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Role of chitinase in the pathogenicity of Beauveria bassiana against the rice earhead bug Leptocorisa oratorius (Order: Hemiptera; Family: Alydidae)

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Abstract

Chitinase is a key virulence factor in entomopathogenic fungi, enabling host invasion through degradation of the chitin-rich insect cuticle. This study evaluated the role of chitinase produced by a native isolate of *Beauveria bassiana* (Bals.) Vuill. (Hypocreales; Cordycipitaceae) against the rice earhead bug, *Leptocorisa oratorius* (Fab.) (Hemiptera: Alydidae). Laboratory bioassays showed stage-dependent susceptibility, with first-instar nymphs exhibiting the highest mortality (84%) at 9 days after treatment, while egg hatchability was reduced to 72%, indicating partial ovicidal activity. Chitinase production was optimized under different cultural and environmental conditions, with maximum activity observed on the fifth day of incubation. Peptone (0.07%), colloidal chitin (0.4%), and $FeSO_4$ (0.015%) significantly enhanced enzyme production. Optimal fungal growth occurred at pH 5 and 28°C, whereas maximum chitinase activity was recorded at pH 5 and 37°C. Partial purification by ammonium sulfate fractionation resulted in the highest specific activity (2.60 U mg^{-1}), purification fold (2.68), and yield (14.34%) at 75% saturation. SDS-PAGE analysis revealed a molecular weight of approximately 60 kDa. The results confirm the importance of chitinase in *B. bassiana* pathogenicity and highlight its potential for developing eco-friendly biopesticides for sustainable management of rice insect pests.

1. Introduction

Entomopathogenic fungi are among the most promising biological control agents for sustainable management of insect pests in agricultural ecosystems. Their ability to infect insects through direct cuticular penetration, regulate pest populations, and reduce dependence on chemical insecticides makes them important components of integrated pest management (IPM) programs (Vega et al 2012; Mascarin& Jaronski 2016; Jaronski 2024).Among these, *Beauveria bassiana* (Bals.) Vuill. (Hypocreales; Cordycipitaceae) is one of the most widely studied and commercially exploited fungal entomopathogens due to its broad host range, ecological adaptability, and high insecticidal potential (Zimmermann 2007; Vega et al 2012). The fungus has been successfully used against numerous insect pests across different orders and is considered an effective and environmentally safe alternative to synthetic insecticides (Mascarin& Jaronski 2016; Shah & Pell 2003).

Infection by *B. bassiana* begins with adhesion of conidia to the insect cuticle, followed by germination, penetration, and colonization of host tissues. The insect cuticle acts as the primary barrier to fungal invasion and is degraded through the combined action of extracellular hydrolytic enzymes, including chitinases, proteases, and lipases (St. Leger et al 1996; Ortiz-Urquiza &Keyhani 2013). These enzymes facilitate breakdown of structural components of the cuticle and enable fungal entry into the host body. Among the cuticle-degrading enzymes, chitinase plays a particularly critical role by hydrolyzing chitin, a major structural component of the insect exoskeleton (Singh et al. 2014; Rathore & Gupta 2015). Variation in enzymatic activity is often associated with differences in virulence among fungal isolates, and enhanced enzyme production has been linked to increased pathogenicity and host mortality (Ortiz-Urquiza & Keyhani 2013; Mascarin et al 2024).The effectiveness of *B. bassiana* also varies among isolates depending on ecological adaptation, environmental tolerance, and compatibility with local host

populations. Native isolates frequently exhibit improved performance under local agro-climatic conditions, highlighting the importance of evaluating indigenous fungal strains for biological control programs (Ramanujam et al 2011; Biryol et al 2025).

Rice (*Oryza sativa* L.) is a major staple crop that sustains nearly half of the world's population, yet its productivity is constrained by several insect pests. Among the rice pests, the rice earhead bug, *Leptocorisa oratorius* (Fab.) (Hemiptera: Alydidae), is a serious sucking pest that attacks developing grains during the milky stage, resulting in chaffy grains, reduced grain weight, and significant yield losses (Baharally & Simon 2014; Tiwari et al 2014; Gupta et al 2018). Management of this pest largely depends on chemical insecticides; however, environmental contamination, pest resistance, and non-target effects necessitate the development of sustainable alternatives. Although *B. bassiana* has demonstrated considerable potential as a biological control agent against various agricultural pests, information on the pathogenicity of native isolates against *L. oratorius* and their associated enzymatic mechanisms remains limited. Understanding stage-specific susceptibility of the pest and the role of chitinase in fungal pathogenicity is essential for developing effective biological control strategies.Therefore, the present study evaluated the pathogenic potential of a native isolate of *Beauveria bassiana* against different life stages of *Leptocorisa oratorius* and characterized its chitinase activity associated with host infection.

2. Materials and Methods

2.1 Collection and laboratory rearing of *Leptocorisa oratorius*

Adults of *Leptocorisa oratorius* (Fab.) of both sexes were collected from rice fields at the Instructional-cum Research Farm, Assam Agricultural University (AAU), Jorhat, Assam, India, using sweep nets and manual hand picking. Collected insects were transported to the laboratory in ventilated plastic containers containing fresh rice panicles. Laboratory culture was established by introducing one gravid male-female pair into a plastic oviposition chamber (20 cm diameter; 8-inch tub) containing rice seedlings maintained in water. The chamber was covered with fine nylon mesh to prevent escape and facilitate oviposition. After egg deposition, adults were removed to avoid cannibalism or disturbance.

