



## Antagonistic Ability Of Entomopathogenic Fungi *Metarhizium anisopliae* AND *Beauveria bassiana* Against Mortality of Wood Grasshopper (*Valanga nigricornis*)

Chairunnisah J. Lamangantjo, Wirnangsi Din Uno, Intan Zulfatadillah Mursali, Maryam Pakaya

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo.  
Jl. Prof. Dr. Ing. BJ. Habibie, Kabupaten Bone Bolango 96554, Gorontalo, Indonesia

\*Corresponding Author : [chairunnisah@ung.ac.id](mailto:chairunnisah@ung.ac.id)

**ABSTRACT.** The entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* are potential biological control agents for suppressing the population of the wood grasshopper (*Valanga nigricornis*). This study aims to evaluate the antagonistic ability of the fungi *M. anisopliae* and *B. bassiana* against the wood grasshopper (*Valanga nigricornis*). This study is a descriptive study with a quantitative approach, applying inoculum suspension at four concentration levels (P1, P2, P3, and P4) and observing insect mortality and colony growth in culture media. The results showed that the P4 suspension treatment produced the highest mortality of 92% with a probit value of 1.28, while mortality in other treatments ranged from 0 to 75%. The highest infection viability showed that in the P4 treatment, the CFU value was  $3.0 \times 10^6$  for *M. anisopliae* and  $1.4 \times 10^6$  for *B. bassiana*. These findings indicate that high inoculum concentrations increase the antagonistic effectiveness and infection ability of entomopathogenic fungi.

**Keywords :** *Antagonistic, Beauveria bassiana, Entomopathogenic, Metarhizium anisopliae*

### INTRODUCTION

Agriculture is a vital sector that is needed by the community to meet food needs (Devlet, 2021). Generally, the needs in the agricultural sector require sufficient water availability, favorable climatic conditions, good biological agents, and minimal disturbance from plant pests (Fanani et al., 2025). In the sustainable agriculture sector, there are many challenges in crop cultivation, one of which is pest attacks that inhibit growth (Bouri et al., 2023). Pest attacks on cultivated plants usually damage important parts of the plant such as leaves, stems, flowers, and fruit (Mani, 2022). Damage to agricultural land is commonly caused by several types of pests such as armyworms, caterpillars, aphids and thrips, as well as other insects that damage plant tissue and become vectors of disease (Paschapur et al., 2025). Among the pests that attack cultivated plants, there is a group of insect pests that has been overlooked and has the potential to cause considerable damage, one of which is the wood grasshopper (Teweloipaky et al., 2024).

Wood grasshopper are insects belonging to the order Orthoptera with high feeding rates, so when there is a population explosion, wood grasshopper can attack plants on a massive scale (Prastyaningsih & Juliarti, 2023). Although some members of Orthoptera are known pests, this type of insect plays a role as decomposers in the ecosystem. However, in the agricultural sector, wood grasshopper have a negative impact if their numbers surge and there is no adequate control (Chatterjee, 2022). At certain times, wood grasshopper can attack agricultural land massively in large numbers, causing severe defoliation of plants, disrupting photosynthesis, and drastically reducing productivity (Mulvihill, 2021). Meanwhile, with increasing awareness of the impact of synthetic pesticide use on the environment and human health, an alternative control solution is the application of biological agents using the entomopathogenic microbes *M. anisopliae* and *B. bassiana* (Gebremariam et al., 2022).

*M. anisopliae* and *B. bassiana* fungi are microbes that are pathogenic to insects that are pests of important crops (Fronza et al., 2017). These fungi have been reported to have the ability to infect various stages of insect metamorphosis, from eggs, larvae, pupae, to imagoes (Grijalba et al., 2018). A study conducted by Pertiwi & Haryadi (2022) showed that *M. anisopliae* and *B. bassiana* are antagonistic to various types of arthropods, including plant-damaging insects, but do not pose a threat to human health, making them safe for farmers to use as an alternative to synthetic pesticides. The fungi *M. anisopliae* and *B. bassiana* can produce an enzymatic compound called cyclic peptide, or destruxin, which is composed of five amino acids: proline, isoleucine, methyl-valine, methyl-alanine, and beta-alanine. Destruxin has an effect that causes abnormalities in the midgut, hemocytes, Malpighian tubules, and muscle tissue of the host (Yin et al., 2021). The pathogenesis of *M. anisopliae* and *B. bassiana* fungi begins with the entry and growth of fungi through the insect's hemocoel, attacking important tissues such as the digestive tract and respiratory system, leading to the death of the target insect (de Paula et al., 2021). The use of *M. anisopliae* and *B. bassiana* fungi as entomopathogenic fungi to control pests has several advantages, namely high reproductive capacity, short life cycle, long-lasting spore formation both in nature and under unfavorable conditions, relative safety, selectivity, easy to produce, and a very low likelihood of resistance (Susandi et al., 2023). Based on the problems described above, considering the impact of wood grasshopper attacks on plants and the potential of *M. anisopliae* and *B. bassiana* fungi as alternatives to synthetic pesticides, it is important to conduct a study on the antagonistic properties of *M. anisopliae* and *B. bassiana* fungi as controllers of the wood grasshopper (*Valanga nigricornis*) population.

