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# Impact of *Beauveria bassiana* on Populations of Insect Pests and Their Natural Enemies on Rice (*Oryza sativa* L.)

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***Abstract.*** *The efficacy of Beauveria bassiana, an entomopathogenic fungus used as a biological control agent against insect pests(phytophagous), was evaluated for its impact on insect pest populations and their natural enemies in rice plants. This study aims to evaluate the role of B. bassiana in influencing insect pest populations and the balance of natural enemy populations during the rice growth stage and its effect on crop yield. The applications were carried out in the Girirejo rice field in North Samarinda, Samarinda, East Kalimantan, Indonesia. The study employed a Randomized Complete Block Design (RCBD) with four treatments and three replications. The treatments consisted of B0 (without B. bassiana), B1 (4.5 x $10^{10}$ conidia $g^{-1}$), B2 (9.0 x $10^{10}$ conidia $g^{-1}$) and B3 (13.5 x $10^{10}$ conidia $g^{-1}$). Data were analyzed by analysis of variance, followed by the Least Significant Difference (LSD) test at 5% error level. Results showed that B. bassiana significantly reduced insect pest populations at 80 and 100 days after planting (DAP) compared to the control. No significant differences were observed in natural enemy populations, although a decline in neutral insects was recorded at 60 DAP. Importantly, B. bassiana application significantly increased dry grain yield without negatively affecting natural enemies. These findings suggest that B. bassiana is a safe and effective biological control strategy for sustainable rice cultivation.*

***Keywords:*** *biological control; entomopathogenic fungi; rice yield; insect pests; natural enemies.*

**Type of the Paper:** Regular Article.

## 1. Introduction

Rice is a staple food for more than half of the world's population, with 90% of global production and consumption occurring in Asia. This underscores the central role of rice in regional and global food security [1]. In Indonesia, rice remains the primary food source for the majority of the population, with per capita consumption reaching 92.39 kg $year^{-1}$ in 2024 [2].

Rice production in Indonesia in 2024 reached 53.14 million tons with an average yield of 5.29 Mg $ha^{-1}$. In East Kalimantan, the production was 249,642.90 Mg with a yield of 3.96 Mg $ha^{-1}$ [3], which remains considerably below the national average. Efforts to achieve optimum rice production in the field always encounter various obstacles. Factors influencing rice productivity in East Kalimantan include climate change, land conditions dominated by acidic soils with



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relatively low nutrient content, limited irrigation systems, the use of less adaptive varieties, the conversion of agricultural land, and disturbances caused by plant pests [4–8].

Phytophagous insect pests have been one of major constraints damaging plant tissues. Pest attacks can inhibit plant growth, reduce the quality and quantity of grain, and result in significant yield losses. In addition to harboring pests, rice paddy agroecosystems also serve as habitats for various arthropod species that perform important ecological functions, including parasitoids, predators, and beneficial organisms such as pollinators and decomposers [9–11]. The presence of these beneficial arthropods is essential for maintaining ecosystem balance and supporting natural biological control in rice cultivation.

Synthetic insecticides remain the primary method for plant pest control and are effective in suppressing pest populations and increasing crop yields. However, their toxic properties can harm humans, livestock, pollinators, natural enemies, and plants; they cause environmental pollution and improper dosage may trigger insect resistance and resurgence [12–15]. Furthermore, continuous pesticide use can destabilize agroecosystems by reducing natural enemy populations, thereby increasing the risk of pest outbreaks [16,17].

In response to these issues, microbial bioinsecticides have gained attention as alternatives or supplements to synthetic chemicals. Their global adoption is driven by demands for residue-free agricultural products, reduced dependence on chemical pesticides, expansion of organic farming, and implementation of integrated pest management [18]. Nevertheless, bioinsecticides must be applied with caution because they generally require more time to kill pests compared to chemical insecticides, during which infected insects may still cause significant crop damage [19].

Microbial biopesticide agents, such as bacteria, viruses, nematodes, protozoa, and fungi are considered effective biological pest control tools [20]. Among them, entomopathogenic fungi are particularly important due to their ability to trigger epizootics in insect populations [21]. These fungi are cosmopolitan in distribution, aggressively infect insect hosts, and more than 750 species across at least 90 genera are currently recognized as insect pathogens, spanning phyla such as Chytridiomycota, Ascomycota, Basidiomycota, and the subphylum Entomophthoromycotina [22–24].

Microbial bioinsecticides, including entomopathogenic fungi, play an important role in biological control within integrated pest management programs. *Beauveria bassiana* is one of the most widely used species because it offers effective pest suppression without harming the environment [14,25]. This fungus can be found and isolated from rhizosphere soils around rice fields in East Kalimantan, and is capable of infecting insect pests [26]. Its application is practical, as the fungus can be administered using conventional sprayers similar to those used for chemical insecticides.

*B. bassiana* has been proven effective against insect pests from multiple orders, including Lepidoptera [27-29] and Hemiptera [26,30]. A key advantage of this fungus is its mode of infection, which occurs via direct penetration of the insect cuticle. Conidia adhere to the epicuticle through hydrophobic, electrostatic, and adhesin-mediated mechanisms [23,31], followed by germination into germ tubes that form penetration structures. Mechanical pressure coupled with cuticle-degrading enzymes enables cuticle breach and access to the haemocoel [32,33].

