Biological control of white grubs (*Lepidiota stigma* L; Coleoptera; Scarabaeidae) with entomopathogenic nematodes and fungus *Metharizium anisopliae* (Metsch)

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**Abstract.** Biological Control Agents (BCA) Entomopathogenic nematodes (*EPn*) *Heterorhabditis* spp. and fungi *Metharizium anisopliae* have been known to control the major pests of sugarcane and cassava, namely the white grub larvae *Lepidiota stigma* L. This study was conducted to test the effectiveness of biological control agents for controlling three species of pests: *L. stigma*, *Galleria melonella*, and *Tenebrio molitor*. We tracked larval mortality for 24, 48, and 72 hours after the initial inoculation. Similarly, organic fertilizer mixed with the fungus *M. anisopliae* (in a 25-L pot) was tested on the larvae. The results showed that entomopathogenic nematodes were effective in killing 100% of *L. stigma* larvae pests, whereas *M. anisopliae* was only 10% effective. When organic fertilizer was mixed with both biological control agents types (nematode and fungi), mortality of the three larvae species reached 100% at a nematode concentration of 10 IJ-1000 mlL. In contrast, *M. anisopliae* only caused 70% mortality at a concentration of 107 spores-L after 72 hours of inoculation. It was difficult to collect white grub larvae of *L. stigma* from the field. From May to June, the larvae were present in the ground at a depth of more than one meter in the soil and therefore it was hard to dig them out.

1. **Introduction**

The demand for cassava in Indonesia has increased every year in response to the demand for its industrially-produced food products, such as tapioca, modified cassava flour, artificial rice, and vegetable oil [1-3]. The main obstacle to the cultivation of sugarcane and cassava is soil fertility i.e., both crops are often cultivated on marginal land where the nutrient very low and so growth is suboptimal [4]. In both cases, most farmers attempt to improve soil fertility using animal manure, which tends to decompose in adequately.

White grubs larvae (Scarabaeidae: Coleoptera), such as *Lepidiota stigma*, *Anomala viridis*, and *Hollotrichia halleri*, are crop pests that commonly attack the plants [5, 6]. These three pest species are so prolific that cassava plants require control measures to prevent crop failure [7]. Presently, the fungus *Metharizium anisopliae* is used as a biological control agent (BCA). This fungus is applied to the soil at the beginning of the rainy season where sugarcane is cultivated [8, 6]. In the present study, we examine the relative effectiveness of alternative, biological control methods that use either entomopathogenic nematodes (*EPn*) in the genus *Heterorhabditis* spp. or the fungus *M. anisopliae*, both of which we mix with organic fertilizer before application to our experimental plants.
2. Material and Methods

EPn used as BCAs were bred en masse and in vitro using the Bedding method described by Chaerani [9], whereas the fungus pathogen of *M. anisopliae* was used as a replacement feed. The BCAs were also formulated with organic fertilizer so that the resulting treatment could be applied to cassava as a combined biopesticide and biofertilizer. The first experiment was a pathogenicity test using EPn (*Heterorhabditis* spp.) and pathogenic fungus on the larvae of *L. stigma*, *Galleria melonella*, and *Tenebrio molitor* (n= 20 insects). The EPn larvae were placed in a petridish under which filter paper was applied. Then, each larva was dropped in a BCA solution with micropipette according to the tested treatment.

We used a completely randomized design (CRD) approach with six treatments (and three replicates) of varying BCA concentration. EPn treatment concentrations were $10^3$, $10^4$, $10^5$, $10^6$, and 100 Infective Juveniles (IJ)/100 ml; the fungus pathogen treatment concentrations were 107, 106, $10^5$, $10^4$, and $10^3$ spores/L. For all treatments, we recorded larval mortality after 24 h, 48 h, and 72 h of inoculation. Our results were analyzed using variant analysis. Then, we applied the Duncan test at 5% level of significance. If a control died, we corrected for that using the formula of Abbot [10].

The second experiment was conducted in a 25-L pot filled with organic fertilizer, Pen, or fungi of *M. anisopliae* and then inoculated with 20 *L. stigma* third instar larvae. Data were collected on *L. stigma* mortality after 4 and 20 d of inoculation. Larvae mortality observations on cassava plants were recorded for five BCA treatments: (A) $10^6$ IJ/L applied via spray, (B) $10^7$ IJ/L applied via watering, (C) $10^8$I/g applied with organic fertilizer, (D) *M. anisopliae* fungus added at $10^9$ spores/L applied via spray, and (E) *M. anisopliae* fungus applied at $10^3$ spores/200 g applied with organic fertilizer. Observations were recorded to determine the intensity of *L. stigma* damage on cassava within extensive $8 	imes 10^2$ m plots of each treatment. All plant samples were acquired diagonally across each plot, from at least 10 samples/plot. For each sample, we recorded symptoms of plant infection, including leaf wilt, leaf yellowing, drying, and mortality.