## MATERIALS AND METHODS

**Research Site.** This research was conducted in November 2023. The research location was in the microbiology laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Gorontalo.

**Material.** The equipment used in the study included Petri dishes, inoculation needles, Erlenmeyer flasks, incubators, ovens, hot plates, autoclaves, vortex mixers, 40 glass bottles, spray bottles, measuring cups, analytical scales, aluminum foil, vortex mixers, tweezers, stirring rods, gauze, label paper, and cameras. The materials used were: *M. anisopliae* and *B. bassiana* fungal isolates, wood grasshopper, distilled water, PDB (Potato Dextrose Broth) medium, 70% alcohol, tissue, and cotton.

**Method.** Pure isolates of *M. anisopliae* and *B. bassiana* fungi were grown in PDB liquid culture medium at 30°C for 2x24 hours. After incubation for 2x24 hours, each culture isolate that had grown in the medium was made into several concentrations of liquid suspension by adding 100 mL of sterile distilled water. The percentage concentrations of the suspensions obtained for the entomopathogenic antagonistic test included 0% (Control); 5% (P1); 10% (P2); 15% (P3); and 20% (P4). Each suspension was placed in a different spray bottle. Next, place 3 wood grasshoppers in each glass container for each concentration treatment and repeat 4 times. The fungal suspension obtained was sprayed on the bodies of the locusts, which is a source of nutrition for wood grasshoppers (*Valanga nigricornis*).

**Data Analyst.** The percentage of wood grasshopper (*Valanga nigricornis*) mortality for each treatment was calculated and observed during different observation periods, namely 1–5 days after application of the entomopathogenic fungal suspension. The statistical data used in this study were analyzed using probit values with a significance level of 5% to determine the  $LT^{50}$  value. The percentage mortality rate of wood grasshoppers (*Valanga nigricornis*) was calculated using the formula developed by Pertiwi & Haryadi (2022) as follows:

$$\text{Mortality (\%)} = \frac{\text{Number of Death}}{\text{Total Amount}} \times 100$$

## RESULTS AND DISCUSSION

### Antagonistic Ability of Entomopathogenic Fungi *M. anisopliae* and *B. bassiana* against the Wood Grasshoppers (*Valanga nigricornis*).

The antagonistic ability of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* is characterized by the mortality rate and probit value. The results of the study show that the highest mortality rate from the antagonistic test of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* was in the P4 suspension treatment with a mortality percentage of 92% and a probit value of 1.28, while the mortality percentage in the P1, P2, and P3 suspension control treatments was in the range of 0–75% (Table 1, Figure 1).

Table 1. Antagonistic Ability of Entomopathogenic Fungi *M. anisopliae* dan *B. bassiana*

Treatment	Number of Deaths		Mortality (%)		Probit Value	
	<i>M. anisopliae</i>	<i>B. bassiana</i>	<i>M. anisopliae</i>	<i>B. bassiana</i>	<i>M. anisopliae</i>	<i>B. bassiana</i>
P0	0	0	0	0	1,280983515	1,2809835
P1	6	7	50	58	2,424170696	2,4779305
P2	8	9	67	75	2,525260351	2,5676209
P3	9	6	75	50	2,567620941	2,4241707
P4	11	11	92	92	2,641183795	2,6411838



