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BIO EFFICACY OF ENTOMOPATHOGENIC FUNGI, *BEAUVERIA BASSIANA* FORMULATIONS AGAINST COFFEE BERRY BORER (*HYPOTHENEMUS HAMPEI*)

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ABSTRACT

The coffee berry borer (*Hypothenemus hampei* Ferrari) (Coleoptera: Curculionidae: Scolytinae) is one of the most destructive pest of coffee worldwide causing significant yield and quality losses. This study evaluates the bioefficacy of *Beauveria bassiana* (UAS, Raichur Bb 5a) against the coffee berry borer (*H. hampei*) under laboratory conditions. Mortality percentage of adults and larvae were recorded at 24, 48 and 72 hours after treatment. The results revealed that in both adults and larvae conidial suspension exhibited significantly higher mean mortality (45.71%) compared to talc-based formulation (25.24%) and control (7.62%) with peak mortality of 62.86% at 72 hours. In larvae also conidial suspension again outperformed talc-based formulation (67.62%), achieving 94.29% overall mean mortality and 100% mortality at 72 hours. These results showed that the *B. bassiana*, an entomopathogenic fungi found to be effective for causing mortality in coffee berry borer (adult and larval). This study highlights the significant potential of this biopesticide applications in coffee pest management.

***Key words*: *Beauveria*, Bioefficacy, Coffee berry borer, Entomopathogenic fungi,**

Introduction

Coffee in India is cultivated in hilly undulating landscapes by varying degrees of slope often under a diverse shade canopy. India ranks as the seventh largest coffee producer in the world accounting for about 3% of global production. The total area under coffee cultivation in the country is approximately 432000 ha, with an annual production of about 374000 tonnes and an average productivity of 814 kg/ha (https://coffeeboard.gov.in/Database). Among the coffee-growing states, Karnataka dominates with around 70% of national production, followed by Kerala (20%) and Tamil Nadu (8%) (https://apps.fas.usda.gov/newgainapi/api). In addition to these traditional coffee belts, Andhra Pradesh has emerged as a non-traditional promising coffee-growing state. Coffee is mainly cultivated in the high-altitude tribal regions of the Araku Valley, Paderu and Chintapalli areas of the Eastern Ghats in Visakhapatnam district. The state currently has an estimated area of about 101000 ha under coffee cultivation with an annual production of approximately 12,265 metric tonnes and an average productivity of around 170 kg /ha (www.deccanchronicle.com/southern-states/andhra-pradesh/aps).

The practice of coffee farming plays a crucial role in the preservation of forest cover and in maintaining the rich biodiversity of flora and fauna within the ecologically significant Western and Eastern hill ranges. The crop is grown at elevations ranging from 500 to 1500 m above mean sea level depending on species, with *Coffea arabica* favoring higher altitudes (1000–1500 m) and *Coffea robusta* occupying lower elevations (500–1000 m). Annual rainfall in coffee-growing regions varies between 1000 and 2500 mm often supplemented by irrigation in areas with erratic rainfall. Optimal temperatures range from 15–25°C for Arabica and 24–30℃ for Robusta, with high relative humidity (70–90%) supporting vegetative growth and flowering. Coffee thrives on deep, fertile, well-drained acidic soils (pH5.5–6.5) and is often cultivated under a diverse shade canopy, which enhances biodiversity, stabilizes microclimate and improves soil organic matter. Sloping topography aids drainage and reduces root disease incidence (Manson *et al*., 2024).

Coffee cultivation in India has been vulnerable to a multiple pests and diseases as in many other coffee-producing countries, production is affected by the coffee berry borer (*Hypoth-enemus hampei*), an insect native to Central Africa (Crowe, 2004). *H. hampei* was first reported in India in 1990 at Gudalur, located in the Nilgiris district of Tamil Nadu and is currently the main pest for coffee production in all coffee growing regions. The adult CBBs feed on the endosperm of coffee berries by boring through the exocarp, mesocarp and endocarp and the female lays eggs inside of it (Sponagel, 1994). With vestigial wings and rudimentary compound eyes, CBB males complete their life cycle within the coffee berries without leaving the berry. After sibling mating, female CBBs fly to a favourable coffee berry and lay eggs inside of it. The penetration hole generated by the CBB females on the surfaces of infested coffee berries makes them easy to be attacked by microorganisms, such as bacteria and fungi subsequently resulting in rot (Damon, 2000).

