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RESEARCH ARTICLE
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Efficacy of a Powdered *Beauveria bassiana* Formulation against Cocoa Pod Borer and Fall Armyworm

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ABSTRACT

This study evaluated the effectiveness of a single Beauveria bassiana powder formulation across two complementary pest–crop systems: cocoa–cocoa pod borer (*Conopomorpha cramerella*) and maize–fall armyworm (*Spodoptera frugiperda*). The objective was to assess its broad-spectrum biocontrol potential by testing different concentrations, application methods, and culture durations under field and laboratory conditions. Field application on cocoa fruits (8–11 cm, two months old) significantly reduced pod borer infestation from 66.66% (control) to 27.77% (150 g formulation). Laboratory and field evaluations on maize indicated that the formulation with 75 g/500 mL water and maize flour achieved the highest spore density and 100% larval mortality at a 21-day culture duration. The treated maize plots also exhibited reduced pest incidence (30.1%) and higher yields (9.70 t/ha) compared to controls (42.1% and 8.83 t/ha). These results demonstrate that a single *B. bassiana* formulation can effectively suppress two major insect pests under distinct ecological conditions, supporting its role as a multi-target and broad-spectrum biocontrol agent. This integrative approach highlights the formulation's potential contribution to sustainable and integrated pest management strategies in both cocoa and maize production systems.

Keywords: *Beauveria bassiana*, Biological control, Formulation, Culture duration, Cocoa pod borer, Fall armyworm.

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INTRODUCTION

Cocoa and maize are pivotal tropical crops that support the livelihoods of millions of smallholder farmers in Southeast Asia, Africa and Latin America. However, their productivity is severely constrained by insect pests that cause substantial yield losses and economic damage. Among the most destructive pests in cocoa production is the cocoa pod borer (*Conopomorpha cramerella*), which can inflict up to 80% yield loss in unmanaged farms due to internal pod tunneling and reduced bean quality. Concurrently, the fall armyworm (*Spodoptera frugiperda*), originally endemic to the Americas, has rapidly spread to Africa and Asia since 2016, becoming a major global pest of maize and other staple crops. The larvae of FAW feed voraciously on leaves and reproductive tissues, causing rapid defoliation and significant grain yield reduction if uncontrolled. However, the productivity of cocoa has been declining due to several factors, with pest infestation being one of the most significant. Among the pests attacking cocoa, the cocoa pod borer (*C. cramerella*) is particularly harmful, causing yield losses of up to 80.0.1% in Indonesia (Wahab et al., 2017) and up to 80–90% in unmanaged cocoa farms across Southeast Asia (Niogret et al., 2019; Saleh et al., 2020). The damage caused by this pest leads to reduced seed weight, lower fat content, and sticky seeds, ultimately affecting the quality of cocoa beans (Pratama et al., 2021).

At the same time, maize (*Zea mays* L.), another strategic crop in the region, faces severe losses caused by the invasive fall armyworm (*S. frugiperda*), which damages leaves and reduces grain yield. This invasive species has spread rapidly from the Americas to Africa and Asia, causing severe yield losses and threatening food security

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(Goergen et al., 2016; Mursyidin et al., 2024). Control of CPB and FAW using conventional synthetic insecticides has been widely practiced, but this approach raises serious environmental and human health concerns, disrupts beneficial arthropods and can lead to resistance development. Synthetic insecticides is unsustainable and may contribute to insecticide resistance, increased production costs, reduced agrobiodiversity, the impoverishment of soil microbiota, water pollution (Suryani et al., 2026). Therefore, there is a need for alternative, safe, and environmentally friendly pest control methods. Recent studies have shown that botanical and biological control agents can significantly affect the feeding behavior, morphology and survival of S. frugiperda, suggesting their potential as eco-friendly alternatives to chemical insecticides (Aleem et al., 2023; Dewi et al., 2024). This has stimulated research into alternative, environmentally sound pest management methods, particularly biological control using microbial agents. Entomopathogenic fungi (EPF) are among the most promising biological control agents due to their ability to infect and kill a broad range of insect hosts with minimal non-target effects.

Beauveria bassiana (Hypocreales: Cordycipitaceae) is one of the most widely studied EPF for insect pest management. It infects insects via cuticular penetration and proliferates internally, causing host mortality through mechanical damage and production of toxic secondary metabolites such as beauvericin and bassianolide. Additionally, *B. bassiana* has several advantages, including its ability to produce durable spores, its selectivity, and its low potential for resistance development (Afifah et al., 2022). Recent work has demonstrated that *B. bassiana* exhibits significant pathogenicity against lepidopteran pests, including FAW, under laboratory and controlled conditions. For example, a study showed that *B. bassiana* formulations could achieve over 50% mortality in invasive FAW larvae at higher conidial concentrations, with antifeedant effects reducing leaf damage significantly, indicating its potential as a target-specific biocontrol agent (Purwanti et al., 2022).