Egg-bearing leaves were maintained until hatching. Newly emerged first instar nymphs were transferred using fine camel hair brush into small water filled glass bottles (50 ml capacity) containing fresh rice leaves and panicles. Each bottle was enclosed within a cylindrical acrylic rearing cage (height 25 cm, diameter 10 cm) with the upper opening covered using muslin cloth to allow ventilation and prevent insect escape. Food material was replaced after each molt to ensure continuous nutrition availability. Insects were observed daily to monitor development from first instar to adult stage. Cultures were maintained for at least one generation prior to experimentation to obtain synchronized cohorts of uniform age.

All insects were maintained under controlled laboratory conditions of 25 ± 2°C temperature, 70 ± 5% relative humidity and 12:12 h (light:dark) photoperiod. These conditions are suitable for laboratory rearing of hemipteran pests (Southwood & Henderson, 2000). Different life stage of *L. oratorius* is provided in Supplementary Fig. 1.

A native isolate of *Beauveria bassiana* (Bals.) Vuill. (Isolate KR855715) was obtained from the Department of Entomology, Assam Agricultural University, Jorhat, Assam, India. The isolate was originally recovered from an infected adult tea mosquito bug collected from the Experimental Garden for Plantation Crops, AAU, Jorhat (25.45° N, 93°30' E) and previously evaluated for pathogenicity (Mudoi et al 2017).

The fungus was maintained on potato dextrose agar (PDA; HiMedia Laboratories, India). Cultures were incubated at 25 ± 2°C and 85% relative humidity for 10 days to obtain profuse sporulation. Subsequent subculturing was carried out periodically to maintain viability.For bioassays, conidia were harvested by gently scraping the sporulating culture and suspending spores in sterile distilled water containing Tween 80 (0.023% v/v) as a surfactant to ensure uniform dispersion (Lacey et al 2015). The suspension was filtered through sterile muslin cloth to remove mycelial fragments.Conidial concentration was determined using a Neubauer hemocytometer and adjusted to 1×10^7 conidia ml^{-1}. Viability was assessed by germination test on PDA, and suspensions showing > 90% germination after 24 h were used for experiments (Goettel & Inglis 1997).

2.3.1 Egg hatchability assay

Freshly laid eggs (< 24 h old) were collected along with egg-bearing rice leaves and placed individually in 100 ml conical flasks containing water and fresh rice panicles. Each flask was enclosed within a ventilated insect chimney covered with muslin cloth.Eggs were sprayed with *B. bassiana* conidial suspension (1×10^7 conidia ml^{-1}) using a calibrated hand atomizer delivering 25 ml per flask. Control groups were treated with sterile distilled water containing Tween 80 (0.023% v/v).Each flask containing 20 eggs constituted one replication, and five replications were maintained per treatment. Egg hatch was recorded daily, and percent hatchability was calculated.

2.3.2 Nymph and adult mortality assay

First instar nymphs of uniform age were transferred to plastic beakers (500 ml) containing rice seedlings. Later instar nymphs and adults were maintained similarly. Each beaker was enclosed within ventilated insect rearing chimneys.Insects were sprayed with conidial suspension (1×10^7 conidia ml^{-1}) at 25 ml per beaker. Control insects received sterile distilled water with Tween 80.Each beaker containing 10 insects represented one replication, with five replications per treatment. Mortality was recorded at 3, 6, and 9 days after treatment (DAT). Dead insects were incubated in moist chambers at 25°C $\pm$ 1to confirm mycosis based on fungal outgrowth.

2.4.1 Preparation of colloidal chitin

Colloidal chitin was prepared from prawn shell powder following acid hydrolysis and precipitation (Roberts & Selitrennikoff 1988).nFive grams of prawn shell powder was slowly added to 90 ml concentrated hydrochloric acid under constant stirring and maintained at 4°C for 24 h. The mixture was filtered into 500 ml ice cold ethanol and left overnight to precipitate chitin. Precipitated chitin was collected by centrifugation (4200 rpm, 10 min, 4°C) and washed repeatedly with distilled water until pH 5.0. The colloidal chitin obtained was stored at 4°C.

2.4.2 Optimization of growth conditions for chitinase production

2.4.2.1 Incubation time:

Chitinase production was evaluated in potato dextrose broth (PDB). Chitinase production is measured by measuring chitinase activity in the cultures. Cultures were incubated for 7 days, and enzyme activity was measured daily to determine optimum incubation period.

2.4.2.2 Nutritional factors:

PDB was supplemented with different concentrations of peptone (0.05%, 0.07%, 0.1%, 0.12% & 0.15%; w/v); colloidal chitin (0.1%, 0.2% 0.3%, 0.4% & 0.5%; w/v) and Ferrous sulphate ($FeSO_4 \cdot 7H_2O$) (0.005%, 0.01%, 0.015% 0.02% & 0.025%; w/v). Spore suspension (0.5 ml) was inoculated into each dilution of 100 ml of supplemented media in 250 ml flasks and incubated at 30°C and 150 rpm for 5 days in a rotary shaker incubator. After sufficient growth obtained, the chitinase activity was estimated in the cell free extract.