The concealed life cycle of the coffee berry borer (CBB) complicates its management. Chemical control is most effective when females remain in the penetration position (AB position) before the endosperm is damaged, but becomes ineffective once they enter the seed (CD position) and offspring begin feeding (Bustillo *et al*., 1998). Insecticides such as endosulfan and organochlorines have been widely used (Damon, 2000), though resistance and environmental concerns have limited their effectiveness. Laboratory studies have shown that cartap hydrochloride and chlorpyriphos can cause complete mortality of CBBs upon direct exposure (Lin & Chen, 2015). Alternative strategies include biological control using ants (Hymenoptera: Formicidae) (Morris *et al*., 2018) and monitoring with alcohol-baited funnel traps (Aristizabal *et al*., 2015). Despite these efforts, CBBs inside infested berries remain difficult to control. Therefore, an alternative management strategy is needed for controlling CBBs.

Microbial control could be an alternative method to manage CBBs. *Beauveria bassiana*, an entomopathogenic fungus belonging to the order Hypocreales and family Cordycipitaceae, is widely used as a biological control agent against a variety of insect pests (Butt *et al.*, 2001). Its infection process begins when conidia adhere to the insect cuticle, germinate, and form specialized structures called appressoria. The combined mechanical pressure of the appressorium and enzymatic activity allows the fungus to penetrate the cuticle. Once inside, fungal mycelia proliferate as hyphal bodies, invading the procuticle and epidermal layers, before spreading through the hemocoel where they secrete toxins that facilitate tissue colonization (Wang *et al.,* 2005; Cho *et al.*, 2006). The insect ultimately dies from a combination of physical tissue damage, toxicosis, dehydration, and nutrient depletion. Following host mortality, hyphae emerge from the cadaver and produce new conidia, completing the infection cycle. Beyond the coffee berry borer (*H. hampei*), *B. bassiana* is effective against a wide range of insect pests, including species in Coleoptera, Lepidoptera and Hemiptera emphasizing its versatility and importance as a sustainable tool in integrated pest management.

Material and methods

The present study entitled "Bioefficacy of *B. bassiana* formulations against Coffee Berry Borer (*H. hampei*)" was conducted at the Horticultural Research Station (HRS), Ambajipeta, Dr. B.R. Ambedkar, Konaseema dist., Dr. Y.S.R. Horticultural University, Andhra Pradesh. It falls under the Agro-climatic Zone 3 of Andhra Pradesh, which is characterized by a tropical climate with distinct wet and dry seasons. The region receives an average annual rainfall of approximately 900 mm and is located at an altitude of 34 meters (112 feet) above mean sea level (MSL). The geographical coordinates of the station are 16.7714°N latitude and 81.8629°E longitude. The area experiences hot and humid summers with temperatures often exceeding 40°C and

Table 1: Mean percent mortality of adult coffee berry borers at different time intervals post-treatment with *B. bassiana* formulations.

Time (Hrs)	Mean mortality %			C.V %	C.D at 5%
	T_1: Talc formulation of *B. bassiana*	T_2: Conidial suspension of *B. bassiana*	T_3: Control		
24	10.00^b	28.57^a	1.43^b	55.10	8.25
48	25.71^b	45.71^a	8.57^c	18.30	5.48
72	40.00^b	62.86^a	12.86^c	21.91	9.49
Overall mean	25.24	45.71	7.62	-	-

Means followed by the same superscript letter in a row are not significantly different (Tukey's HSD, p > 0.05).

mild winters.

Bio efficacy characterization

A completely randomized design (CRD) assay was conducted to evaluate the pathogenicity of *B. bassiana* (UAS, Raichur Bb 5a) against the coffee berry borer (*H. hampei*) under controlled laboratory conditions. The experiment comprised three treatments: T – talc-based formulation of *B. bassiana* (1 × 10^8 cfu ml^{-1}), T, – conidial suspension of *B. bassiana* (1 × 10^8 cfu ml^{-1}) and T1 – control (sterile distilled water). Each treatment was replicated seven times, with ten individuals (adults or larvae) per replication. Test insects were collected from physiologically mature coffee berries exhibiting characteristic symptoms of CBB infestation. Prior to treatment, the insects were surface sterilized by immersion in 1% sodium hypochlorite solution for 2 minutes and subsequently rinsed with sterile distilled water to eliminate potential microbial contaminants. Adults and larvae were treated separately with their respective formulations. Mortality observations were recorded at 24, 48, and 72 hrs post-treatment under *in vitro* conditions to assess the comparative bioefficacy of the two *B. bassiana*.