Beyond pest mortality, entomopathogenic fungi may provide additional plant-beneficial effects. Recent research has shown that *B. bassiana*, not only as an entomopathogen but also as an endophytic fungus capable of colonizing plant tissues and providing systemic protection against diverse pests (Mascarin & Jaronski, 2016; Bitencourt et al., 2024). Such versatility strengthens its role as a sustainable and eco-friendly alternative within modern IPM frameworks. Strains of *B. bassiana* can colonize maize endophytically and promote plant growth, highlighting a dual role as both biocontrol agent and growth promoter. In hydroponic systems, inoculated maize showed enhanced vigor compared to controls, suggesting that EPFs can confer benefits beyond pest suppression (Liu et al. 2022).

Despite these promising results, the translation of laboratory efficacy into field success has been inconsistent due to environmental challenges faced by fungal spores in the field, such as UV degradation, desiccation, and variable microclimatic conditions. The formulation of EPFs plays a critical role in mitigating these stresses and improving field persistence. Recent advances in formulation technology include encapsulation, use of organic carriers, nutrient enrichment, and adjuvants designed to enhance adhesion, moisture retention, and ecological stability. These innovations have been explored primarily in single pest–crop systems, but comparative evaluations of standardized formulations across multiple host–pest systems remain limited. Traditionally, research on *B. bassiana* has focused on its effects on single pests such as coffee berry borer, cotton leafworm, and cabbage pests, demonstrating broad applicability but often in isolated contexts. For instance, studies have characterized local isolates for control of lepidopteran pests, documenting high virulence against *Spodoptera litura* and other crop-damaging species (Islam, 2023). Moreover, entomopathogenic fungi have been investigated in mixed biocontrol approaches, showing synergistic effects when combined with parasitoids, further emphasizing their versatility in integrated pest management (Zhang et al., 2024). Recent studies have demonstrated the resilience of *B. bassiana* under fluctuating environmental conditions, making it a viable option for diverse agricultural settings (Daud et al., 2020). Furthermore, recent innovations have explored the synergistic combination of *B. bassiana* with plant-based nanoemulsions to enhance its virulence and stability. Suryani et al. (2024) reported that the integration of *Mirabilis jalapa* nanoemulsion with *B. bassiana* significantly increased the mortality of *S. frugiperda* larvae, indicating that formulation improvement can strengthen its biocontrol efficacy under field conditions.

The cocoa pod borer, in particular, presents unique challenges due to its cryptic feeding habits inside pods, reducing the efficacy of foliar sprays and requiring targeted biological strategies. Indonesian studies have evaluated *B. bassiana* against CPB and reported significant larval and pupal mortality under laboratory conditions, highlighting the fungus's capacity to infect CPB when delivered at effective concentrations (Hardiansyah et al. 2023).

In addition to pest suppression, entomopathogenic fungi have been evaluated in field conditions against FAW using various agents. A recent field study reported that *B. bassiana*-based agents reduced FAW larval populations significantly, leading to lower infestation in treated maize plots, demonstrating sustainable suppression potential under real agroecological conditions (Azazy et al., 2025).

However, despite demonstrated efficacy in both laboratory and some field trials, few studies have systematically evaluated whether one standardized powdered formulation of *B. bassiana* can deliver consistent performance across both cocoa and maize systems targeting two biologically distinct pest species (CPB and FAW) under similar treatment regimes. Such cross-system validation is particularly relevant for smallholder farmers managing mixed crop systems in tropical environments, where a single biocontrol product could reduce operational complexity and cost. Therefore, the primary knowledge gap addressed in this study is the lack of

empirical evidence for the cross-ecological applicability of a single powdered *B. bassiana* formulation in controlling pests with different biology and feeding behavior under both controlled (laboratory) and field conditions. Addressing this gap will provide insight into formulation robustness and its potential integration into broader, scalable Integrated Pest Management (IPM) strategies that could benefit diverse tropical cropping systems. Sustainable crop protection strategies increasingly emphasize environmentally friendly approaches, including the use of biological agents and improved pathogen management practices to reduce yield losses caused by pests and diseases (Kochorov et al., 2025). In this context, the present study investigates the efficacy of a standardized powdered *Beauveria bassiana* formulation against CPB and FAW by combining laboratory bioassays with field infestation and yield assessments. By doing so, this research aims to generate comprehensive data on cross-host performance, contributing to the development of sustainable biological control tools that are ecologically robust, economically feasible, and applicable to multiple crop–pest scenarios.

