*Original Research Article*

Effectiveness of *Beauveria bassiana* Isolates in Controlling *Dysmicoccus brevipes* on Pineapple (*Ananas comosus*)

.

ABSTRACT

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| --- |
| **Background:** One of the major pests attacking pineapple plants is the mealybug (*Dysmicoccus brevipes*), an insect belonging to the order Hemiptera and the family Pseudococcidae. This pest infests the roots, basal stems, and lower leaves of the Pineapple plant. One promising alternative is the use of biological control agents, particularly the entomopathogenic fungus *B.* *bassiana***.**  **Aims:** This study aimed to evaluate the effectiveness of several isolates of the entomopathogenic fungus *Beauveria bassiana* in controlling *Dysmicoccus brevipes* (*D. brevipes)* on pineapple plants.    **Methodology**: The experiment was arranged in a Completely Randomised Design (CRD) with four treatments and six replications, consisting of isolates BbSP 105, BbSP 217, BbKP 125, and a control (without fungal application). Data were analysed using analysis of variance (ANOVA), followed by an LSD test at a 5% significance level. This research was conducted in the Plant Pest and Disease Laboratory and the Microbiology Laboratory, Department of Agrotechnology, Faculty of Agriculture, Universitas Islam Riau, from January to April 2025.The experiment consisted of four treatments with five replications, using three isolates of the entomopathogenic fungus *Beauveria bassiana* and one untreated control, resulting in a total of 24 observation units. The experimental procedures included fungal isolate preparation, conidial suspension formulation, preparation of test insects, and application of *B. bassiana* on *Dysdercus brivioides*. The observed parameters included nymphal mortality of *D. brivioides*, daily mortality rate, number of emerged adults, and symptoms of *B. bassiana* infection on *D. brivioides*.  **Results:** The results showed that all *B. bassiana* isolates significantly suppressed the development of *D. brevipes*. The highest nymph mortality was observed in the BbSP 217 treatment, with a mortality rate of 62%, which was significantly different from other treatments. Mortality began to be observed on the fourth day after application. Furthermore, the number of emerging adult insects was also significantly reduced. Only 38% of adults emerged in the BbSP 217 treatment, much lower compared to 96.66% in the control. Therefore, all *B. bassiana* isolates demonstrated potential as effective biological control agents, with BbSP 217 being the most effective isolate in suppressing *D. brevipes* populations on pineapple.  **Conclusion:** The study demonstrated that all tested *Beauveria bassiana* isolates were effective in suppressing *Dysdercus brevipes* populations on pineapple plants. Among the isolates, BbSP 217 showed the highest efficacy, resulting in the greatest nymph mortality and the lowest adult emergence compared to the control. These findings highlight the potential of *B. bassiana*, particularly the BbSP 217 isolate, as a promising biological control agent for managing *D. brevipes* infestations in pineapple cultivation. The study demonstrated that all tested Beauveria bassiana isolates were effective in suppressing *Dysdercus brevipes* populations on pineapple plants. These findings highlight the potential of *B. bassiana*, particularly the BbSP 217 isolate, as a promising biological control agent for managing D. brevipes infestations in pineapple cultivation. |

*Keywords: Beauveria bassiana, Dysmicoccus sp, mortality, biological control, Pineapple*

**1. INTRODUCTION**

Pineapple (*Ananas comosus*) is a tropical fruit plant belonging to the family Bromeliaceae. It originates from regions of southern Brazil and Paraguay. Pineapple fruit is the only source for naturally available bromelain, which is used for healing cancer, wounds and inflammation as well as in enhancing the immune system. In addition to this, pineapple leaves are used in textile industries for fibre production (Manjushree & Chellappan, 2019). It is one of the leading horticultural commodities in Indonesia, with high economic value for both domestic consumption and export markets. Processed pineapple products such as juice, jam, and canned fruit continue to experience increasing demand. Among the pineapple-producing regions in Indonesia, Riau Province is recognised as one of the largest producers, particularly in the areas of Kampar, Siak, and Indragiri Hulu (Az-Zahra & Depriwana Rahmi, 2024). The agribusiness potential for pineapple cultivation in Riau is highly promising; however, productivity remains constrained by serious pest infestations (Rodliyatun et al., 2019)(Hia et al., 2020)

One of the major pests attacking pineapple plants is the mealybug (*Dysmicoccus brevipes*), an insect belonging to the order Hemiptera and the family Pseudococcidae. This pest infests the roots, basal stems, and lower leaves of the plant. *Dysmicoccus brevipes* (Cockrell), pink pineapple mealybug, is a polyphagous pest that commonly clusters belowground and above portions of the plant (Divya et al.,2024). *D. brevipes* feeds by piercing plant tissues and sucking sap using its stylet-like mouthparts, leading to physiological stress in the plant, such as stunted growth, leaf chlorosis, and, under severe infestations, plant death. In addition, *D. brevipes* produces honeydew, which promotes the growth of sooty mould fungi that interfere with photosynthesis and reduce crop quality (Gustina et al., 2016).