2.4.2.3 Environmental parameters:

Along with the different concentrations of media-ingredients the growth temperature and media pH were optimized. The cultures were incubated at different growth temperature, 25ºC, 28ºC, 30ºC, 35ºC, 37ºC, 40 ºC and media pH, 2, 3, 4, 5 and 6 for 5 days and subsequently tested for the chitinase activity.

Optimization of both cultural and reaction parameters was carried out using a one-factor-at-a-time (OFAT) approach. Each variable was independently evaluated while maintaining other factors constant. The optimum levels of nutritional (peptone, colloidal chitin, $FeSO_4$) and environmental parameters (pH and temperature) were selected based on maximum chitinase activity.

After 5 days fermentation, culture was centrifuged (4000 rpm, 10 min, 4°C) and supernatant obtained served as crude enzyme. Chitinase activity was determined following Elawati et al (2018) and estimated using the following formula.

$$Unit\ of\ activity\ \left(\mathrm{U\ ml}^{-1}\right) = \frac{Absorbance\ control - Absorbance\ sample}{0.001} \times \frac{1}{60}$$

Optimization of reaction conditions was carried out to determine the optimum temperature and pH for chitinase activity of *B. bassiana*. These parameters differ from growth conditions, as they influence enzyme efficiency during the assay rather than enzyme production during fungal cultivation. The chitinase activity was evaluated at different reaction temperatures (20ºC, 25ºC, 30ºC, 35ºC, 37ºC, 40ºC & 45ºC) and pH levels (3 to 8) under standardized assay conditions, and the optimum conditions were selected based on maximum enzyme activity.

For purification of chitinase from *B. bassiana*, the fungus was cultured under optimized nutritional and physicochemical conditions and harvested after five days of incubation, corresponding to maximum enzyme activity. The culture was centrifuged (4000 rpm, 10 min at 4^{O}C) and the supernatant obtained was used as the crude enzyme source. The crude enzyme was subjected to ammonium sulphate ($(NH_4)_2SO_4$) fractionation at different saturation levels (55%, 65%, 75%, 85%, and 95% w/v) at 4°C, incubated overnight, and centrifuged (4,500 rpm, 30 min). Each fraction was subjected to dialysis against 1,000 mL of 0.1 M citrate buffer (pH 5.0) using a dialysis membrane with a molecular weight cut-off of 10 kDa. Dialysis was carried out at 4°C with six buffer changes over a 24 h period. Dialyzed samples were stored at 4°C until further use.

2.8 Protein Estimation

Protein concentration was measured using the Lowry method (Lowry et al 1951) with BSA as standard. Reagents were prepared as follows: 2% Na_2CO_3 in 0.1 N NaOH (Reagent A), 0.5% $CuSO_4 \cdot 5H_2O$ in 1% sodium potassium tartrate (Reagent B), and freshly mixed Reagents A and B (50:1) as alkaline $CuSO_4$ solution (Reagent C). Folin-Ciocalteau reagent (1 N, Reagent D) was diluted from commercial stock. BSA stock (1 mg/mL) was prepared in distilled water, and standards of 0–160 µg/mL were made in duplicate. To each standard, unknown, and blank, 5 mL Reagent C and 0.5 mL Reagent D were added, mixed, and incubated in the dark for 30 min. Absorbance was measured at 660 nm, and protein concentrations were calculated from a standard curve. These values were used to determine chitinase specific activity (U/mg), fold purification, and yield (%) using: specific activity = total activity / total protein, purification fold = final / initial specific activity and yield (%) = (final / initial total activity) × 100.

Purified enzyme was analyzed through SDS-PAGE according to Laemmli (1970). SDS-PAGE was performed by casting a 12% resolving gel (10 ml) using distilled water (3.3 ml), Tris buffer (1.5 M, pH 8.8; 2.5 ml), acrylamide–bisacrylamide (4.0 ml), 10% SDS (100 µl), 10% APS (100 µl), and TEMED (4 µl), followed by polymerization for 20–30 min. A 4% stacking gel (5 ml) was then prepared (distilled water 1.68 ml, Tris buffer 1.25 ml, acrylamide–bisacrylamide 2.0 ml, 10% SDS 50 µl, 10% APS 25 µl, TEMED 7 µl), poured over the resolving gel, and allowed to polymerize after comb insertion. The gel was assembled in an electrophoresis unit with 1× running buffer. Protein samples were mixed with sample buffer (2:1), boiled, combined with loading dye, and 25 µl was loaded per well. Electrophoresis was carried out at 150 V for 45–60 min. The gel was stained with Coomassie Brilliant Blue, destained overnight (methanol:acetic acid:water, 50:10:40), and protein bands were visualized for molecular weight estimation.