MATERIALS & METHODS

Study Area

The field experiments were conducted in cocoa plantations located in Polewali Mandar Regency and maize fields in Gowa Regency, South Sulawesi, Indonesia. The cocoa experimental site was located at approximately 3°24′S and 119°15′E, at an altitude of 50–75 m above sea level, characterized by a tropical humid climate with average annual rainfall of 2,000–2,500 mm and average temperature ranging from 26–32°C. The maize field experiment in Gowa Regency (5°11′S; 119°27′E) was conducted under similar tropical conditions with well-drained loamy soil. These agroecological descriptions are provided to support environmental context influencing fungal performance.

Application of *B. bassiana* Powder on Cocoa Fruits

The entomopathogenic fungus *B. bassiana* used in this study was obtained from a laboratory culture collection at Hasanuddin University Makassar. The isolate was previously identified morphologically and confirmed through microscopic examination of conidial structures. Conidia were cultured on Potato Dextrose Agar (PDA) medium and incubated at 27 ± 1°C for 14 days until sporulation was complete. For powder formulation preparation, harvested conidia were air-dried under sterile conditions and mixed with carrier materials at specified ratios. Conidial density was determined using a hemocytometer and adjusted to the desired concentration before application. Spore viability was assessed prior to field use by calculating germination percentage after 24 h incubation and only batches with ≥90% viability were used for experiments.

This method was adapted and modified from Herawati and Majid (2017) and Pratama et al. (2021). The *B. bassiana* formulation was prepared by dissolving the appropriate weight of powder in 1 L of distilled water and filtered before spraying. A portable sprayer was used to apply the suspension evenly to cocoa fruits measuring 8–11 cm (approximately two months old). Each treatment consisted of 20 trees, and five fruits per tree were randomly selected for observation. The treatments included: P0: control (no *B. bassiana* application); P1: 50 g formulation/L water; P2: 150 g formulation/L water.

The experiment followed a Randomized Complete Block Design (RCBD) with three replications. Each block contained three treatments randomly assigned to plots. This design allowed comparison among treatments while accounting for environmental variability in the field. Spraying was carried out in the afternoon (14:00–17:00 WITA) during dry weather, with three applications at 10-day intervals. Each fruit received approximately 150 mL of suspension.

Cocoa pod damage was evaluated both externally and internally to ensure accuracy. External damage was identified by small, dark holes or frass at the pod surface, whereas internal damage was assessed by opening the pods to count infested seeds and evaluate damage intensity. Damage was categorized into four severity levels (A–D) following Herawati and Majid (2017). This modification was made to address the reviewer's concern that external symptoms alone might be unreliable indicators of CPB infestation.

The percentage of affected fruits was calculated using Equation (1):

$$T = \frac{A}{B}\, x\, 100\% \quad (1)$$

Where T is the percentage of affected fruits, A is the number of affected fruits, and B is the total number of fruits observed. Damage intensity was assessed during harvest by scoring the damage to the cocoa beans. The severity of damage was classified into four categories: A is No damage (healthy beans); B is Light damage (less than 12% of the beans are affected); C: Moderate damage (12%-54% of the beans are affected). D is Severe damage (more than 54% of the beans are affected and cannot be separated from the fruit pulp) Herawati and Majid (2017).

Experimental Design

The experiment on cocoa was arranged in a randomized block design. Each treatment consisted of four replicates, with each replicate comprising 10 cocoa pods randomly selected within a block. Treatments included: P0 (control), P1 (100 g/L), and P2 (150 g/L) powdered formulation. Applications were performed using a hand sprayer to ensure uniform coverage of pod surfaces. Applications were conducted in the early morning (07:00–09:00) to minimize UV degradation and enhance fungal persistence. Treatments were applied at 7-day intervals for a total of three applications. Infestation levels were assessed by counting damaged pods. To improve diagnostic accuracy, pods were dissected after harvest to confirm internal larval presence and feeding damage. Infestation percentage was calculated based on confirmed CPB attack symptoms.

The maize experiment was conducted using a completely randomized design. Treatments consisted of different formulation combinations (75 g/500 mL with maize flour carrier) and culture duration treatments (14, 21, and 28 days). Each treatment was replicated four times, with each replicate consisting of 10 maize plants. Larval mortality was observed in laboratory bioassays. Third instar FAW larvae were used for laboratory assays to ensure uniform susceptibility. Each replicate included 10 larvae placed individually in plastic containers and treated with sprayed conidial suspension. Mortality was recorded daily for seven days, and dead larvae were surface-sterilized and incubated to confirm mycosis. Field infestation was assessed visually. Field observations were conducted at 7-day intervals by assessing leaf damage using a standardized damage scale. Final yield was measured at harvest and converted to tons per hectare.