Control activities using *Beauveria bassiana* have long been implemented by the East Kalimantan Food Crop and Horticulture Protection Center. However, research on this entomopathogenic fungus in East Kalimantan, particularly in the Samarinda region, has not yet been comprehensively conducted to evaluate the impacts of its field-scale application on insect pest populations and natural enemies. This study provides new insight by analyzing how *B. bassiana* shapes the balance among insect pests and their natural enemies across vegetative and generative growth stages of rice. By linking shifts in arthropod community structure to rice yield performance, the research demonstrates the ecological safety and practical value of *B. bassiana* as a sustainable component of Integrated Pest Management (IPM) in tropical rice ecosystems.

## 2. Materials and Methods

### *2.1. Research location and experimental design*

The field research was conducted for five months on the Girirejo rice fields in North Samarinda, Samarinda, East Kalimantan. The rice variety used was Mekongga, which was planted in 12 experimental plots, each measuring 4 m x 3 m. Rice planting was conducted using the Jajar Legowo 6:1 technique with a spacing of (0.2 m x 0.4 m). Each plot was separated by a distance of 1 m. Planting was performed by transferring seedlings from the nursery to the field, placing them into holes 3–4 cm deep. Seedlings were transplanted at 18 days old.

This experiment was arranged in a Randomized Complete Block Design (RCBD) with four treatments and three replications, consisting of B0 (without *B. bassiana treatment*), B1 ($4.5 \times 10^{10}$ conidia $g^{-1}$ equivalent to 1 g $L^{-1}$ of water), B2 ($9.0 \times 10^{10}$ conidia $g^{-1}$ equivalent to 2 g $L^{-1}$ of water) and B3 ($13.5 \times 10^{10}$ conidia $g^{-1}$ equivalent to 3 g $L^{-1}$ of water. The *Beauveria bassiana* isolate used in this study was obtained from the Laboratory of the Food Crop and Horticulture Protection Center in East Kalimantan. The isolate was propagated in the laboratory as conidial powder. Prior to application, conidial viability was tested using the germination method on PDA medium. The suspension was only used if conidial viability reached ≥ 90% germination.

The application of *B. bassiana* was carried out at 10, 30, 50, 70 and 90 days after planting (DAP). The application is applied in the afternoon by spraying. Data were collected at 20, 40, 60, 80 and 100 days after planting. Before field application, the conidial powder was formulated

directly by mixing it with water at the required concentration, then homogenized and poured into the knapsack sprayer tank.

The standard spray volume was 500 mL /m². For a 4 × 3 m (12 m²) plot, approximately 6 L of suspension was required per plot. The final suspension concentration in the sprayer tank corresponded to the designated treatment.

Harvesting was conducted 110 days after planting, when flag leaves remained green or panicles began to yellow. At this stage, the stalks appeared bent, and the grains were filled and hardened.

### *2.2. Observation of pests and natural enemies*

Observation data include the number of insect populations caught. The canopy arthropod sampling was conducted by hand picking with a standard duration of 10 minutes per plot. Sampling of predators active at ground level was carried out using pitfall traps deployed for 24 hours, starting at 17:00, with five traps per plot. Arthropod samples actively flying were taken using a sweep net of ten double swings per plot [34]. For insects active at night, one light trap per plot was operated for four hours (18:00–22:00) [35,36]. All insects collected from each trap were placed in bottles containing 70% alcohol, and their populations were calculated based on their roles in the rice agroecosystem, namely as pest arthropods, natural enemies, and neutral arthropods. To distinguish the roles of the three arthropod groups, reference literature was used [37,38]. Arthropod populations were observed at 20, 40, 60, 80, and 100 days after planting (DAP).

### *2.3. Data analysis*

Observation data were analyzed by analysis of variance, followed by a comparison of the Least Significant Difference (LSD) test at 5% error level. Data analysis was conducted using R software version 4.6.1 as a statistical computing environment, with RStudio as an Integrated Development Environment (IDE) to facilitate manuscript management, visualization, and interpretation of results.

## 3. Results and Discussion

### *3.1. Arthropod diversity in the rice field of the experimental area*

The arthropod community in the experimental rice field showed a balanced presence of phytophagous insects, natural enemies, and neutral groups, indicating a stable agroecosystem where biological control can function effectively. Landscape heterogeneity enhances natural pest suppression by supporting predator diversity [39]. Similarly, the framework of landscape agroecology highlights how methodological design and spatial configuration of habitats can strengthen ecological processes and sustain agroecosystem resilience [40]. However, responses of crop pests and their predators to landscape composition are not always consistent, underscoring

the complexity of ecological interactions and the need for context-specific management strategies [41].

