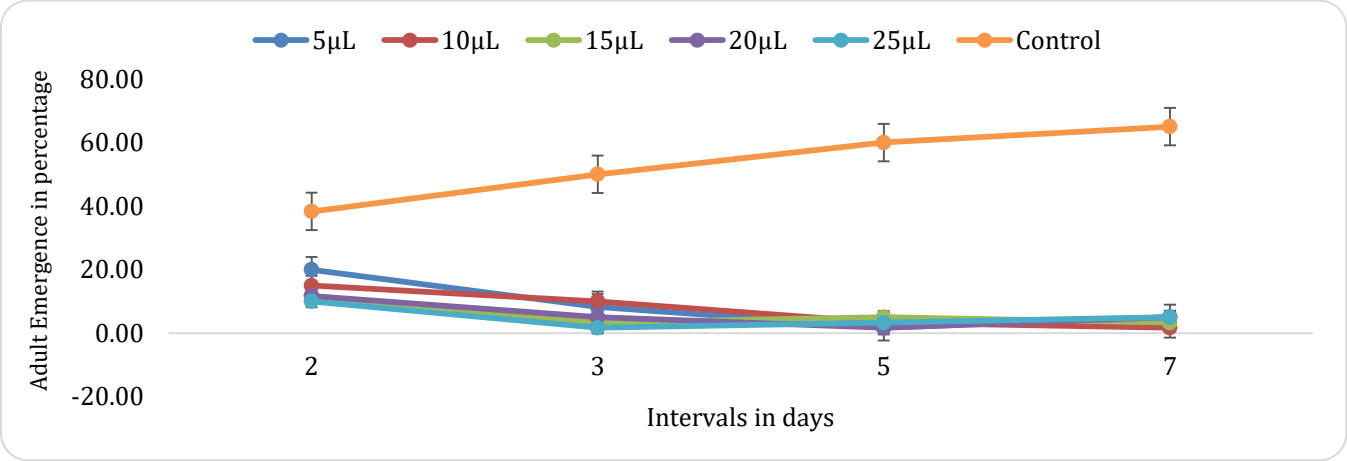

Figure 5. Percentage adult emergence of *B. dorsalis* at different intervals.

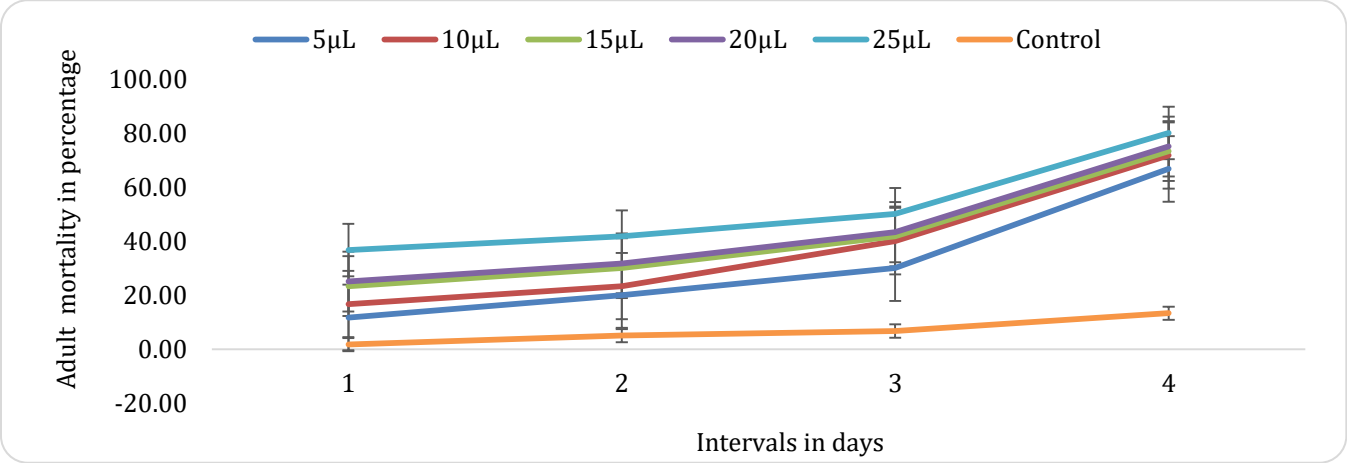

Figure 6. Percentage adult mortality at different intervals.

DISCUSSION

The current study was conducted for the production and extraction of cuticle degrading enzymes from various isolates of *B. bassiana*. After the extraction of crude enzymes, the liquid media was subjected to SDS-PAGE analysis, which revealed different bands on the gel, indicating the presence of various enzymes with different sizes in kilo Daltons (kDa).

The bands with sizes of 19 and 47 kDa showed the presence of protease enzymes in the *B. bassiana* culture. These protease enzymes exhibited a pH range of 7 to 12 and a temperature range of 35 to 45°C. They play a vital role in the hydrolysis and degradation of insect cuticles by inactivating antifungal proteins in the insects' epidermal layer. The molecular weight of the proteases was determined by comparing them to standardized enzyme markers. During the analysis, lipase enzymes with molecular weights of 24 kDa and 30 kDa were also observed, indicating the presence of lipase enzymes. Lipases facilitate the degradation of insect integuments

(Zibaee et al., 2018). The study involved the analysis of *B. bassiana* and *M. anisopliae* using chromatography and electrophoresis. The results revealed the presence of protease enzymes with extracted protein molecular weights of 15 kDa and 11 kDa for *B. bassiana* and *M. anisopliae*, respectively. Furthermore, the study discovered that during the early stages of infection, *B. bassiana* caused epicuticle degradation. However, once degraded, the cuticle's function was no longer necessary, and the entomopathogenic fungi only broke down lipid substrates during the cuticle penetration process (Zhang et al., 2012).