Preparation of *B. bassiana* Conidial Suspension

A virulent strain of *B. bassiana* (UAS, Raichur, Bb 5a) was cultured on Potato Dextrose Agar (PDA) plates and incubated at 25 ± 2 °C for 10–14 days to obtain abundant sporulation. The conidial suspension was prepared by adding 10 mL of sterile distilled water containing 0.02% Tween 80 to each sporulated plate and gently scraping the surface with a sterile glass rod to dislodge conidia. The resulting suspension was filtered through a double layer of sterile muslin cloth to remove mycelial fragments and debris. The concentration of conidia in the filtrate was determined using a Neubauer hemocytometer under a compound microscope. The conidial suspension (conidia/mL) was calculated using the formula:

Conidia/mL=Average count per square × Dilution factor × 10^4 (Conidia/mL)

The stock suspension was then adjusted with sterile 0.02% Tween 80 solution to achieve a final concentration of 1 × 10^8 cfu/mL for biassay studies. Prior to use, conidial viability was assessed by germination test on water agar after 18 h incubation at 25°C and only suspensions with germination rates above 90% were utilized in the experiments.

Inoculation of Coffee berry borer

Adult coffee berry borers (*H. hampei*) were inoculated by immersion in the respective *B. bassiana* conidial suspensions for 2 minutes. Control insects were immersed in sterile 0.02% Tween 80 solution. Following inoculation, both treated and control adults were transferred to sterile Petri dishes, with each experimental unit consisting of ten insects. Four mature coffee berries were placed in each Petri dish to serve as both food and shelter for the insects. To maintain adequate humidity, cotton swabs moistened with sterile distilled water were placed inside each dish. Each treatment was replicated three times, and the Petri dishes were incubated at 27 ± 2 °C under controlled laboratory conditions. Mortality was recorded at 24-hour intervals until at least one treatment reached 100% mortality. Dead insects were transferred individually to moist chambers (sterile Petri dishes lined with moistened filter paper) to confirm fungal sporulation. The entire bioassay was repeated twice on different dates to verify reproducibility of results.

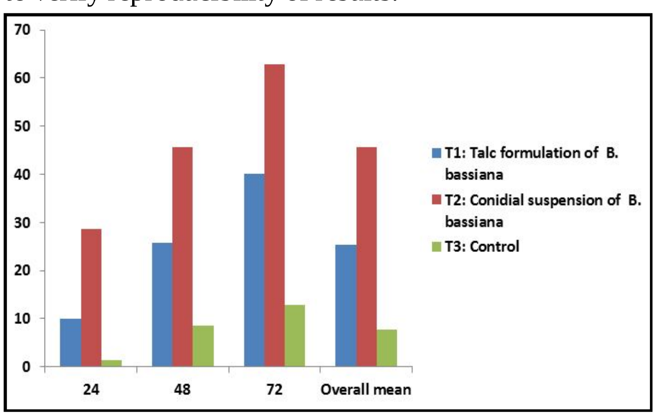

Fig. 1: Mean percent mortality of adult coffee berry borers at different time intervals post-treatment with *B. bassiana* formulations.

Table 2: Mean percent mortality of larval coffee berry borers at different time intervals post-treatment with B. *bassiana* formulations.

Time (Hrs)	Mean mortality %			C.V %	C.D at 5%
	T_1: Talc formulation of *B. bassiana*	T_2: Conidial suspension of *B. bassiana*	T_3: Control		
24	60.00^{b}	85.71^{a}	7.14^{c}	21.27	12.17
48	67.14^{b}	97.14^{a}	18.57^{c}	12.05	8.25
72	75.71^{b}	100.00^{a}	28.57^{c}	11.25	8.61
Overall mean	67.62	94.29	18.10	-	-

Means followed by the same superscript letter in a row are not significantly different (Tukey's HSD, p > 0.05).

Data analysis

The percentage mortality data were subjected to arcsine square-root transformation prior to analysis of variance (ANOVA) (Python) and treatment means were compared at a significance level of $p < 0.01$ to determine differences in pathogenic efficacy among formulations.