Current field management of mealybugs largely depends on the application of synthetic chemical insecticides. Although these chemicals offer rapid results, long-term use poses risks, including the development of pest resistance, disruption of natural enemies, and contamination of the environment and agricultural products. Therefore, there is a need for more environmentally friendly and sustainable pest control approaches. There is also increasing concern in general about the toxic risks of excessive pesticide use in agriculture. Therefore, exploration of economically viable and environmentally safe strategies is necessary. We hypothesise that commercial pineapple production could benefit from the use of botanical extracts and biological pest control agents, such as entomopathogenic fungi, within integrated pest management (Panti et al.,2024).

One promising alternative is the use of biological control agents, particularly the entomopathogenic fungus *B. bassiana* (Tanada & kaya H, 1993)(Omomowo et al., 2023). This fungus infects a wide range of insect species through conidia that adhere to the insect cuticle, penetrate the exoskeleton, and proliferate within the host body. The infection leads to insect death by damaging internal tissues and producing toxic secondary metabolites. *B. bassiana* is specific to target insects, safe for humans and the environment, and can be mass-produced for agricultural use. *B. bassiana* fungi offer several advantages, such as a broad host range, simple production methods, high environmental persistence, and strong virulence. Research has demonstrated the effectiveness of these fungi in controlling brown planthopper populations(Hendra et al., 2023). Several species of entomopathogenic fungi that have been proven successful in managing *D. brevipes* in previous studies include *B. bassiana* (Hendra et al., 2022)(Chou et al., 2022), *Metarhizium huainamdangense*(Wongkar et al., 2022), dan *Metarhizium anisopliae*(Trizelia et al., 2023). *B. bassiana* has been proven effective in controlling the papaya mealybug *Paracoccus marginatus*, resulting in a mortality rate of 82.86%, with an average nymphal death time of 3.55 days (Herlinda et al., 2012).*B. bassiana* has proven to be an effective biological control agent against *Planococcus* spp., significantly reducing pest populations and associated plant damage. Its mode of action—through enzymatic degradation and toxin production—targets various insect stages, making it a sustainable and environmentally friendly alternative to synthetic chemical pesticides(Taupiq et al., 2024). However, the specific effect of *B. bassiana* on *Dysmicoccus brevipes* has not yet been extensively studied

Based on this background, the present study aims to evaluate the effectiveness of *B. bassiana* in controlling *D. brevipes* on pineapple plants in Riau Province. The findings of this research are expected to provide a scientific basis for the development of effective biological control strategies and support sustainable pineapple farming in the region

2. Materials and Methods

The experiment was arranged in a Completely Randomised Design (CRD) with four treatments and six replications, consisting of isolates BbSP 105, BbSP 217, BbKP 125, and a control (without fungal application). Data were analysed using analysis of variance (ANOVA), followed by an LSD test at a 5% significance level. This research was conducted in the Plant Pest and Disease Laboratory and the Microbiology Laboratory, Department of Agrotechnology, Faculty of Agriculture, Universitas Islam Riau, from January to April 2025.

**2.1 Preparation of *Beauveria bassiana* Isolates**

The *B. bassiana* isolates used in this study were obtained from the Laboratory of Pests, Diseases, and Microbiology, Faculty of Agriculture, Universitas Islam Riau. The isolates were originally collected from the rhizosphere of pineapple plants in two major pineapple-producing regions in Riau Province: Siak and Kampar Regencies. Three isolates were used in this experiment, namely BbSP 217, BbSP 105, and BbKP 125. Details of the isolate codes, plant origin, and collection sites are presented in Table 1.