**Table 1**. Arthropods and their Role in the paddy land.

| Class | Ordo | Family/Genus | | Role |
|---|---|---|---|---|
| Insecta | Lepidoptera | Crambidae | *Scirphopaga innotata* | Pest |
| | | | *Scirpophaga incertulas* | Pest |
| | | | *Cnaphalocrocis medinalis* | Pest |
| | | Nymphalidae | *Junonia atlites* | Pollinator |
| | | Uraniidae | *Urapteroides* sp. | Pollinator |
| | Coleoptera | Coccinellidae | Menochilus sexmaculatus | Predator |
| | | Carabidae | *Ophionea nigrofasciata* | Predator |
| | | | *Pterostichus melanarius* | Predator |
| | | Scarabaeidae | *Leucopholis* sp. | Neutral |
| | | Dytiscidae | *Hydrophillus triangularis* | Neutral |
| | | Chrysomelidae | *Agelastica* sp. | |
| | Hemiptera | Alydidae | *Leptocorisa acuta* | Pest |
| | | Cimicidae | *Scotinophara coarctata* | Pest |
| | | Lygaeidae | *Paraeucosmetus pallicornis* | Pest |
| | | Delphacidae | *Nilaparvata lugens* | Pest |
| | | Cicadellidae | *Nephotettix virescens* | Pest |
| | | | *Cofana* sp. | Pest |
| | | Pentatomidae | *Perillus bioculatus* | Pest |
| | | Gerridae | *Gerris* sp. | Pest |
| | Orthoptera | Acrididae | *Oxya chinensis* | Pest |
| | | | *Locusta migratoria* | Pest |
| | | Gryllidae | *Metioche vittaticollis* | Pest |
| | | Tettigonidae | *Conocephalus longipennis* | Pest |
| | | Pyrgomorphinae | *Atractomorpha* sp. | Pest |
| | | Gryllotalpidae | *Gryllotalpa* sp. | Pest |
| | Blattodea | Blaberidae | *Pseudophoraspis* sp. | Neutral |
| Insecta | Diptera | Muscidae | Musca domestica | Neutral |
| | | Tipulidae | *Tipula oropezoides* | Detritivore |
| | | Culicidae | *Culex* sp. | Neutral |
| | | Stratiomyidae | *Hermetia illucens* | Neutral |
| | | Chironomida | *Chironomus* sp. | Neutral |
| | Odonata | Libellulidae | *Neurothemis* sp. | Predator |
| | | | *Pantala flavescens* | Predator |
| | | Coenagrionidae | *Agriocnemis pygmea* | Predator |
| | Hymenoptera | Vespidae | *Vespa* sp. | Predator |
| | | Apidae | *Bombus* sp. | Pollinator |
| | | | *Xylocopa aestuans* | |
| | | Ichneumonidae | *Lissopimpla excelsa* | Predator Predator |
| | | Formicidae | *Odontomachus simillimus* | |
| Arachnida | Araneae | Oxyopidae | *Oxypes javanus* | Predator |
| | | Tetragnathidae | *Tetragnatha maxillosa* | Predator |
| | | Araneidae | *Araneus inustus* | Predator |
| | | Lycosidae | *Lycosa pseudoannulata* | Predator |
| Entognatha | Collembola (subclass) | Not identified | Not identified | Detritivore |

The balance between herbivores and natural enemies is a critical factor in maintaining agroecosystem stability. Recent studies highlight that landscape complexity and ecological engineering practices enhance natural enemy diversity and strengthen biological control services

[42]. Such ecological intensification strategies, including habitat diversification, reduce pest outbreaks and improve resilience in rice agroecosystems [43].

Based on their functional roles within the ecosystem, the identified arthropods were classified into three primary categories: phytophagous arthropods (pests), natural enemies (predators and parasitoids), and neutral arthropods (Table 1). This functional group classification is crucial for developing sustainable pest management strategies, such as ecological engineering, which aims to enhance ecosystem services provided by beneficial arthropods [44,45].

**Phytophagous insects (Pests)**. Phytophagous arthropods are major pests in rice agroecosystems, feeding on plant tissues and reducing yields. Three main orders were identified: Lepidoptera, Hemiptera, and Orthoptera, with key families including Pyralidae and Crambidae (Lepidoptera), Delphacidae, Cicadellidae and Alydidae (Hemiptera), and Acrididae (Orthoptera). These families are well-known rice pests capable of causing significant yield losses if uncontrolled [46,47]. Among them, Pyralidae, Delphacidae, and Alydidae are consistently reported as serious threats across Asia, inflicting direct damage through feeding and indirect damage via disease transmission [47,48]. Current management emphasizes integrated pest management (IPM) strategies to reduce dependence on broad-spectrum insecticides and maintain ecological balance [49,50].

**Natural enemy arthropods**. Natural enemies are essential for biological control, regulating pest populations through predation and parasitism. This group exhibited considerable diversity across several insect orders and the class Arachnida. Predators were identified in Coleoptera, Odonata, Hemiptera, and Diptera. Importantly, spider families within the Order Araneae, such as Lycosidae, are recognized as dominant generalist predators in rice paddies. Recent studies confirm that diverse spider communities are critical for maintaining stable and effective pest suppression throughout the crop cycle [51].

The Order Hymenoptera contained both predators (e.g., Formicidae) and parasitoids (e.g., Ichneumonidae). Parasitoid wasps are highly effective biological control agents, as many specialize in attacking specific pest life stages, such as the eggs or larvae of planthoppers and lepidopteran [50].

**Neutral insects**. Neutral arthropods are organisms that are neither directly harmful as pests nor beneficial as natural enemies, but perform other important ecological functions in the food web [52]. The primary role of this group is as detritivores, or decomposers. Organisms such as members of the subclass Collembola and the orders Coleoptera and Blattodea act as "ecosystem engineers" in the soil. They accelerate the decomposition of plant debris and other organic matter, a process fundamental to nutrient cycling and soil fertility.

Collembola, for example, strongly influence soil microbial communities and associated functions, thereby enhancing decomposition efficiency [53]. Arthropods more broadly are recognized as key agents in maintaining soil fertility through their role in organic matter breakdown and nutrient release [54]. Recent perspectives also describe these organisms as "tiny architects of fertile soils," highlighting their critical contribution to sustaining ecosystem health and productivity in rice paddy systems [55]. In addition to detritivores, the neutral category can also include other insects whose presence is temporary or whose specific role in the ecosystem is not yet understood.