2.10 Statistical analysis

All experiments were conducted following a completely randomized design (CRD). Data were subjected to two-way analysis of variance (ANOVA), considering developmental stages and days after treatment (DAT) as factors. Mean separation was performed using Duncan's Multiple Range Test (DMRT) at $P < 0.05$ (Gomez & Gomez 1984). The corresponding F-values, degrees of freedom (df), and significance levels are presented in the Results section

3. Results

The mortality of *Leptocorisa oratorius* varied significantly across developmental stages and increased progressively with time after treatment (DAT). Egg hatchability was recorded at 72.00%, indicating partial ovicidal effect of the treatment. A highly significant difference in mortality among stages was observed ($P < 0.05$), indicating stage-dependent susceptibility to the treatment. Among the different stages, the first instar nymphs exhibited the highest susceptibility, recording mortality of 66.00%, 72.00%, and 84.00% at 3, 6, and 9 DAT, respectively, which were significantly higher than most other stages. The second instar also showed comparatively high mortality (62.00–80.00%) and remained statistically at par with the first instar at later observation intervals (Table 1.). In contrast, later instars and adults exhibited lower susceptibility. The third and fourth instars showed moderate mortality ranging from 46.00% to 70.00%, with gradual increases over time. The fifth instar recorded mortality between 42.00% and 62.00%, while the adult stage showed the lowest mortality, ranging from 38.00% at 3 DAT to 58.00% at 9 DAT, and remained significantly lower than early instars.

A consistent increase in mortality from 3 to 9 DAT was observed across all stages, with the highest relative increase recorded in adults (52.63%) and fourth instar (52.17%), followed by fifth instars (47.62%) and third instars (43.48%). Early instars showed comparatively lower percentage increases, reflecting already high initial mortality levels. The results demonstrate that the treatment was more effective against early nymphal stages, while later instars and adults were relatively less susceptible, though mortality increased with exposure duration.

Table 1

Effect of *Beauveria bassiana* (1×10^7 conidia/ml) on egg hatchability and stage wise mortality of *L. oratorius* across different days after treatment (DAT)

Stages	Egg hatchability (%)	Mortality (%)			% increase in mortality (3-9DAT)
		3DAT	6DAT	9DAT	
Egg	72.00 ± 3.74 (58.05)	-	-	-	
Firstinstar	-	66.00 ± 2.45 ^{c}	72.00 ± 2.00 ^{c}	84.00 ± 4.00^{c}	27.27
Secondinstar	-	62.00 ± 3.74^{bc}	72.00 ± 5.83 ^{c}	80.00 ± 4.47^{bc}	29.03
Thirdinstar	-	46.00 ± 4.00 ^{a}	54.00 ± 4.00 ^{b}	66.00 ± 5.10 ^{ab}	43.48
Fourthinstar	-	46.00 ± 4.00 ^{ab}	60.00 ± 3.16 ^{b}	70.00 ± 3.16^{abc}	52.17
Fifthinstar	-	42.00 ± 3.74 ^{a}	56.00 ± 2.44 ^{b}	62.00 ± 4.90 ^{a}	47.62
Adult	-	38.00 ± 3.74 ^{a}	46.00 ± 5.10 ^{a}	58.00 ± 5.83 ^{a}	52.63

No. of replications = 5; number of nymphs and adults per replication = 10; number of eggs per replication = 20. Values represent mean ± SE. Means within a column followed by different letters differ significantly according to DMRT at P<0.05. Two–way ANOVA revealed significant effects of developmental stage and days after treatment on mortality (P<0.001)

Chitinase production by *Beauveria bassiana* varied significantly with incubation time, nutrient composition, pH, and temperature.

3.2 Effect of incubation time

Chitinase activity increased progressively during the initial growth period, reaching a peak of 0.25 U ml^{-1} on the 5th day of incubation, followed by a sharp decline thereafter (Fig. 1a). The lowest activity was recorded on the 7th day (0.08 U ml^{-1}), indicating that enzyme production is closely associated with the active growth phase of the fungus.

3.3 Effect of nitrogen source (peptone)

Supplementation of the basal medium with peptone significantly enhanced chitinase production. The highest enzyme activity (1.05 U ml^{-1}) was recorded at 0.07% peptone, followed by 0.1% (0.93 U ml^{-1}), after 5 days of incubation. Further increase in peptone concentration resulted in a marked decline in enzyme activity, with the lowest activity (0.07 U ml^{-1}) observed at 0.05%, indicating an optimum nitrogen requirement for enzyme synthesis (Fig. 1b).

3.4 Effect of colloidal chitin

Colloidal chitin acted as an effective inducer of chitinase production. Enzyme activity remained relatively stable (0.72–0.73 U ml^{-1}) up to 0.2% concentration, followed by a significant increase, reaching a maximum of 1.09 U ml^{-1} at 0.4% chitin. A subsequent decline in activity at higher concentrations (0.93 U ml^{-1} at 0.5%) suggests possible substrate inhibition (Fig. 1c).

3.5 Effect of ferrous sulphate ($FeSO_4 \cdot 7H_2O$)

The addition of ferrous sulphate significantly influenced chitinase production. Enzyme activity increased with increasing iron concentration, reaching a maximum of 1.31 U ml^{-1} at 0.015% $FeSO_4 \cdot 7H_2O$, followed by a decline at higher concentrations (Fig. 1d). This indicates a stimulatory effect of iron at optimal levels, beyond which inhibitory effects may occur.