Görgün and Zengin claim that native-PAGE analyses were conducted without the use of SDS-PAGE. To identify the esterase bands in the samples, the gels were colored with α-naphthyl acetate. The same gels were stained with Coomassie Brilliant Blue and then subjected to silver staining to follow the development of several purification steps. Similar to the native-PAGE examinations, the samples were exposed to denatured SDS-PAGE (Singh et al., 2021).

The complexity of chitinase has been explored by Petrisor et al. (2017), who reported that the fungus secretes several chitinase enzymes. *B. bassiana* has two different chitinases that have been identified as being regulated and triggered by chitin breakdown products. Extracellular acidic chitinases have also been discovered on the cuticle surfaces of hosts during fungal invasion. Chitinolytic activity has been demonstrated in several entomopathogenic fungi and is thought to be significant for pathogenicity. However, the enzymes implicated in pathogenicity have been poorly described using crude chitinase preparations. Various molecular masses of chitinases were observed in different isolates of *M. anisopliae* and *B. bassiana*, including 43.5-kDa, 33-kDa, 45-kDa, and 110-kDa (Kim et al., 2010).

One of the most important components of an insect's cuticle is protein. Chitinase is one of the most significant and early enzymes involved in invasion, appearing after the proteolytic destruction of the target proteins and the ultimate exposure of chitin in the host cuticle. Extracellular proteases can be found in insect hemolymph. Several *B. bassiana* isolates showed significantly varying protease activity, and there were variations in the peak protease production period (Cheong et al., 2020). High levels of protease release were observed three days after inoculation in media containing various nitrogen sources, demonstrating that the media's elements do not necessarily determine when protease synthesis is at its peak. The proteolytic activity of cultures of *B. bassiana* isolates reduced with increasing culture age; this was most likely brought on by nutrient limitation or culture autolysis. Almost all isolates of *B. bassiana* included a 66-kDa protease, as well as two trypsin-like proteases with molecular weights of 30 kDa and 27 kDa. In *B. bassiana* conidia, a metalloprotease measuring 103 and 12 kDa was found.

An effective biocontrol agent is *B. bassiana*. It has been used for a very long time in agroecosystems and has proven to be quite successful in containing insect outbreaks. Due to its remarkable ability to enter host insects through the cuticle, this microbe heavily relies on a collection of hydrolyzing enzymes. Proteases are essential enzymes in the fungal penetration through the insect cuticle because studies have revealed that they are released early in the pathogenic process. Nearly all-virulent entomopathogenic fungi have higher proteolytic activity for the two proteases, trypsin-like (Pr2) and subtilisin-like (Pr1), which directly affect host insects. The genetic engineering of entomopathogenic fungi has emphasized the significance of proteases in virulence and how they can be used in the creation of novel mycoinsecticides.

The majority of research on the effects of entomopathogenic fungi has focused on the potential for inoculum production and death in target insects in order to distinguish between different fungal species or strains. It is necessary to take into account additional pathogenic elements, such as enzyme secretion capacity, and to make an effort to achieve their overexpression to ensure effectiveness. Proteases would be good candidates because they ensure fungal penetration, in addition to having direct toxicity to insects. Moreover, genetic engineering has become the mainstay of research on pathological management of insect pests, with the goal of making entomopathogenic fungi more virulent and effective at destroying insects. The findings of this investigation will aid our understanding of the level of protease production in various *B. bassiana* isolates, as well as the incubation time and Pr1 and Pr2 production by *B. bassiana* isolates. The role of proteases, lipases, and chitinase in the virulence of entomopathogenic fungi will now be the subject of fresh research.

CONCLUSION

Beauveria bassiana is an amorphous entomopathogenic

fungus capable of controlling many insect pests. *B. bassiana* is pathogenic to insects because it contains cuticle-degrading enzymes. The extracted cuticle-degrading enzymes were analyzed using the SDS-PAGE technique. After analysis, they were divided into three cuticle-degrading enzymes based on their molecular masses: protease, lipases, and chitinases, each having different molecular masses measured in kilo Daltons. The bands representing their molecular masses were clearly visible after destaining the gel, confirming the presence of these cuticle-degrading enzymes. These enzymes can enhance the activity of *B. bassiana* when mixed with the mycelial body of less pathogenic strains of *B. bassiana*. Therefore, these enzymes may play a significant role in causing pathogenicity against many insect pests. Protease, lipase, and chitinase, among other enzymes, are present in *B. bassiana* and can destroy the antifungal protein found in *B. dorsalis* cuticle. Utilizing *B. bassiana* can be a powerful biological control agent to manage *B. dorsalis*.

AUTHORS' CONTRIBUTION

S conceived the idea; S, MAQ and SS conducted research; FA, US and S worked on SDS-PAGE analysis; S, AM, HN, MK and U wrote the manuscript. All the authors proofread and approved the manuscript.

CONFLICT Of INTEREST

The authors declare no conflict of interest.

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