*3.2. Impact of B. bassiana application on the population of pest insects and natural enemies.*

The results show that the arthropods collected consisted of pest insects, natural enemies (parasitoids and predators), and other neutral insects. The ANOVA results indicated that during the early observation stages (20, 40, and 60 days after planting, DAP), there were no significant differences among treatments with varying *Beauveria bassiana* concentrations in pest insect density. The low F-values combined with p-values greater than 0.05 confirm that the application of this entomopathogenic fungus did not exert a significant effect during the vegetative phase of rice growth. This outcome may be associated with relatively low pest populations and environmental conditions that were not yet favorable for fungal infection and development (Table 2).

**Table 2.** Density pest insects under different *Beauveria bassiana* concentration treatments (individuals/plot, Mean ± SE, n=3).

| Treatment | Rice Plant Age (DAP) | | | | |
|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 |
| B0 | 13.00 ± 4.04 | 23.67 ± 6.69 | 19.67 ± 2.85 | 37.67 ± 5.90 a | 69.33 ± 4.81 a |
| B1 | 8.00 ± 1.00 | 18.33 ± 3.28 | 19.67 ± 8.67 | 25.00 ± 2.52 b | 38.33 ± 2.96 b |
| B2 | 6.33 ± 0.33 | 20.67 ± 1.76 | 25.00 ± 8.66 | 26.67 ± 4.37 b | 36.67 ± 4.26 b |
| B3 | 11.00 ± 1.15 | 25.00 ± 3.21 | 17.67 ± 3.76 | 20.00 ± 5.69 b | 35.00 ± 4.04 b |
| F-value | 1.67 | 1.23 | 0.30 | 8.75 | 18.09 |
| p-value | 0.271 | 0.379 | 0.826 | 0.013 | 0.002 |
| LSD 0.05 | $7.99^{ns}$ | $3.97^{ns}$ | $19.91^{ns}$ | $8.71^{*}$ | $13.34^{*}$ |

Remarks: Means followed by different letters within the same column are significantly different at $p<0.05$ (LSD test). * = significant at 5% level; NS = non-significant.

In contrast, significant differences were observed during the generative phase (80 and 100 DAP). At 80 DAP, the untreated control (B0) exhibited the highest pest population (37.67 individuals/plot), which was significantly greater than B1 (25.00 individuals/plot), B2 (26.67 individuals/plot), and B3 (20.00 individuals/plot). Similarly, at 100 DAP, B0 produced the highest pest density (69.33 individuals/plot), whereas treatments with *B. bassiana* (B1 = 38.33 individuals/plot, B2 = 36.67 individuals/plot, and B3 = 35.00 individuals/plot) significantly suppressed pest populations. However, no significant differences were detected among the three

fungal concentrations (B1, B2, and B3), suggesting that increasing concentration does not necessarily enhance efficacy (Table 2).

These results suggest that the application of *B. bassiana* was associated with a reduced insect pest population during the generative phase (80–100 DAP). Previous studies have reported that *B. bassiana* can reduce insect populations through infection processes involving conidial attachment, cuticle penetration, and host colonization [18]. However, because insect cadavers were not collected and fungal infection was not confirmed through re-isolation or microscopic observation in the present study, the observed population reduction cannot be directly attributed to *B. bassiana* infection.

In contrast, Rosfiansyah et al. [50] provided clear laboratory evidence in their study on the rice pest Leptocorisa acuta, where *B. bassiana* successfully killed the insects and fungal mycelium visibly surrounded the cadavers. This finding supports the infection mechanism of *B. bassiana* and strengthens the assumption that the population decline observed in the present study may also be related to the activity of this entomopathogenic fungus. Nevertheless, future research should incorporate pathogen confirmation procedures such as cadaver collection, fungal re-isolation, and microscopic observation to validate the causal role of *B. bassiana* in pest mortality.

The observed changes in pest populations during later growth stages may be influenced by ecological factors such as canopy density and plant–insect interactions. Previous studies have reported that suppression was stronger in older plants, likely due to denser canopy microclimates that increase humidity and favor fungal development [56]. Environmental factors such as humidity, temperature, and host density also influence infection success [57]. In the present study, microclimatic parameters (temperature, relative humidity, and rainfall) were not monitored; therefore, their contribution to the observed reductions cannot be determined. Future research should include systematic microclimate monitoring to validate the relationship between environmental conditions and the efficacy of *B. bassiana*.

The lack of significant differences among B1, B2 and B3 suggests that higher concentrations may not improve efficacy, highlighting the need for optimized application rates to balance cost and effectiveness [58]. Moreover, effectiveness is strongly linked to microclimate conditions; in less humid environments, fungal performance may decline, requiring integration with habitat management strategies such as ecological engineering [42] Thus, *B. bassiana* represents a promising eco-friendly alternative to chemical insecticides, reducing risks of resistance and environmental pollution [59].

The effect of *B. bassiana* application on the average population of natural enemy arthropods was evaluated at 20, 40 and 60, 80 and 100 days after planting (DAP) (Table 3). The population

of natural enemies remained relatively stable and showed no significant differences between treatments with and without *B. bassiana*.