3.6 Effect of pH and temperature on chitinase production (Growth conditions)

The pH and temperature of the growth medium significantly influenced chitinase production by *B. bassiana*. Maximum enzyme activity (2.23 U ml^{-1}) was observed at pH 5, while lower activities were recorded at both acidic and alkaline conditions, indicating that slightly acidic pH favors enzyme production. Similarly, chitinase production increased with temperature, reaching a peak at 28°C, followed by a decline at higher temperatures (Fig. 1e, f). This suggests that fungal growth and enzyme synthesis are optimal under moderately warm and slightly acidic conditions.

3.7 Optimization of reaction conditions (Enzyme activity)

In contrast to growth conditions, reaction temperature and pH influenced the catalytic efficiency of the enzyme during assay. Chitinase activity remained relatively low and stable (0.12–0.14 U ml^{-1}) across the temperature range of 20–35°C, followed by a sharp increase to a maximum (2.23 U ml^{-1}) at 37°C. Thereafter, a steep decline in activity was observed, reaching 0.09 U ml^{-1} at 45°C (Fig. 2).Similarly, reaction pH markedly affected enzyme activity. Chitinase activity increased sharply from 0.33 U ml^{-1} at pH 3 to a peak of 2.23 U ml^{-1} at pH 5, followed by a gradual decline to 0.71 U ml^{-1} at pH 8 (Fig. 2).

3.8 Purification *B. bassiana*-chitinase

For purification of chitinase from *B. bassiana*, the fungus was cultured under optimized nutritional and physicochemical conditions and harvested after five days of incubation, corresponding to maximum enzyme activity. The culture was centrifuged (4000 rpm, 10 min at $4^{O}C$) and the supernatant obtained was used as the crude enzyme source. The crude enzyme was subjected to $(NH_4)_2SO_4$ fractionation at different saturation levels (55%, 65%, 75%, 85%, and 95% w/v) at 4°C, incubated overnight, and centrifuged (4,500 rpm, 30 min). Each fraction was subjected to dialysis against 1,000 mL of 0.1 M citrate buffer (pH 5.0) using a dialysis membrane with a molecular weight cut-off of 10 kDa. Dialysis was

carried out at 4°C with six buffer changes over a 24 h period. Dialyzed samples were stored at 4°C until further use.

The dialyzed fractions were analyzed for chitinase activity and protein content. Based on these values, specific activity (U mg^{-1}), purification fold, and yield (%) were calculated, and the fraction showing maximum activity was considered for further analysis.

3.9 Specific activity

The chitinase activity of crude extract and different $(NH_4)_2SO_4$ fractions varied across the saturation levels. The crude enzyme exhibited the highest total activity (1115 U ml^{-1}) compared to all fractionated samples. Among the $(NH_4)_2SO_4$ fractions, the highest enzyme activity was observed at 75% saturation (160 U ml^{-1}), followed by 95% (120 U ml^{-1}), 85% (72 U ml^{-1}), 65% (65 U ml^{-1}), and 55% (50 U ml^{-1}) (Table 2).

The specific activity of crude *B. bassiana* chitinase was 0.97 U mg^{-1}. Among the fractions, the highest specific activity was recorded in the 75% fraction (2.60 U mg^{-1}), followed by 95% (2.35 U mg^{-1}), 65% (2.16 U mg^{-1}), 55% (2.00 U mg^{-1}), and 85% (1.30 U mg^{-1}) (Table 2).

Table 2
Ammonium Sulfate ($(NH_4)_2SO_4$) Fractionation and corresponding protein content, activity, and purification of *Beauveria bassiana* chitinase

$(NH_4)_2SO_4$ (%)	Total activity (U)	Protein concentration (mg/ml)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
55	50	0.05	25	2.00	2.06	4.48
65	65	0.06	30	2.16	2.22	5.82
75	160	0.12	60	2.60	2.68	14.34
85	72	0.11	55	1.30	1.34	6.45
95	120	0.10	51	2.35	2.42	10.76
Crude sample	1115	2.30	1150	0.97	1	100

3.10 Purification fold

The purification fold increased with $(NH_4)_2SO_4$ fractionation, reaching a maximum of 2.68-fold at 75% saturation. This was followed by 2.42-fold at 95%, 2.22-fold at 65%, 2.06-fold at 55%, and 1.34-fold at 85% saturation (Table 2).

3.11 Yield

The yield of chitinase varied among the different fractions. The highest yield (14.34%) was obtained at 75% $(NH_4)_2SO_4$ saturation, while the lowest yield (4.48%) was recorded at 55% saturation (Table 2).

3.12 Molecular weight

The purity of the enzyme was examined by SDS-PAGE and determined the molecular weight at the highest purified fraction *i.e.* 75% ammonium salt fraction (Fig. 3). The molecular weight of purified chitinase was around 60 kDa.