Laboratory Bioassays: Growth Media Testing

Insect Source and Rearing

Eggs and larvae of *S. frugiperda* were collected from infested maize fields in Gowa Regency and reared in the laboratory for two generations on fresh maize leaves to ensure healthy, uniform test populations. Second-instar larvae were used for bioassays.

Laboratory Bioassay

The experiment was arranged in a Completely Randomized Design (CRD) with four treatments and five replications, each consisting of 20 larvae. The treatments were: P0 (Control): No *B. bassiana* formulation; P0 (control, distilled water only); P1: *B. bassiana* 25 g/500 mL + 5 g rice flour; P2: *B. bassiana* 50 g/500 mL + 5 g kaolin; P3: *B. bassiana* 75 g/500 mL + 5 g maize flour.

Larvae were placed in sterile Petri dishes lined with moistened filter paper and maintained at 27 ± 1°C, 75 ± 5% RH, with 12:12 h light:dark. Each larva was treated with 1 mL of suspension using a micropipette to ensure even coverage, then provided fresh maize leaves daily. Larval mortality was recorded daily for 11 days and calculated using Equation (2):

$$M = \frac{a}{a+b} x\ 100\% \quad (2)$$

Where *M* represents larval mortality, *a* is the number of dead larvae and *b* is the number of living larvae. Data were subjected to analysis of variance (ANOVA), and means were separated using Duncan's Multiple Range Test (DMRT) or Tukey's Honest Significant Difference (HSD) test at α = 0.05, depending on design structure. DMRT was used for CRD and Tukey's HSD for RCBD.

Field Application in Maize

To ensure replication and address field variability, the maize field trial used a Randomized Complete Block Design (RCBD) with three replications per treatment. Each plot measured 5 × 10 m, with a planting density of 75 × 25 cm and 100 plants per plot. Three treatments were applied: P0 (control, untreated seed), P1 (B. bassiana 50 g/500 mL suspension), P2 (B. bassiana 75 g/500 mL suspension).

Seeds were soaked in their respective suspensions for 30 minutes prior to planting. No chemical pesticides were applied during the trial. Pest infestation (%) and yield (tons/ha) were calculated using Equations (3) and (4).

$$P = \frac{a}{b}\ x\ 100\% \quad (3)$$

Where *P* represents the percentage of pest damage, *a* is the number of damaged leaves and *b* is the total number of leaves observed.

Maize yield was calculated using the following equation 4.

$$Y = \frac{10{,}000m^2}{L(m^2)}\ x\ \frac{X\ (kg)}{1{,}000\ kg} \quad (4)$$

Where *Y* is the yield in tons per hectare, *X* is the yield in kilograms, and *L* is the plot area in square meters.

To improve statistical robustness, the data were analyzed using ANOVA followed by Tukey's HSD at α = 0.05 instead of an independent t-test as used previously. This modification ensured that the analysis matched the experimental design and replicated field structure. The study site had an average daily temperature of 29°C and relative humidity of 78%.

Culture Duration and Spore Assessment

To separate the effects of concentration and carrier material, a factorial design was proposed for future studies as recommended by the reviewer. However, in the present work, culture duration effects were evaluated using a Completely Randomized Design (CRD) with four levels (7, 14, 21, and 28 days) and three replications. The percentage of mortality was calculated using the equation 5.

$$Mo = \frac{k}{kn} x\ 100\% \quad (5)$$

Where *Mo* represents mortality percentage, *k* is the number of dead larvae, and *kn* is the total number of larvae. Spore density was quantified using a hemocytometer, and spore viability was determined after 24 h incubation at 27 ± 1°C. The spore density was calculated using the following equation 6.

$$S = \frac{t}{n\ x\ 0.25} x\ 10^6 \quad (6)$$

Where S: Spore density per mL of solution; t: Total number of spores observed in the sample grid; n: Number of grids used in the haemocytometer; 0.25: Correction factor for the small scale of the sample grid; 10^6: Standard effective spore concentration.

RESULTS & DISCUSSION

Effect of *B. bassiana* on Cocoa Pod Borer Infestation

The application of powdered *B. bassiana* significantly reduced CPB infestation compared to the control treatment.

ANOVA indicated a significant treatment effect ($P<0.05$) on CPB infestation percentage. The highest

suppression was observed at 150 g/L (P2), reducing infestation from 66.66% in the control to 27.77%. This represents approximately 58.3% relative suppression under field conditions. The reduction in infestation confirms that the powdered formulation was able to establish infection pressure under tropical field conditions despite environmental constraints such as rainfall and temperature fluctuations. The ability of *B. bassiana* to infect CPB, a cryptic pest feeding internally within cocoa pods, suggests that spore adhesion and cuticular penetration occurred during larval movement outside or at pod surfaces prior to entry. These findings align with previous laboratory-based studies reporting CPB larval mortality following exposure to *B. bassiana*. However, unlike controlled laboratory conditions, the present study demonstrates field-level suppression, providing stronger ecological relevance and practical applicability. This distinction is important because field persistence and environmental resilience often limit fungal efficacy in tropical systems.