**Table 3**. Density of natural enemies under different *Beauveria bassiana* concentration treatments (individuals/plot, Mean ± SE, n=3).

| Treatment | Rice Plant Age (DAP) | | | | |
|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 |
| B0 | 9.00 ± 3.21 | 24.00 ± 7.02 | 22.00 ± 2.08 a | 35.33 ± 5.24 | 16.33 ± 0.33 |
| B1 | 10.67 ± 2.33 | 20.00 ± 3.00 | 24.67 ± 3.71 a | 25.00 ± 7.09 | 15.33 ± 2.33 |
| B2 | 11.67 ± 2.85 | 11.67 ± 5.24 | 10.00 ± 1.53 b | 19.33 ± 1.86 | 10.33 ± 1.76 |
| B3 | 11.67 ± 3.53 | 17.67 ± 7.75 | 17.00 ± 4.51 ab | 15.33 ± 1.45 | 16.00 ± 1.53 |
| F-value | 0.30 | 1.81 | 3.53 | 2.95 | 3.05 |
| p-value | 0.82 | 0.25 | 0.09 | 0.12 | 0.11 |
| LSD 0.05 | $7.93^{ns}$ | $13.26^{ns}$ | $11.88^{ns}$ | $17.50^{ns}$ | $5.56^{ns}$ |

Remarks: Means followed by different letters within the same column are significantly different at $p<0.05$ (LSD test). * = significant at 5% level; NS = non-significant.

The results of the study showed that the natural enemy populations (predators and parasitoids) at various rice growth stages were relatively stable and showed no significant differences between the *B. bassiana* treatment and the control. This indicates that the application of this entomopathogenic fungus is selective for target insects (pest), but relatively safe for non-target organisms such as predators and parasitoids.

This phenomenon is supported by previous studies, which demonstrated that most natural enemies tend to exhibit low susceptibility to entomopathogenic fungi, primarily because their behavioral patterns, morphological traits, and ecological niches reduce direct exposure compared to phytophagous insects [60,61]. Some predators, such as spiders and hemipteran insects, have foraging behaviors that reduce direct contact with *B. bassiana* conidia [56]. In addition, parasitoids are frequently located inside their hosts, thereby limiting their exposure to conidia, a phenomenon that has been systematically reviewed and confirmed to reflect their compatibility and reduced susceptibility to entomopathogenic fungi [61].

The sustainability of natural enemy populations after *B. bassiana* application is an advantage in Integrated Pest Management (IPM) implementation. The combination of entomopathogenic fungi and natural enemies can increase the effectiveness of pest control and maintain the balance of the rice field ecosystem [18,59].

The results also showed that *B. bassiana* application caused fluctuations in neutral arthropod populations. At 20, 40, 80, and 100 days after planting (DAP), populations did not differ significantly between treatments (Table 4). However, at 60 days after planting, significant differences were found, with the lowest population in treatment B1 (6.33 individuals/plot), significantly different from the control (19.00 individuals/plot) and treatment B2 (14.67 individuals/plot).

**Table 4**. Density of neutral insects under different *Beauveria bassiana* concentration treatments (individuals/plot, Mean ± SE, n=3).

| Treatment | Rice Plant Age (DAP) | | | | |
|---|---|---|---|---|---|
| | 20 | 40 | 60 | 80 | 100 |
| B0 | 11.67 ± 1.86 | 10.00 ± 2.65 | 19.00 ± 3.06 a | 18.67 ± 4.91 | 11.33 ± 2.03 |
| B1 | 11.00 ± 3.79 | 5.33 ± 2.40 | 6.33 ± 2.03 b | 17.00 ± 4.04 | 14.33 ± 6.57 |
| B2 | 13.00 ± 3.51 | 4.00 ± 1.53 | 14.67 ± 3.48 a | 9.00 ± 1.00 | 7.33 ± 1.86 |
| B3 | 11.33 ± 5.61 | 8.00 ± 2.65 | 12.00 ± 2.31 ab | 12.67 ± 2.40 | 13.00 ± 2.31 |
| F-value | 0.20 | 1.82 | 5.09 | 1.80 | 0.77 |
| p-value | 0.89 | 0.24 | 0.04 | 0.25 | 0.55 |
| LSD 0.05 | 6.72 | 6.90 | 8.13 | 11.25 | 11.97 |

Remarks: Means followed by different letters within the same column are significantly different at $p<0.05$ (LSD test). * = significant at 5% level; NS = non-significant.

In general, the impact of *B. bassiana* on neutral arthropods was relatively limited and temporary. This fungus prefers phytophagous insects, so neutral arthropods rarely experience direct infection [60,62]. The fluctuating dynamics of neutral populations are thought to be influenced by changes in the microhabitat after application and indirect interactions with other organisms. As plants age, populations tend to recover. This finding supports reports that long-term application of *B. bassiana* does not significantly reduce the diversity of non-target arthropods [63,64].

Although neutral arthropods do not directly control pests, they still play a role in maintaining ecosystem balance, for example as part of the food chain and providing other ecosystem services [64]. Therefore, the use of *B. bassiana* can be considered an environmentally friendly biological control strategy.

### *3.3. Effect of B. bassiana application on the population proportion of rice pest arthropods during Vegetative and Generative periods*

The descriptive analysis of arthropod population proportions at different rice growth stages indicated observable shifts in composition. In the vegetative phase (Fig. 1), the arthropod population composition tended to be balanced. Phytophagous insect pests and natural enemies (predators and parasitoids) accounted for more than 35% of the relative proportion, while neutral arthropods were below 30%. In the *B. bassiana* treatment with a concentration of $9.0 \times 10^{10}$ conidia/g, the proportion of insect pests was observed at more than 40%, while the proportion of natural enemies and neutral arthropods decreased below 30%.