Results and Discussion

The bioefficacy of two formulations of *B. bassiana*a, talc-based formulation (T_1) (UAS, Raichur, Bb 5a) and a conidial suspension (T_2) was evaluated against the coffee berry borer in laboratory conditions, with an untreated control (T_3) for comparison. Mortality was assessed in both adult and larval stages at 24, 48 and 72 hours post-treatment with data recorded as cumulative percent mortality out of 10 individuals per replicate across seven replicates. Statistical analysis involved one-way ANOVA at each time point to compare treatments, followed by Tukey's Honestly Significant Difference (HSD) test for post-hoc pairwise comparisons ($p < 0.05$). Coefficient of Variation (CV %) and Critical Difference (CD at 5%) values were calculated to assess data variability and the minimum difference required for significance, respectively.

Mortality in Adults of Coffee Berry Borers

Mortality rates in adults of coffee berry borers increased progressively over the observation period for all treatments indicating a time-dependent effect of the fungal pathogen (Table 1 and Fig. 1). At 24 hours post-treatment, the conidial suspension (T_2) induced the highest mean mortality of 28.57%, which was significantly greater than both the talc formulation (T_1) at 10.00% and the control (T_3) at 1.43% ($p < 0.05$, as indicated by distinct Tukey HSD grouping letters). The CV% at this time point was 55.10%, reflecting moderate variability, with a CD (5%) of 8.25% required for significant differences between any two means.

By 48 hours, mortality escalated across treatments, with T_2 again showing the superior efficacy at 45.71%, significantly outperforming T_1 (25.71%) and T_3 (8.57%). The control exhibited minimal natural mortality, underscoring the pathogenic role of *B. bassiana*. Variability decreased (CV% = 18.30%) and the CD (5%) was 5.48%, allowing for clearer differentiation among treatments.

At 72 hours, the trend persisted, with T_2 achieving 62.86% mortality, followed by T_1 at 40.00% and T_3 at 12.86%. All treatments were significantly different from each other ($p < 0.05$), with a CV% of 21.91% and CD (5%) of 9.49%. The overall mean mortality across the three time points was highest for T_2 (45.71%), followed

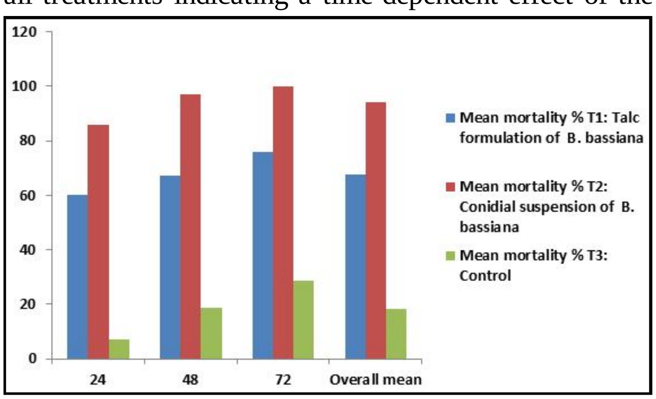

Fig. 2: Mean percent mortality of larval coffee berry borers at different time intervals post-treatment with *B. bassiana* formulations.

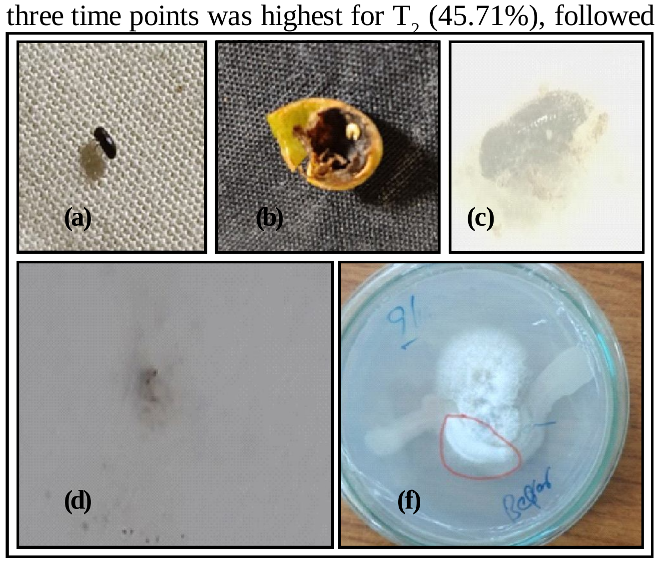

Fig. 3: Coffee Berry Borer (*Hypothenemus hampei*) a) Healthy adult, b) Healthy larvae, c) Infected adult, d) infected larvae, e) Recovery of *B. bassiana* culture from Infected adult