4. Discussion

The increasing demand for eco-friendly and sustainable agriculture has necessitated the reduction of chemical pesticide use, thereby promoting biological alternatives such as entomopathogenic fungi. Among these, *Beauveria bassiana* has gained considerable importance due to its effectiveness against a wide range of insect pests and its adaptability to diverse agroecosystems (Zimmermann 2007; Vega et al 2012; Mascarin and Jaronski 2016; Jaronski 2024). The infection process of *B. bassiana* largely depends on its ability to penetrate the insect cuticle, which is primarily composed of chitin and proteins. This penetration involves both mechanical force exerted by fungal hyphae and enzymatic degradation mediated by chitinases, proteases, and lipases (St. Leger et al 1996; Ortiz-Urquiza and Keyhani 2013). Among these enzymes, chitinase plays a critical role in degrading the cuticular barrier and facilitating host invasion, thereby directly influencing fungal virulence (Singh et al 2014; Rathore and Gupta 2015; Zhang et al 2022).

In the present study, *B. bassiana* exhibited significant pathogenicity against different developmental stages of *Leptocorisa oratorius*, with higher susceptibility observed in nymphal stages compared to adults. The reduced egg hatchability and progressive increase in mortality across stages indicate successful fungal infection. The greater susceptibility of nymphs may be attributed to their relatively softer cuticle and weaker defense mechanisms, which is consistent with earlier findings (Mehinto 2014; Chandran and Subramanian 2016). Similar stage-dependent susceptibility patterns have been widely reported, supporting the effectiveness of entomopathogenic fungi in integrated pest management systems.

The effectiveness observed in the present study may also be attributed to the use of a native isolate of *Beauveria bassiana*, which is likely better adapted to local agro-climatic conditions and host populations. Native isolates are often reported to exhibit enhanced virulence, environmental tolerance, and persistence under field conditions compared to non-native or commercially introduced strains. This improved performance is primarily due to their long-term adaptation to local environmental factors such as temperature, humidity, and host availability. Moreover, native strains may possess greater compatibility with indigenous insect populations, resulting in higher infection efficiency and survival. Similar observations have been reported by Ramanujam et al (2011) and more recently by Biryol et al (2025), emphasizing the importance of region-specific isolates in biological control programs.

Optimization of cultural conditions revealed that incubation time, nutrient composition, pH, and temperature significantly influenced chitinase production. Maximum enzyme activity was observed on the 5th day of incubation, followed by a decline, indicating that enzyme production is closely associated with the active growth phase of the fungus. Similar observations have been reported by Mishra et al (2013) and Priya et al (2016), suggesting that chitinase production is growth-phase dependent.

Among the nutritional factors, peptone significantly enhanced chitinase production, with optimum activity observed at 0.07%, followed by a decline at higher concentrations. This may be attributed to nitrogen catabolite repression, where excess nitrogen suppresses enzyme synthesis. Similar enhancement of chitinase production by organic nitrogen sources has been reported in *Trichoderma harzianum* (Sandhya et al 2004). Colloidal chitin, acting as both substrate and inducer, exhibited a concentration-dependent effect, with maximum activity at 0.4%, followed by a decline at higher concentrations, possibly due to substrate inhibition (Dhar and Kaur 2010; Karthik et al 2022). Likewise, iron supplementation enhanced enzyme activity up to an optimum concentration, beyond which inhibitory effects were observed, indicating its role as a cofactor in enzymatic processes.

The influence of physicochemical factors further indicated that slightly acidic pH (pH 5) and moderate temperature (28°C) were optimal for chitinase production. These findings are in agreement with earlier reports (Zhang et al 2004; Patidar et al 2005; Priya et al 2016). Recent studies also highlight that environmental conditions play a crucial role in regulating fungal metabolism and enzyme synthesis (Singh et al 2021; Kumar et al 2023).In contrast, reaction conditions determine enzyme efficiency rather than production. The present study demonstrated that chitinase activity was highest at 37°C and pH 5, indicating that catalytic efficiency differs from production conditions. Such differences are commonly observed due to variations in enzyme structure and stability under assay conditions. Similar findings have been reported by Pinnamaneni et al (2010) and supported by recent enzymological studies (Zhao et al 2022). The sharp decline in activity beyond the optimum temperature further suggests thermal denaturation of the enzyme.

Partial purification using ammonium sulphate fractionation revealed that the 75% saturation fraction yielded maximum enzyme activity, specific activity, purification fold, and yield. This indicates that chitinase precipitates optimally at this saturation level, which is consistent with earlier reports (Ueda et al 2005; Gangwar et al 2016). The increase in specific activity confirms effective enrichment of the enzyme following removal of impurities.

SDS-PAGE analysis revealed a distinct protein band corresponding to approximately 60 kDa. This molecular weight is consistent with previous reports of chitinase from *Beauveria bassiana* (Kang et al 1999; Fleuri et al 2009), indicating structural similarity and conservation of the enzyme across different isolates.

5. Conclusion

The study highlights that chitinase produced by *Beauveria bassiana* plays a crucial role in insect infection and can be effectively enhanced and partially purified under optimized conditions. The high activity and specificity of the enzyme, particularly in the 75% ammonium sulfate fraction, suggest its potential as a bioactive component for enzyme-based biopesticides. These findings provide a foundation for future development of chitinase driven biopesticides, offering a sustainable, targeted, and environmentally friendly approach for managing rice earhead bug and other insect pests. Further research on large-scale production, formulation, and field application could make chitinase based biopesticides a viable tool in integrated pest management. In addition, combining chitinase with other entomopathogenic factors or microbial metabolites could enhance its efficacy against a broader spectrum of pests. The development of stable, effective chitinase formulations may also reduce reliance on chemical pesticides, contributing to safer and more sustainable agricultural practices.