Conversely, in the generative phase (Fig. 2), the arthropod population composition showed descriptive changes. The proportion of insect pests increased to more than 45% in all treatments, while natural enemies decreased to less than 30% and neutral arthropods decreased to less than 25%. This condition is related to the rice panicle formation phase, where the phytophagous arthropod population is primarily dominated by pests from the order Hemiptera (e.g., Alydidae),

with attacks increasing in intensity during the generative phase. This descriptive tendency aligns with reports that the generative phase is a critical period for the increase in the population of major rice pests [47,49].

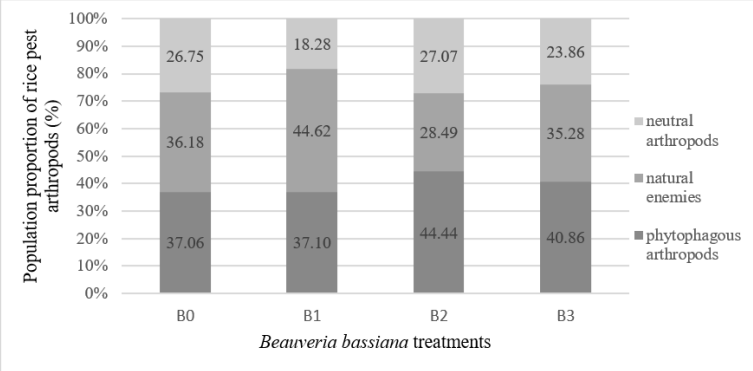



**Fig. 1**. Effect of *B. bassiana* on the proportion of arthropods in paddy vegetative life.

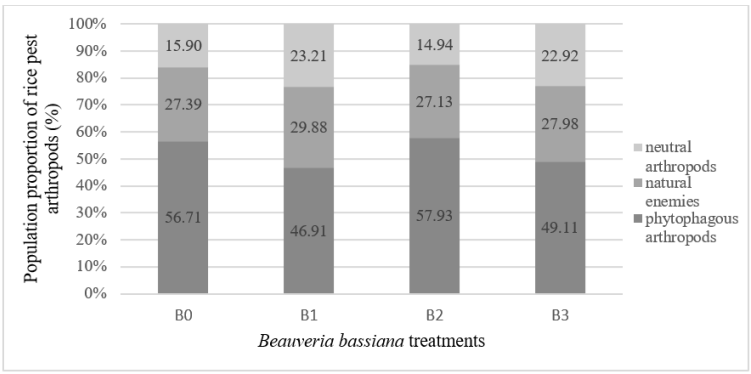



**Fig. 2**. Effect of *B. bassiana* on the proportion of arthropod populations in paddy generative life.

Changes in arthropod composition are also closely related to the dynamics of rainfed rice field ecosystems. Rice field ecosystems undergo rapid changes due to soil cultivation, fallow periods, and harvests, which affect the availability of habitat and food sources for arthropods. In the study area, the rice fields were fallow after harvest before planting, with a planting pattern of only one crop per year. This situation suppresses pest populations and reduces the density of natural enemies during the early vegetative phase, resulting in slower recovery of predator and parasitoid populations. A similar phenomenon was also reported by Savary et al. [49], who highlighted that rainfed rice fields and long fallow systems can delay the recovery of natural enemy communities during the early stages of plant growth.

The low population density of natural enemies is also influenced by environmental factors that are less conducive to their development, while microclimatic conditions and host availability are more favorable for pest development [39,65]. However, the presence of neutral arthropods has the potential to support ecosystem stability. Increasing the population of neutral arthropods can act as an alternative food source, thus supporting the long-term sustainability of predator and parasitoid populations [11]. Therefore, although *B. bassiana* is effective in suppressing pest

populations, agroecosystem management that considers habitat availability and non-target arthropod diversity is still necessary to maintain the ecological balance of rice fields.

*3.4. Effect of B. bassiana application on arthropod dynamics on rice crops*

Observations of pest arthropod population dynamics were conducted at 20-day intervals (Fig. 3). The population of pest arthropods in the control treatment (without *B. bassiana*) increased rapidly from 60 to 100 days after planting (DAP). In contrast, treatments with *B. bassiana* were also associated with an increase in pest populations; however, the rate of increase was relatively slower. The population peak in all treatments occurred at 100 DAP, corresponding to the panicle-filling and ripening stages, when rice plants are highly susceptible to pest attack.