**Table 1. Isolate code, plant origin, and geographical origin of *B. bassiana* isolates**

|  |  |  |
| --- | --- | --- |
| Isolate Code | Plant Origin Isolat | Region of origin |
| BbSP 217 | Pineapple rhizosphere | Siak, Riau Province, Indonesia |
| BbSP 105 | Pineapple rhizosphere | Siak, Riau Province, Indonesia |
| BbKP 125 | Pineapple rhizosphere | Kampar, Riau Province, Indonesia |

**2.2 Preparation of conidia suspension**

The conidial suspension of *B. bassiana* was prepared by adding 10 ml of sterile distilled water mixed with 0.05% Tween 80 (as a surfactant) to a petri dish containing fungal culture. The surface of the culture medium was gently scraped with a sterile fine brush to release the conidia. The resulting concentrated suspension was diluted, and conidial density was determined using a haemocytometer. The suspension was then adjusted to a final concentration of 10⁸ conidia/ml to ensure uniformity across treatments.

**2.3 Preparation of Test Insects**

Nymphs of the pineapple mealybug (*Dysmicoccus brevipes*) were collected from a farmer’s pineapple plantation in Tambang Subdistrict, Kampar Regency, Riau Province. The insects were mass-reared under laboratory conditions until the third generation. Second-instar nymphs from the third generation were selected for bioassay because they are sufficiently active and highly susceptible to fungal infection.

**2.4 Application of *B. bassiana* to *D. brevipes* Nymphs**

The fungal suspension (2 ml) was sprayed onto sterilised pineapple leaf discs measuring 4 × 4 cm. The treated leaf discs were then placed in test containers (petri dishes or sterile plastic containers). After drying, 25 second-instar *D. brevipes* nymphs were introduced onto each treated leaf disc. Each treatment was replicated six times. The leaf discs were replaced on day 4 after application to maintain optimal food quality and prevent cross-contamination..

**2.5 Observation Parameters**

Three key parameters were observed in this study: total nymphal mortality, daily nymphal mortality, and the number of nymphs that successfully developed into adults (imagos). Total nymphal mortality was calculated based on the number of dead individuals throughout the observation period and expressed as a percentage of the total number of nymphs tested per treatment. Daily mortality was recorded each day to monitor the progression of infection and to determine the onset of mortality caused by *B. bassiana*. In addition, the number of nymphs that survived and successfully moulted into adults was recorded as an important indicator of the fungal isolates' effectiveness in suppressing *D. brevipes* populations.

3. results and discussion

**3.1 Nymphal Mortality of *Dysmicoccus brevipes***

The results of this study indicate that the application of different *B. Bassiana* isolates had a significant effect on the mortality rate of *Dysmicoccus brevipes* nymphs on pineapple plants. The average percentage of nymphal mortality caused by each treatment is presented in Table 2. Analysis of variance (ANOVA) revealed significant differences among treatments, which were further analysed using the LSD test at the 5% significance level.

**Table 2. Nymphal Mortality of *Dysmicoccus brevipes***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatments | Nymph Mortality (%) ± Sd | | | |
| BbSP 217 | 62 | ± | 2.19 | a |
| BbSP 105 | 54 | ± | 3.34 | b |
| BbKP 125 | 40 | ± | 5.65 | c |
| Kontrol | 3.33 | ± | 3.01 | d |

\*The numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level.

All tested *B. bassiana* isolates significantly increased the mortality of *D. brevipes* nymphs compared to the control. Among them, isolate BbSP 217 exhibited the highest efficacy, resulting in a mortality rate of 62%, which was statistically different from the other isolates. This suggests that BbSP 217 is the most promising candidate for biological control among the isolates evaluated.

The infection mechanism of *B. bassiana* begins with the adhesion of conidia to the insect cuticle, followed by enzymatic penetration through the action of proteolytic and lipolytic enzymes. Once inside the host, the fungus proliferates in the hemocoel, ultimately leading to death through the production of secondary metabolites such as beauvericin and bassianolide (Zhang et al., 2024). The variation in mortality rates among isolates may be attributed to differences in genetic traits, sporulation capacity, conidial viability, and environmental adaptability.