Declarations

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Data Availability: *The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.*

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Figures

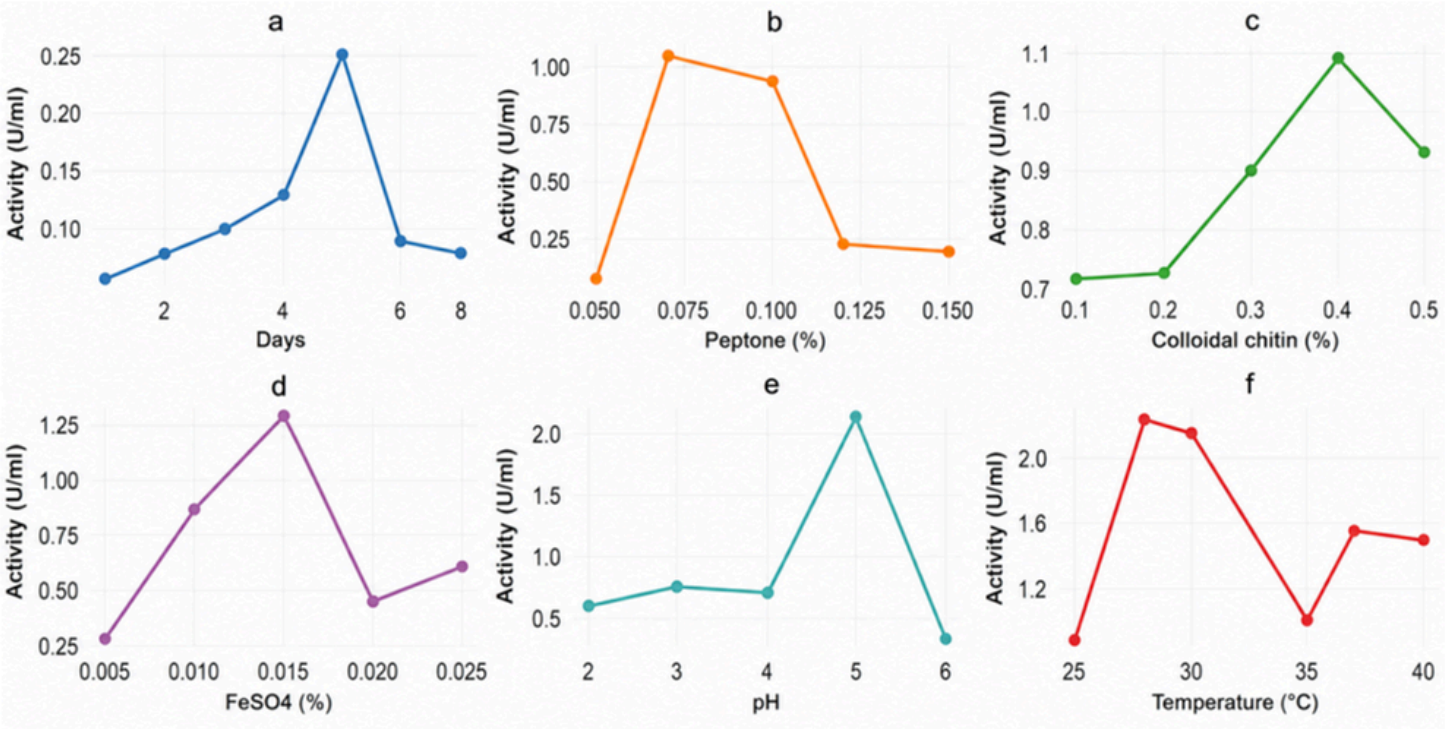


Figure 1

Effect of incubation time and physicochemical factors on chitinase activity of *Beauveria bassiana*. (A) Effect of incubation time (days); (B) effect of peptone concentration (%); (C) effect of colloidal chitin (%); (D) effect of $FeSO_4$ concentration (%); (E) effect of growth medium pH; and effect of temperature (°C) on chitinase activity. Values represent mean enzyme activity (U/ml) based on three replications