The results of the cocoa pod borer (CPB) infestation percentage on cocoa fruits are shown in Table 1. To maintain consistency, all abbreviations are now standardized in English (e.g., CPB = Cocoa Pod Borer, FAW = Fall Armyworm). Without the application of *Beauveria bassiana* (control, P0), infestation increased significantly from the first to the fourth week, reaching 66.66% in the fourth week, confirming high pest pressure under untreated conditions. Environmental factors such as temperature and humidity influence pest population dynamics, where unstable temperature ranges increase CPB activity (Herawati & Majid, 2017).

Table 1: Percentage of cocoa fruits attacked by CPB after application of *B. bassiana*

Treatment	Week 1	Week 2	Week 3	Week 4
P0 (control)	13.88 ± 1.20^{a}	36.10 ± 2.31^{a}	47.21 ± 3.44^{a}	66.66 ± 2.91^{a}
P1 (50 g)	5.55 ± 0.41^{b}	11.10 ± 1.22^{b}	13.88 ± 2.02^{b}	47.21 ± 1.77^{b}
P2 (150 g)	0 ± 0.00^{c}	2.77 ± 0.18^{c}	11.10 ± 0.74^{b}	27.77 ± 1.63^{c}

Values (mean ± SD) bearing different letters in a column indicate significant differences based on ANOVA followed by Duncan's Multiple Range Test (DMRT) at α = 0.05.

The results of ANOVA showed a significant effect of B. bassiana concentration on infestation percentage ($F = 6.73$, $P<0.05$). The treatment details (P0–P2) have been clarified and correspond to those described in the Methods section. The lowest infestation level was recorded in P2 (150 g *B. bassiana)*, demonstrating that increased fungal concentration enhances infection potential through higher conidial adhesion and faster host penetration. These findings align with previous reports by Pratama et al. (2021), who found a 61.6% reduction in CPB infestation using 6 g/10 L formulations.

Higher concentrations increase conidia density, improving adhesion and penetration efficiency, thus accelerating insect mortality within 3–5 days. The infestation intensity also confirmed this, with P0 (control) having the highest infestation (19.51%), while P2 (150 g) had the lowest (0.51%). These results are consistent with Herawati & Majid (2017), who demonstrated that increasing *B. bassiana* concentration reduced CPB infestation by up to 61.6%. Compared to chemical insecticides, *B. bassiana* provides sustainable suppression without environmental risks, and its infective persistence under humid tropical conditions offers long-term pest control advantages (Saranraj & Jayaparakash, 2017; Afifah et al., 2022).

Effect on FAW Larval Mortality

Fig. 1 shows the spore density of *B. bassiana* formulations at various concentrations.

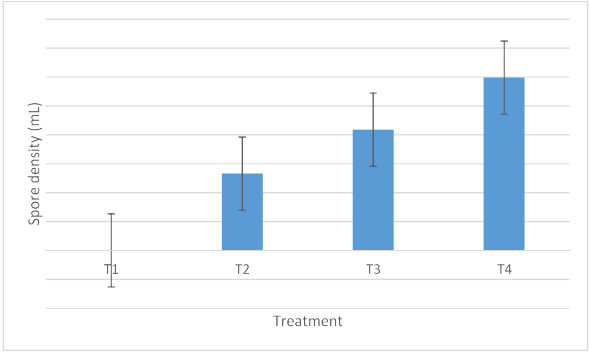

Fig. 1: Spore Density of *Beauveria bassiana* Flour Formulation (10^7/mL); T1 = Control (no *B. bassiana*), T2 = 25 g/500 mL + 5 g rice flour, T3 = 50 g/500 mL + 5 g kaolin, and T4 = 75 g/500 mL + 5 g maize flour.

The formulation containing 75 g/500 mL of water + 5 g of corn flour demonstrated the highest spore density and larval mortality of *Spodoptera frugiperda* (Table 2). Significant mortality began on day 7 after application. The improved carrier media stability enhanced conidial dispersal, ensuring more uniform infection.

Table 2: Mortality (%) of FAW larvae after application of *B. bassiana* formulations

Treatment	Day 1	Day 3	Day 5	Day 7	Day 10
T1 (Control)	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	0 ± 0.00^{a}	3.00 ± 0.08^{a}
T2	0 ± 0.00^{a}	16.0 ± 1.6^{b}	40.0 ± 2.4^{b}	64.0 ± 3.2^{b}	84.0 ± 2.9^{b}
T3	0 ± 0.00^{a}	8.0 ± 0.8^{b}	48.0 ± 2.6^{b}	72.0 ± 2.8^{b}	80.0 ± 3.0^{b}
T4	0 ± 0.00^{a}	16.0 ± 1.7^{b}	48.0 ± 2.4^{b}	96.0 ± 1.9^{c}	96.0 ± 2.1^{c}

Values (mean ± SD) bearing different letters in a column/treatments ($F = 12.31$, $P<0.05$) indicate significant differences based on ANOVA followed by Duncan's Multiple Range Test (DMRT) at α = 0.05.