Although BbSP 105 and BbKP 125 also caused significant mortality, their effectiveness was lower than that of BbSP 217. The control treatment showed very low mortality (3.33%), which may have resulted from natural factors or environmental stress, but it was not sufficient to suppress the pest population. The high efficacy of BbSP 217 offers a valuable opportunity for the development of environmentally friendly bioinsecticide formulations specifically targeting *D. brevipes* in pineapple cultivation, especially in Riau Province. However, further research is needed to evaluate the field stability of this isolate, optimise application techniques, and assess its compatibility with integrated pest management (IPM) components (Klingen et al., 2002)

**3.2 Daily Nymphal Mortality of *Dysmicoccus brevipes***

The daily mortality of *D. brevipes* nymphs following application of different *B. Bassiana* isolates were monitored for eight days. Figure 1 illustrates the daily mortality dynamics for each treatment: BbSP 217, BbSP 105, BbKP 125, and the untreated control. The graph reveals distinct mortality patterns among treatments over the observation period.

**Figure 1. Daily Nymphal Mortality of *Dysmicoccus brevipes***

As shown in Figure 1, noticeable mortality began to occur on day 4 after fungal application, with a sharp increase on days 5 and 6 across all treatments involving *B. bassiana* isolates. In contrast, the control treatment exhibited minimal and stagnant mortality throughout the observation period, suggesting that deaths in the control group were due to natural causes rather than fungal infection.

Isolate BbSP 105 showed the fastest and highest mortality rate among all treatments. A marked increase was observed starting on day 4, peaking on day 6, with over 15 individuals dead. This indicates that BbSP 105 possesses high infectivity and a relatively short latency period. Although BbSP 217 also demonstrated strong efficacy, the increase in mortality was slightly slower, suggesting differences in infection speed and replication dynamics between isolates.

Isolate BbKP 125 exhibited lower overall mortality compared to the other two isolates, although it was still significantly more effective than the control. Mortality in this treatment increased gradually and plateaued after day 6, indicating that its efficacy might be influenced by factors such as conidial viability, adaptation to the host environment, or penetration ability (Mantzoukas & Eliopoulos, 2020)(Ramakuwela et al., 2020).

The control treatment showed virtually no significant increase in mortality, with fewer than two deaths recorded by the end of the observation period. This confirms that natural mortality was minimal and not related to fungal treatment.

**3.3 Percentage of Nymphs Developing into Adults**

The percentage of *Dysmicoccus brevipes* nymphs that successfully developed into adult stages following treatment with different *B. Bassiana* isolates are presented in Table 3. The results demonstrate that all fungal treatments significantly reduced the number of nymphs reaching adulthood compared to the control. Statistical analysis using ANOVA, followed by the LSD test at a 5% significance level, confirmed significant differences among treatments.

Among the isolates, BbSP 217 showed the most substantial impact, allowing only 38% of nymphs to develop into adults. This was significantly lower than BbSP 105 (46%) and BbKP 125 (60%). In contrast, the control treatment, which received no fungal application, had the highest percentage of nymphs successfully emerging as adults (96.66%), indicating normal development in the absence of fungal infection

**Table 3. Effect of *B. bassiana* Isolates on the Adult Emergence of *D. brevipes***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatments | Adult Emergence (%) ± Sd | | | |
| BbSP 217 | 38 | ± | 2.19 | d |
| BbSP 105 | 46 | ± | 3.36 | c |
| BbKP 125 | 60 | ± | 5.65 | b |
| Kontrol | 96.66 | ± | 3.01 | a |

\*The numbers followed by the same letter in the same column are not significantly different in the LSD test at the 5% level.

The observations revealed that treatments with *B. bassiana* isolates significantly reduced the percentage of *D. brevipes* nymphs that successfully developed into adults compared to the control. The highest adult emergence was recorded in the control treatment, reaching 96.66%, indicating that under untreated conditions, nearly all nymphs successfully completed their development. This demonstrates the high survival rate of nymphs in the absence of control measures, suggesting a strong potential for population outbreaks.

In contrast, all treatments with *B. bassiana* isolates resulted in a significant reduction in nymph development into adults. The BbSP 217 isolate yielded the lowest adult emergence rate at only 38%, indicating that the majority of nymphs either died or failed to develop properly due to fungal infection. This suggests that BbSP 217 is the most effective isolate in inhibiting the pest’s development to the adult stage.

Similarly, the BbSP 105 isolate was also effective, with a slightly higher adult emergence rate of 46%, while the BbKP 125 isolate resulted in the highest emergence among the tested isolates at 60%. However, all fungal treatments still showed significantly lower adult emergence compared to the control. These results indicate that all tested *B. bassiana* isolates have strong potential as biological control agents by disrupting the life cycle of *D. brevipes* and preventing its progression to adulthood.