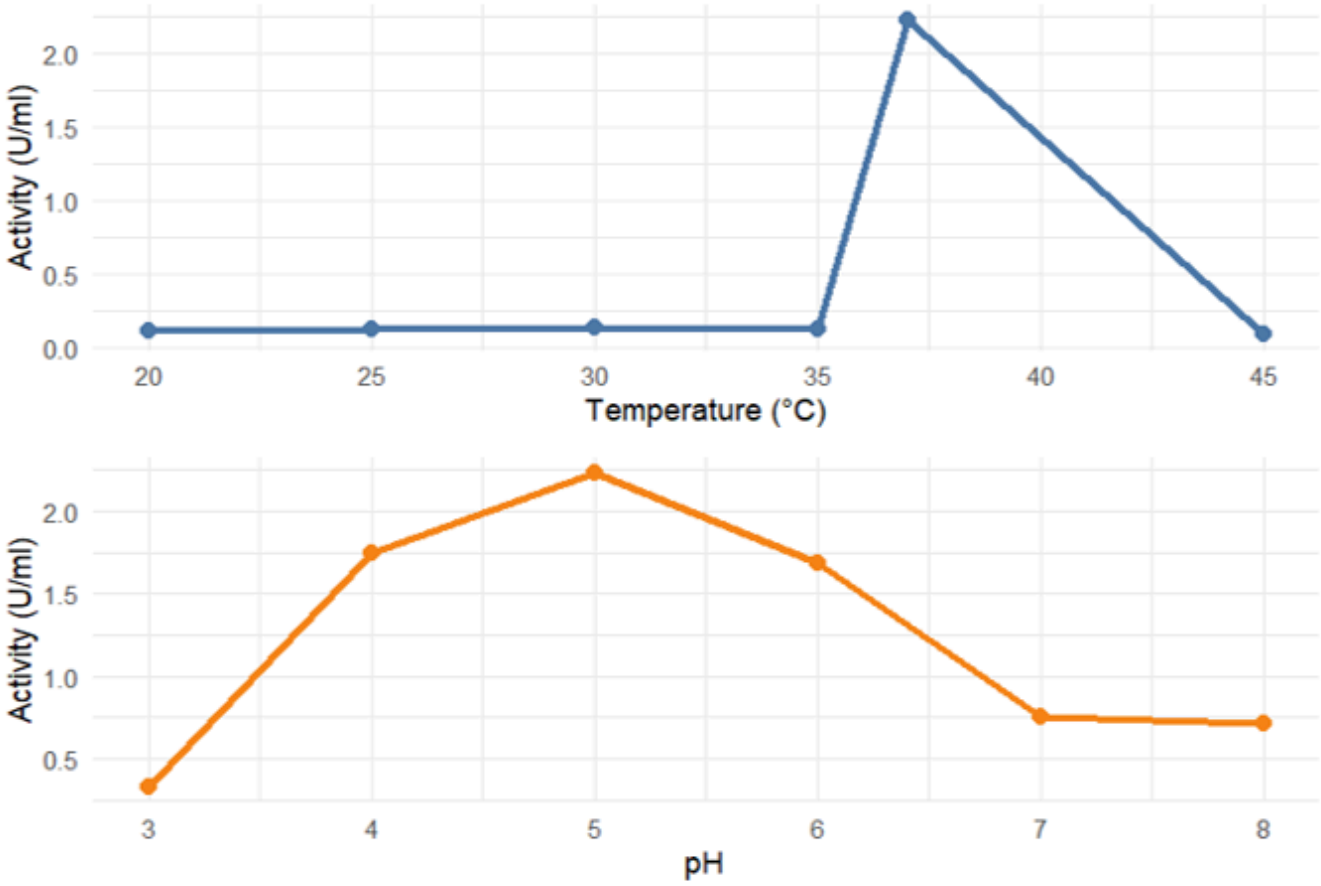


Figure 2

Relative chitinase activity (%) of *Beauveria bassiana* as influenced by reaction temperature and pH

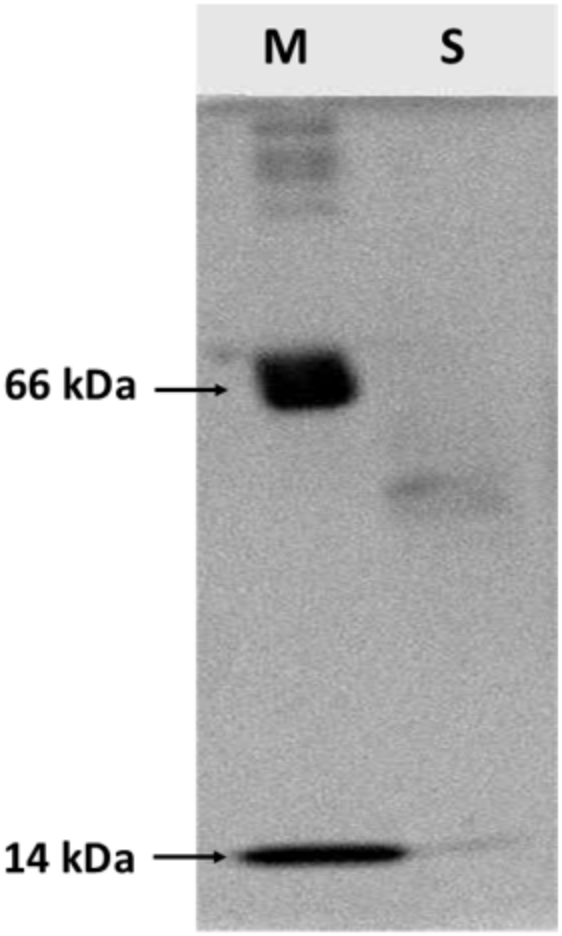


Figure 3

SDS PAGE Gel showing molecular weight of sample (S) as 60 kDa compared to the marker (M): Lysozyme (14kDa) and BSA (66kDa)