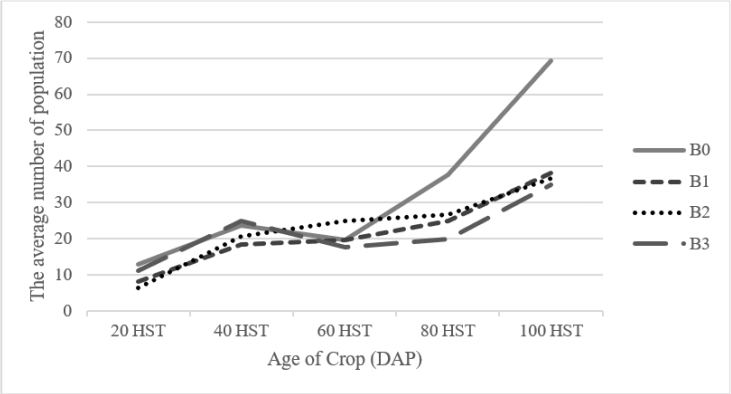



**Fig. 3**. Effect of *B. bassiana* on population dynamics of arthropod pests.

The dynamics of natural enemy populations also fluctuated across treatments (Fig. 4). In most treatments, except for *B. bassiana* 13.5 × $10^{10}$ conidia $g^{-1}$, the peak of natural enemy arthropods occurred at 80 DAP. The highest abundance of natural enemies was observed in the control (without *B. bassiana*). Afterward, populations declined at 100 DAP. Interestingly, in the B. bassiana 13.5 × $10^{10}$ conidia $g^{-1}$ treatment, natural enemies peaked earlier at 40 DAP, decreased at 60–80 DAP, and then increased again at 100 DAP. This descriptive tendency suggests that high concentrations of *B. bassiana* may temporarily suppress natural enemies but allow recovery in the later growth stages.

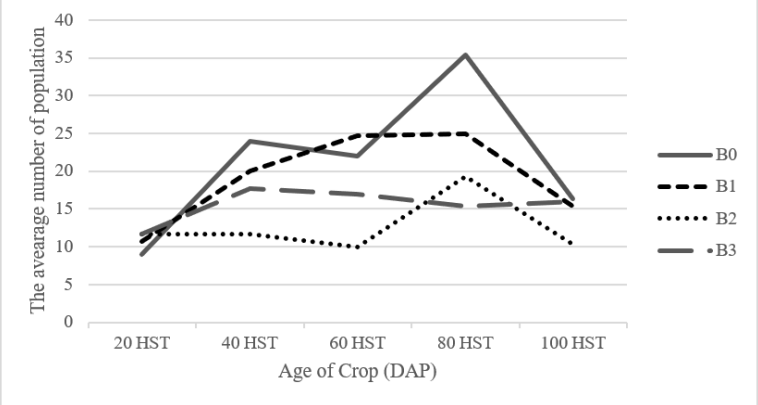



**Fig. 4**. Effect of *B. bassiana* on the dynamics of natural enemy populations.

For neutral insect arthropods, population fluctuations were observed in all treatments (Fig. 5). The peak population occurred at different plant ages depending on the treatment: at 60 DAP in the control and B. bassiana 9.0 × $10^{10}$ conidia $g^{-1}$, at 80 DAP in the *B. bassiana* 4.5 × $10^{10}$ conidia $g^{-1}$ treatment, and at 100 DAP in the *B. bassiana* 13.5 × $10^{10}$ conidia $g^{-1}$ treatment. These results indicate that neutral arthropods, which play an indirect role in stabilizing agroecosystems, are also influenced by fungal applications and environmental conditions.

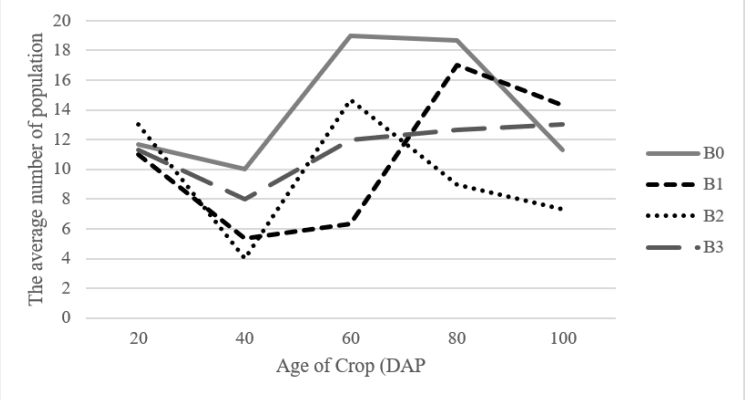



**Fig. 5.** Effect of *B. bassiana* on the population dynamics of neutral arthropods.

From an ecological perspective, rice field insects comprising pests, natural enemies, and neutral species occupy dynamic roles that contribute to the balance and resilience of agroecosystems [66] The suppressive effect of *B. bassiana* during the generative phase reflects not only direct pathogenicity toward phytophagous insects but also indirect ecological influences within the arthropod community. Following conidial adhesion and penetration of the insect cuticle, a reduction in pest abundance likely decreases feeding pressure and alters nutrient and resource flow within the rice canopy, thereby creating a more favorable microenvironment for plant growth.

At the same time, the stability of natural enemy populations suggests that predators and parasitoids experience limited exposure to conidia because their foraging behavior and trophic position minimize direct contact with *B. bassiana*, enabling them to continue exerting top-down control over pest populations. These interactions are crucial for sustaining agroecosystem stability, as the diversity of flora and fauna underpins food web complexity, energy flow, and long-term ecological resilience [60].

Agroecosystem vulnerability to pest outbreaks is strongly influenced by landscape simplification. High-input monoculture farming reduces habitat heterogeneity and limits alternative food resources such as pollen, nectar, and alternative hosts, which causes predator and parasitoid populations to decline and weakens natural pest suppression [41]. Conversely, monoculture systems provide abundant and concentrated food sources for herbivorous insects, enabling them to reproduce efficiently, particularly in intensively fertilized and irrigated fields [67].