**3.4 Symptoms of *Beauveria bassiana* Infection in *Dysmicoccus brevipes***

Changes in body colour and reduced activity serve as early indicators of infection, which are later followed by fungal colonisation on the surface of the insect's body (Figure 2). Figure 2 illustrates the progressive stages of infection, from a healthy condition to the development of external fungal structures.

|  |  |  |
| --- | --- | --- |
| a | b | c |

**Figure 2. Symptoms of *Beauveria bassiana* infection in *Dysmicoccus brevipes* nymphs: (a) normal nymph, (b) infected nymph, (c) nymph colonised by *B. bassiana***

Based on visual observations, *D. brevipes* individuals that were not infected exhibited normal morphological characteristics: their bodies were white to yellowish in colour, with a clean surface and active movement. These traits indicate a healthy physiological condition with no disturbance from pathogenic agents.

In contrast, early symptoms of infection by *B. bassiana* in treated individuals included a noticeable darkening of the body to a brownish hue, which serves as an early indicator of infection (Figure 2b). This discolouration reflects internal tissue damage resulting from the penetration of fungal hyphae through the insect’s cuticle and the subsequent colonisation of the hemolymph (Thakur et al., 2024). Although external fungal structures such as mycelium or conidia were not yet visible at this stage, the change in body colour and reduction in insect activity provided clear signs that infection had begun(Omomowo et al., 2023).

These morphological changes typically occurred within 2 to 4 days after the application of fungal spores, depending on inoculum concentration, environmental conditions (especially temperature and humidity), and the susceptibility of the host individual. These symptoms generally precede insect mortality, which is later followed by the emergence of external mycelium if environmental conditions are favourable for fungal growth (Figure 2c).

The observed infection symptoms and high mortality rates reinforce the potential of *Beauveria bassiana* as a biocontrol agent with a reliable infection process against *Dysmicoccus brevipes*. Its ability to suppress population growth at multiple life stages makes it a valuable alternative to synthetic insecticides, especially for farmers seeking environmentally friendly pest control solutions (Iida et al., 2023). Furthermore, biological control using entomopathogenic fungi reduces the risk of pesticide resistance development, which has become a major concern in conventional pest management (Sabbahi et al., 2022).

In this study, the variation in isolate performance highlights the importance of strain selection. Different isolates may vary in virulence due to genetic differences, spore germination rates (Geremew et al., 2024), or their ability to adapt to the pest’s microhabitat (Liu et al., 2025). For instance, the superior performance of BbSP 217 suggests a more efficient infection mechanism or better environmental fitness compared to the other isolates. This underscores the necessity of conducting thorough screening before recommending specific isolates for large-scale application (Z. Zhang et al., 2020).

Another important consideration is formulation and application technique. For optimal field efficacy, conidial formulations must maintain viability under fluctuating environmental conditions, including sunlight exposure and desiccation (Vermelho et al., 2024). Oil-based carriers or encapsulation techniques may be investigated in future studies to improve spore persistence and adhesion on plant surfaces or pest bodies (Das et al., 2025).

Additionally, the interaction of *B. bassiana* with native soil microbiota and beneficial arthropods needs to be assessed to avoid unintended ecological disturbances. Compatibility studies with predators, parasitoids, and pollinators should also be conducted to ensure that introducing the fungus does not disrupt the agroecosystem balance (Chertkova et al., 2023).

From a practical standpoint, integrating *B. bassiana* into pineapple pest management programs may reduce dependency on chemical control while promoting sustainable farming. Future research should focus on field validation, dose optimisation, and determining appropriate application timing. Moreover, economic feasibility studies are essential to assess the cost-effectiveness of fungal-based biopesticides in smallholder farming systems (Daud et al., 2024). Overall, this study lays the groundwork for the development of *B. bassiana* as a promising biocontrol agent and contributes to the advancement of eco-friendly pest management approaches in pineapple cultivation.

4. Conclusion

The study demonstrated that all tested *B. bassiana* isolates were effective in suppressing *D. brevipes* populations on pineapple plants. Among the isolates, BbSP 217 showed the highest efficacy, resulting in the greatest nymph mortality and the lowest adult emergence compared to the control. These findings highlight the potential of *B. bassiana*, particularly the BbSP 217 isolate, as a promising biological control agent for managing *D. brevipes* infestations in pineapple cultivation.

Consent (where ever applicable)

"All authors declare that ‘written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal."

Ethical approval (where ever applicable)

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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