This indicates that higher spore density promotes greater conidial contact with the insect cuticle, increasing the probability of germination and infection. The addition of maize flour as a carrier not only supports spore adhesion but also provides nutritional substrates that maintain conidial viability and enhance infectivity. These results align with the findings of Herlinda et al. (2006), who reported that carrier enrichment increases formulation density and spore concentration, thereby enhancing fungal virulence. Recent studies also emphasize that optimizing formulation components — such as carrier type and encapsulation matrix — is critical to sustain high conidial viability and achieve effective pest mortality (Felizatti et al., 2021; Bitencourt et al., 2024).

The progressive increase in larval mortality observed from day 7 onward suggests that infection dynamics followed typical entomopathogenic progression — involving conidial adhesion, germination, penetration through the cuticle, and colonization of hemocoel tissues — leading to systemic fungal infection and death. Thus,

the superior performance of the T4 formulation can be attributed to both the higher concentration of conidia and the synergistic effect of the maize flour carrier, which supports spore stability and efficient host infection.

The highest larval mortality occurred in the 75 g/500 mL + maize flour formulation, suggesting that maize flour enhanced fungal adherence and spore survival on the larval cuticle. Formulation strategies that maintain high viable conidial loads on the cuticle (e.g., microencapsulation, oil carriers) significantly increase infection probability compared to unformulated spores (de Jesus Seabra et al., 2024). This supports the importance of formulation optimization for maintaining high conidial viability and ensuring effective pest mortality.

Re-isolation of *S. frugiperda* cadavers revealed color changes to black, tissue hardening and mummification covered by white mycelium (Fig. 2a). Fig. 2 shows (b) re-isolation on PDA, (c) pure culture formation, and (d) microscopic morphology with septate mycelium and round conidia.

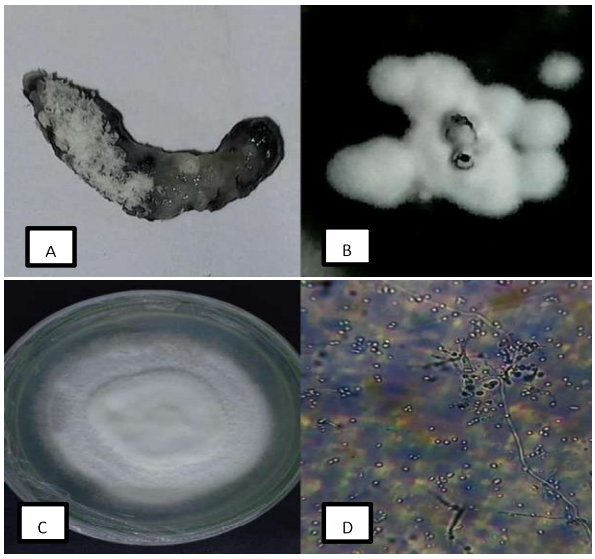

Fig. 2: Infected Insects, Re-isolation, Purification, and Identification (a) Cadaver resulting from *B. bassiana* infection, (b) re-isolation of *B. bassiana* from the cadaver grown on PDA medium, (c) purification of *B. bassiana* isolates and microscopic observation of *B. bassiana* at magnification 40x.

Field Infestation and Yield Performance in Maize

Field-treated maize plots exhibited lower FAW infestation compared to the control. Infestation decreased from 42.1% in untreated plots to 30.1% in treated plots. Although this reduction was moderate compared to laboratory mortality results, it reflects realistic field suppression under natural environmental variability. Grain yield was higher in treated plots (9.70 t/ha) compared to control (8.83 t/ha). However, yield improvement should be interpreted cautiously, as yield variation may also be influenced by environmental, soil, and agronomic factors beyond pest suppression alone. The primary outcome of interest in this study is pest suppression efficacy rather than direct yield enhancement.

Pest infestation in *B. bassiana*-treated maize plots decreased from 42.1% (control) to 30.1%. The observed increase in yield (9.70 t/ha vs. 8.83 t/ha in control) was partly attributed to reduced leaf damage (Fig. 3); however, other factors such as soil fertility, rainfall, and genetic variability may also contribute to the difference. Although seed treatment can lead to endophytic colonization (Posada & Vega, 2005; Jaber & Enkerli, 2016), we did not perform re-isolation from surface-sterilized tissues in this study; therefore, claims of systemic colonization remain provisional and require confirmation in follow-up experiments.