In this context, the integration of *B. bassiana* into rice pest management plays an ecologically important role because it selectively targets phytophagous insects while maintaining beneficial arthropods within acceptable ecological thresholds. Therefore, habitat diversification strategies such as intercropping, maintaining vegetation refuges, and ecological engineering are essential to support the full effectiveness of *B. bassiana* within Integrated Pest Management (IPM) frameworks and ensure sustainable and environmentally friendly rice production [18,59].

*3.5. Effect of B. bassiana application on crop yield*

The application of *Beauveria bassiana* significantly affected the crop yield of the Mekongga rice variety (Fig. 6). Crop yield ranged from 2.41 ± 0.05 to 4.75 ± 0.17 Mg ha$^{-1}$. The highest crop yield was obtained in treatment B2 (4.75 ± 0.17 Mg ha$^{-1}$), which was significantly higher than those of B3 (4.07 ± 0.09 Mg ha$^{-1}$), B1 (3.83 ± 0.06 Mg ha$^{-1}$), and the untreated control B0 (2.41 ± 0.05 Mg ha$^{-1}$) according to the LSD test at the 5% significance level. Treatments B1 and B3 did not differ significantly from each other, whereas both treatments produced significantly higher grain yields than the untreated control (B0).

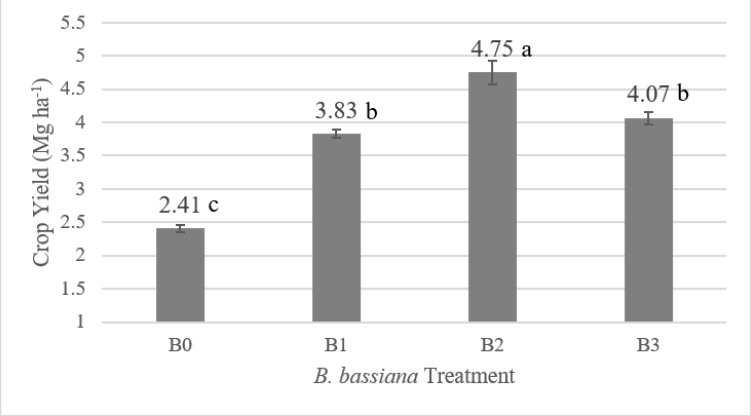



**Fig. 6**. Effect of *B. bassiana* on the rice yield (ton/ha). Bars represent mean ± SE (n = 3). Different lowercase letters above the bars indicate significant differences among treatments according to the LSD test ($LSD_{0.05}$ = 0.36 Mg ha$^{-1}$, $P < 0.05$).

The increased yields indicate that *B. bassiana* application effectively suppresses phytophagous pest populations, thus reducing crop damage during the generative phase, which in turn increases panicle number and grain weight [68].

However, the different results between doses (B1, B2, and B3) suggests that *B. bassiana's* effectiveness does not always increase with increasing spore concentration. This phenomenon is consistent with reports that entomopathogenic fungal applications have an optimum point, where intermediate doses are often more efficient than doses that are too low or too high [69]. In addition to concentration, application success is also influenced by microclimatic conditions, conidial viability, and the timing of application, which coincides with the plant's generative phase [68].

Practically, these findings suggest that *B. bassiana* application can increase rice productivity, but its effectiveness is influenced by a combination of the optimal dose, inoculum quality, and environmental conditions at the time of application. Therefore, appropriate formulation and ecologically based application strategies are essential to maximize the potential of *B. bassiana* as both a biocontrol agent and a growth stimulant.

Entomopathogens such as *B. bassiana* are biological agents that play a crucial role in sustainable agricultural systems. Their application has been proven effective in suppressing phytophagous pest populations without disrupting natural enemies or neutral arthropods [70,71]. In rice paddy ecosystems, humid environmental conditions support the success of fungal applications. Furthermore, the prolonged existence of monoculture systems often places ecological pressure on predators, making the role of entomopathogenic fungi increasingly relevant in maintaining agroecosystem balance [66].

Given its selective and environmentally friendly nature, *B. bassiana* can be recommended as part of an IPM strategy that supports sustainable rice production while reducing the negative impacts of chemical insecticide use [72].

## 4. Conclusions

The application of *Beauveria bassiana* affected rice arthropod dynamics, with a stronger suppressive effect on phytophagous arthropods during the generative phase (80–100 days after planting) while maintaining natural enemy populations. Among the tested concentrations, B2 ($9.0 \times 10^{10}$ conidia $g^{-1}$) was the most effective treatment, providing greater stability of natural enemy populations and producing the highest rice yield. These findings indicate that *B. bassiana* at the B2 concentration has potential as an effective biological control agent that suppresses rice pests while preserving ecological balance and supporting crop productivity.

## Abbreviations

DAP Days After Planting
IPM Integrated Pest Management
LSD Least Significant Difference
RCBD Randomized Complete Block Design

## Data availability statement

Data will be shared upon request by the readers.

## CRediT authorship contribution statement

**Rosfiansyah**: Conceptualization, Methodology, Writing – original draft and Formal analysis. **Didin Kuswoyo**: Data Curation. **Sopialena**: Investigation, Supervision and Writing – review and editing.

## Declaration of Competing Interest

The authors of this manuscript declare no conflict of interest or competing interest.

## Declaration of Use of AI in the Writing Process

The authors used Microsoft Copilot and Grammarly to improve grammar, paraphrase text, and enhance language clarity, and Mendeley to manage references and citations during the preparation of this manuscript. After using these tools, the authors carefully reviewed and edited the content and take full responsibility for the content of the publication.

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