Figure. 1 Morphology of wood grasshopper infected with fungus

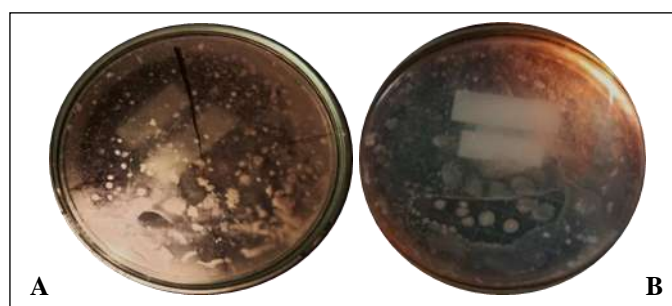
Gebremariam et al. (2022) reported that a single application of *B. bassiana* against *Bemisia tabaci* resulted in mortality of up to 90% at a concentration of  $1 \times 10^8$  conidia/mL, while *M. anisopliae* on the same insect species only reached 88%. The antagonistic power of these entomopathogenic fungi is influenced by spore physiology, including viability, virulence, and adhesion ability. This is supported by Ramdani et al. (2022), who stated that *M. anisopliae* and *B. bassiana* secrete protease and lipase enzymes that play an important role in penetrating insect cuticles, so that the colonization rate will be higher in suspensions with greater spore density. Similar findings were reported by Bagariang et al. (2023), who showed that entomopathogenic fungi have a spore growth mechanism on the cuticle, and then the hyphae secrete chitinase, lipase, and protease enzymes to break down the insect cuticle. After spore penetration, the hyphae develop into the blood vessels and produce toxins such as beauvericin, beauverolite, isoralite, and oxalic acid, which can increase pH and blood clotting and stop blood circulation.

### Viability of Entomopathogenic Fungal Infections *M. anisopliae* and *B. bassiana* in Wood Grasshopper (*Valanga nigricornis*).

The viability of entomopathogenic fungi *M. anisopliae* and *B. bassiana* was demonstrated by the number of colonies growing at various dilution levels and the CFU/gram value of each sample. The results showed that the highest viability of *M. anisopliae* and *B. bassiana* fungal isolates was found in the P4 suspension treatment with CFU values of  $3.0 \times 10^6$  and  $1.4 \times 10^6$ , respectively, thus having the potential to produce higher infectivity against the wood grasshopper (*Valanga nigricornis*) (Table 2).

Table 2. Viability of entomopathogenic fungal infection *M. anisopliae* dan *B. bassiana*

Sample	Dilution	<i>M. anisopliae</i>		<i>B. bassiana</i>	
		Total Coloni	CFU/gr	Total Coloni	CFU/gr
P1	10 <sup>-1</sup>	281	5,5×10 <sup>5</sup>	224	6,0×10 <sup>5</sup>
	10 <sup>-2</sup>	205		198	
	10 <sup>-3</sup>	144		157	
P2	10 <sup>-1</sup>	356	2,6×10 <sup>6</sup>	247	5,8×10 <sup>5</sup>
	10 <sup>-2</sup>	301		203	
	10 <sup>-3</sup>	255		151	
P3	10 <sup>-1</sup>	398	3,0×10 <sup>6</sup>	264	1,4×10 <sup>5</sup>
	10 <sup>-2</sup>	361		252	
	10 <sup>-3</sup>	300		319	
P4	10 <sup>-1</sup>	491	3,0×10 <sup>6</sup>	303	1,4×10 <sup>6</sup>
	10 <sup>-2</sup>	432		289	
	10 <sup>-3</sup>	295		247	

Figure. 2 Isolate Morphology; (A) *M. anisopliae*, (B) *B. bassiana*

The high mortality rate in the P4 suspension of *M. anisopliae* and *B. bassiana* was supported by the highest CFU values, which were  $3.0 \times 10^6$  and  $1.4 \times 10^6$ , respectively. This shows that the higher the concentration of viable spores available, the greater the likelihood of the fungus effectively infecting the host insect. Tobing et al. (2015) reported that the highest insect mortality in the *M. anisopliae* and *M. anisopliae* treatments was at a concentration of  $10^8$ /ml with a mortality rate of 100%. These findings are reinforced by a report by Yasin et al. (2024), which shows that treatment with a solid conidia formulation of *M. anisopliae* with  $CFU > 10^6$  resulted in mortality of more than 90% in various Orthoptera species within 10 days after application. According to Dakhel et al. (2019), the success of *M. anisopliae* infection is largely determined by the density of conidia applied, as high inoculum concentrations accelerate spore penetration through the host cuticle and increase the chances of hemolymph colonization. Mascarin & Jaronski (2016) stated that the success of *B. bassiana* as a biological agent depends on the ability of propagules to maintain viability at the time of application and during contact with insect hosts. In the context of biological control, these results prove that the use of high suspension concentrations significantly increases the effectiveness of biocontrol against wood grasshopper pests. Thus, the P4 suspension treatment, which showed the highest conidia viability, can be recommended as an effective dose for field application in the management of wood grasshopper (*Valanga nigricornis*) populations.

## CONCLUSION

The results showed that both fungal isolates had high biological control potential, with effectiveness levels influenced by the concentration of the inoculum suspension. The P4 suspension treatment was found to produce the highest mortality rate of 92% with a probit value of 1.28, which was consistent with the infection viability results at the highest CFU levels of  $3.0 \times 10^6$  for *Metarhizium anisopliae* and  $1.4 \times 10^6$  for *Beauveria bassiana*, respectively.



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