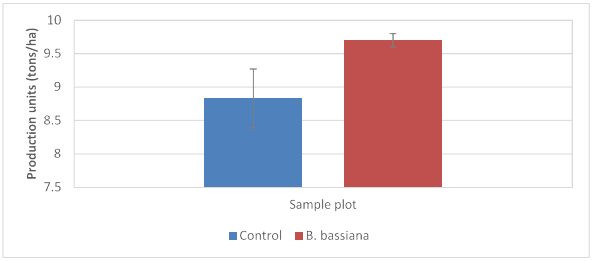

Fig. 3: Fresh Weight Production of Maize.

To validate endophytic behavior, we have acknowledged that re-isolation from surface-sterilized plant tissues was not conducted and recommend it for future studies.

Effect of Culture Duration

Culture duration significantly affected fungal virulence against *S. frugiperda*. The highest larval mortality was observed for 21-day and 28-day cultures, with 100% and 93.3% mortality, respectively (Table 3). Laboratory bioassays showed increased larval mortality with increasing culture duration. Peak mortality (up to 100%) was recorded in the 21-day culture treatment combined with maize flour carrier, indicating optimal virulence at this physiological stage of fungal development. Although 28-day cultures showed high spore density, mortality was slightly reduced, suggesting that virulence may depend not only on conidial concentration but also on metabolic activity and secondary metabolite production. The superior performance of maize flour as a carrier likely contributed to enhanced conidial stability and moisture retention, facilitating improved spore adhesion to larval cuticle. Carrier-based enhancement is a critical determinant of field applicability, particularly under tropical conditions characterized by high UV exposure and rapid desiccation. These results are consistent with previous findings that entomopathogenic fungi can induce significant mortality in FAW larvae under laboratory conditions.

Table 3: Average Percentage of *S. frugiperda* Mortality in Relation to *B. bassiana* Culture Duration

Treatment	Mortality Days- (%)						
(Day)	1	2	3	4	5	6	7
7	0	0	3.3^{a}	13.3	26.6^{a}	46.6^{a}	63.3^{a}
14	0	0	3.3^{a}	16.6	33.3^{a}	50^{a}	70^{a}
21	0	0	10^{a}	23.3	53.3^{b}	93.3^{b}	100^{b}
28	0	3.3	13.3^{b}	26.6	50^{b}	76.6^{b}	93.3^{b}

Numbers followed by the same letter in the same column indicate that the results are not significantly different based on the Tukey test at the 0.05 level.

Importantly, this study demonstrates that formulation optimization (carrier and incubation period)

substantially influences pathogenic performance, reinforcing the necessity of formulation standardization before large-scale deployment.

Spore viability tended to increase with culture duration, reaching its highest at 28 days. Although the numerical data are not presented here, the trend indicates that longer incubation promotes conidial maturity and higher germination potential. These changes may explain the enhanced virulence observed at 21–28 days. These findings align with Wang et al. (2022), who reported that fungal viability directly influences insect mortality, and with Meena et al. (2015), who found that mannitol metabolism contributes to conidial resilience under stress and optimal mortality at 21–28 days may be linked to the maturity of conidia and accumulation of secondary metabolites such as beauvericin, which enhance virulence. Compared with conventional insecticides, *B. bassiana* offers eco-friendly advantages—its infection cycle does not disrupt non-target organisms, and it can adapt across varied temperature and humidity ranges (Daud et al., 2020; Dannon et al., 2020). The infection process includes conidial adhesion, germination, penetration via enzyme secretion, and systemic colonization, ultimately leading to host death. The combination of enzymatic degradation (chitinase, protease) and mycotoxin production (beauvericin, cyclosporine) contributes to the superior biocontrol potential of *B. bassiana*. In addition, endophytic and entomopathogenic fungi have been reported to significantly increase insect mortality and disrupt pest development in several crop systems (Syahrawati et al., 2025). Entomopathogenic microorganisms have been widely investigated as biological control agents for *Spodoptera frugiperda* in maize production systems (Zerbo et al., 2025). Recent research shows that volatile organic compounds (VOCs) emitted by *Beauveria bassiana* can stimulate plant defense responses by inducing phytohormones such as salicylic acid and jasmonic acid and increasing the expression of defense-related genes (Adame-Garnica et al., 2026). EPF (Entomopathogenic Fungi) orchestrates systemic acquired resistance (SAR) and induced systemic resistance (ISR) via salicylic acid (SA) and jasmonic acid (JA) signalling pathways, respectively. Through these mechanisms, EPF promotes plant resilience against both biotic (pathogens and pests) and abiotic stresses (drought, nutrients, heat, and salinity), while improving nutrient uptake, hormone production, and soil quality. In addition, the secondary metabolites produced by EPF strengthen plant defense and stress tolerance.