by T_1 (25.24%) and lowest for T_3 (7.62%), demonstrating that the conidial suspension formulation provided consistently greater bioefficacy than the talc-based one, though both were markedly superior to the control (Fig. 3).

Mortality in larvae of Coffee Berry Borers

Similar to adults, larval mortality increased over time, but with more pronounced differences among treatments, reflecting potentially higher susceptibility of larvae to fungal infection (Table 2 and Fig. 2). At 24 hours, T_2 recorded the highest mean mortality of 85.71%, significantly exceeding T_1 (60.00%) and T_3 (7.14%) ($p < 0.05$). The CV% was 21.27% with a CD (5%) of 12.17%, indicating reliable differentiation.

At 48 hours, T_2 approached near-complete mortality at 97.14%, significantly higher than T_1 (67.14%) and T_3 (18.57%). Variability was low (CV% = 12.05%), and the CD (5%) was 8.25%.

By 72 hours, T_2 achieved 100.00% mortality, while T_1 reached 75.71% and T_3 with 28.57%, with all treatments significantly distinct ($p < 0.05$). The CV% was 11.25% and CD (5%) was 8.61%. Overall means across time points were 94.29% for T_2, 67.62% for T_1 and 18.10% for T_3 confirming the conidial suspension's superior performance (Fig. 3).

The rapid escalation in larval mortality particularly with T_2 suggests that conidia in suspension may penetrate larval cuticles more effectively, leading to faster mycosis compared to the talc formulation. The control's higher mortality in larvae (relative to adults) could indicate environmental factors, but it remained substantially lower than treated groups.

Both *B. bassiana* formulations recorded significant bioefficacy against coffee berry borer, with the conidial suspension (T_2) surpassing the talc formulation (T_1) in both life stages. These results support the potential of *B. bassiana* as a biopesticide for integrated pest management in coffee plantations, ensuring further field trials to validate laboratory outcomes.

Present findings are in agreement with previous studies on *B. bassiana* against coleopteran pests, which reported higher infection rates in larvae compared to adults due to cuticle differences and increased susceptibility of younger stages (Vega *et al.,* 2003; Posada *et al.,* 2005). These findings are consistent with studies on entomopathogenic fungi effecting CBB (Chang *et al.*, 2023) who reported local isolates of *B. bassiana* against the coffee berry borer exhibited significantly higher virulence showing strong pathogenicity in laboratory bioassays. They also observed that mortality increased with time of exposure and that pre-infestation application of fungal conidia enhanced efficacy. Hence, *B. bassiana* can be used for the management of coffee berry borer.

Conclusion

The present laboratory study conclusively demonstrated the superior bioefficacy of *B. bassiana* (UAS, Raichur Bb 5a) conidial suspension (1×10^8 cfu ml^{-1}) over its talc-based formulation against both adult and larval stages of the coffee berry borer (*H. hampei*). The conidial suspension resulted in significantly higher cumulative mean mortality of 45.71% in adults and 94.29% in larvae across 24-72 h post-treatment, with peak efficacies of 62.86% and 100% at 72 h, respectively. In comparison, the talc formulation recorded 25.24% and 67.62% mortality in adults and larvae, while the untreated control exhibited negligible natural mortality (7.62% in adults; 18.10% in larvae). Larvae were more susceptible than adults, likely due to thinner cuticular barriers and enhanced conidial adhesion and penetration in the suspension treatment. The progressive increase in mortality with confirmed fungal sporulation on cadavers validated the pathogenic mechanism of *B. bassiana* through cuticular invasion, mycelial proliferation, and toxin-mediated lethality. Overall, the results establish *B. bassiana* conidial suspension as a highly effective and environmentally benign biopesticide for the integrated management of *H. hampei* in coffee agroecosystems, warranting further field validation to confirm its efficacy and optimize application strategies under natural conditions.

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