The findings of this study highlight the potential of a single powdered *B. bassiana* formulation to function effectively across two distinct crop–pest systems. Cross-system validation is particularly significant because CPB and FAW differ markedly in feeding behavior, habitat, and exposure patterns. CPB larvae develop inside cocoa pods, limiting direct fungal contact, whereas FAW larvae feed externally on maize foliage. Demonstrating efficacy in both systems suggests broad functional adaptability of the formulation.

Environmental persistence remains a major constraint for entomopathogenic fungi. In tropical agroecosystems, high temperature and UV radiation can reduce conidial viability rapidly. The observed suppression levels indicate that the powdered formulation maintained sufficient infectivity under these conditions. This suggests that the carrier system and formulation strategy successfully mitigated environmental stress factors. The difference between laboratory mortality (up to 100%) and field suppression (~30–58%) reflects expected ecological variability. Such differences emphasize the importance of integrating entomopathogenic fungi within IPM frameworks rather than relying on them as standalone eradication tools. Fungal biocontrol agents function best as population regulators that reduce pest pressure below economic thresholds rather than achieving complete elimination. Another important aspect is the physiological state of fungal cultures. The observation that 21-day cultures induced higher mortality than 28-day cultures suggests that optimal virulence may correspond to peak metabolic activity rather than maximal spore density. Secondary metabolites such as beauvericin and bassianolide may play synergistic roles in accelerating host mortality. Therefore, culture age standardization is critical for consistent product performance. From an economic perspective, the ability to apply a single formulation to both cocoa and maize systems offers practical advantages. Smallholder farmers in tropical regions often manage multiple crops simultaneously. Developing a multi-target biocontrol formulation reduces logistical complexity, production costs, and storage requirements. This cross-host applicability enhances scalability and supports sustainable pest management strategies.

Conclusion

This study demonstrates that *Beauveria bassiana* is a promising biological control agent for two major pests, the cocoa pod borer (*Conopomorpha cramerella*) and the fall armyworm (*Spodoptera frugiperda*). This study provides cross-system experimental validation of a standardized powdered *Beauveria bassiana* formulation against two economically important tropical pest species. Application of *B. bassiana* powder at 150 g/L significantly reduced cocoa pod borer infestation, achieving the lowest fruit damage and infestation intensity under field conditions. In maize, the formulation containing 75 g/500 mL water with 5 g corn flour resulted in the highest larval mortality of *S. frugiperda* and improved yield under field conditions. Importantly, optimal fungal performance was observed at culture durations of 21–28 days, indicating that virulence is influenced not only by conidial density but also by physiological maturity and viability of the propagules. Entomopathogenic fungi such as *Beauveria* species have shown promising effectiveness against various agricultural pests under field conditions (Fardoun et al., 2025). Endophytic and entomopathogenic fungi are increasingly recognized for their multiple roles in pest suppression, plant defense induction, and growth promotion within integrated pest management systems (Hendra et al., 2026). These results confirm the potential of *B. bassiana* as a multi-target biological control agent. The consistent efficacy of a single powdered formulation across two

distinct crop–pest systems highlights its formulation stability and practical scalability for broader agricultural application. Rather than functioning as a standalone eradication strategy, this formulation demonstrates potential as a population-suppressive component within Integrated Pest Management (IPM) programs, contributing to reduced reliance on synthetic insecticides and supporting sustainable crop protection. However, this study has several limitations. First, the culture duration experiment used standardized spore densities, which may not fully represent natural conidial production dynamics. Second, endophytic colonization of *B. bassiana* in maize tissues was not verified through re-isolation methods. Field trials were also conducted in a single location and season, which may limit generalization across agroecological zones. Future research should quantify actual conidial productivity across different culture ages, verify endophytic establishment using re-isolation or molecular approaches, and conduct multi-location and multi-season field evaluations to strengthen ecological robustness. Further large-scale validation and compatibility testing with other IPM components, such as parasitoids and botanical insecticides, are recommended to facilitate integrated deployment of *B. bassiana*-based formulations in tropical farming systems.

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Ethics Statement: This study did not involve human participants or vertebrate animals. Experimental work was conducted using insect pests and microbial biological control agents under standard laboratory and field procedures; therefore, formal ethical approval was not required.

Author's Contribution: I.D. Daud conceptualization, supervised, designed the experiments, analyzed the data, and prepared the initial draft of the manuscript. Melina contributed to the field experiments and laboratory analyses. V.S. Dewi assisted with statistical analysis, literature review, and manuscript refinement. A.I. Suryani reviewed and edited manuscript. All authors designed the experiments and analyzed data

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