3. Results and Discussion

The Entomopathogenic nematodes *Heterorhabditis* spp. showed that 100% mortality occurred after 48 h when a concentration of $10^7$ IJ/100 ml was applied. In contrast, when using *M. anisopliae* fungi as a BCA, mortality was very low (10%) after 48 h. This was because it took a long time (at least 7 d) for the larvae to die after symptoms occurred (Table 1). Overall, the higher the concentration of Biological control agents (entomopathogenic nematodes or fungus) applied, higher was the larval mortality. Statistically, the $10^6$ IJ/200 ml and $10^7$IJ/100 ml treatments differed significantly from the other treatments. This could have been because our application regime of Entomopathogenic nematodes followed technical advice that recommended a dosage of $10^7$I/10 ml over a 500 m² area.

| Concentration | % Mortality of | % Mortality of |
|---------------|---------------|---------------|
| IJ/100 ml     | larval pests | of larval pests |
|               | 1 to EPn | to *M. anisopliae* |
|               | Gm | Tm | Ls | Gm | Tm | Ls |
| $10^3$        | 10 | 10 | 0 | 0 | 0 | 0 |
| $10^4$        | 30 | 40 | 60 | 0 | 0 | 0 |
| $10^5$        | 70 | 70 | 80 | 0 | 0 | 0 |
| $10^6$        | 90 | 100 | 100 | 10 | 10 | 10 |
| $10^7$        | 100 | 100 | 100 | 10 | 10 | 10 |
| $10^8$ (control) | 0 | 0 | 0 | 0 | 0 | 0 |

1Larval pests: Gm (*Galleria melonella*), Tm (*Tenebrio molitor*), and Ls (*Lepidiota stigma*)
For the EPn (Heterorhabditis spp.), the lethal concentration for 50% mortality (LC\textsubscript{50}) for T. molitor occurred at 8.7 \times 10^4 IJ/100 ml (Y=0.325x+3.393), while the G. melonella Lethal Time for 50% mortality (LT\textsubscript{50}) was 35 h.

Our pot experiments showed that the pathogenicity of EPn (applied at 10^6 IJ/L and watered with organic fertilizer) showed the highest result that could cause >80% mortality (Table 2).

**Table 2.** Effectiveness of entomopathogenic nematodes in organic fertilizer on mortality of the larval pests Galleria melonella (Gm), Tenebrio molitor (Tm), and Lepidiota stigma (Ls) after 72 h of inoculation

| Concentration | Larval mortality1 (%) by species2 |
|---------------|----------------------------------|
|               | Gm      | Tm      | Ls        |
| 10^3          | 70 b    | 80 ab   | 71 bc     |
| 10^4          | 75 b    | 75 b    | 70 bc     |
| 10^5          | 90 a    | 80 ab   | 86 b      |
| 10^6          | 80 ab   | 90 a    | 100 a     |
| 10^7          | 100 a   | 100 a   | 100 a     |
| 10^0 (control)| 0       | 0       | 0         |

1 % Mortality with the same letters (in the same column) did not significantly differ from one another, according to the DMRT test at 5% level of significance.

Our application of biological control agents as on cassava showed that the intensity of L. stigma damage to plants (30–90 d after planting) was lower when IJ were applied to plants by watering than when they were applied by spraying (Table 4). This probably occurred because biological control agents more effectively controls larval pests of L. stigma in the field. In addition, the application of biological control agents as via watering probably is more viable and higher in biological control agents [10]. The application of entomopathogenic nematodes with soil organic fertilizer probably permitted Entomopathogenic nematodes to persist and remain infective in the soil [11]. We expect that M. anisopliae fungus can be used effectively in rainy seasons because wet, humid conditions can allow fungus to proliferate [6, 8]. However, our insect mortality experiment was conducted in a laboratory by wetting our experimental media.

**Table 3.** Effectiveness of Metarhizium anisopliae (Ma) fungus (mixed into organic fertilizer) on mortality of the larval pests Galleria melonella (Gm), Tenebrio molitor (Tm), and Lepidiota stigma (Ls) after 72 h of inoculation

| Concentration of Ma (spores/L) | Larval mortality1 (%) by species |
|-------------------------------|---------------------------------|
|                               | Gm    | Tm    | Ls    |
| 10^3                          | 40 cb | 50 ab | 60 ab |
| 10^4                          | 50 b  | 55 b  | 65 ab |
| 10^5                          | 60 ab | 60 ab | 70 a  |
| 10^6                          | 70 a  | 60 ab | 75 a  |
| 10^7                          | 70 a  | 70 a  | 70 a  |
| 10^0 (control)                | 0     | 0     | 0     |
Table 4. Damage intensity of *Lepidiota stigma* on cassava plants after 30–90 d

| Treatment | Intensity of plant damage (%) by age of plant after planting | Mean (%)<sup>2</sup> |
|-----------|-------------------------------------------------------------|---------------------|
|           | 30  | 45  | 60  | 75  | 85  | 90  |
| A         | 20  | 20  | 10  | 10  | 5   | 0   | 10.8 d |
| B         | 10  | 10  | 10  | 10  | 5   | 0   | 7.5 e  |
| C         | 20  | 30  | 20  | 20  | 5   | 0   | 15.8 b |
| D         | 30  | 30  | 20  | 20  | 10  | 10  | 20.0 a |
| E         | 10  | 30  | 10  | 10  | 10  | 0   | 12.5 c |
| F         | 20  | 30  | 10  | 10  | 10  | 10  | 15.0 b |

<sup>1</sup>Treatments: (A) EPn application of 10<sup>6</sup> IJ/l by spraying, (B) EPn application of 10<sup>6</sup> IJ/l by watering, (C) EPn application with organic fertilizer at 100 IJ/g, (D) *M. anisopliae* application at 10<sup>9</sup> spores/L by spraying, (E) *M. anisopliae* application at 10<sup>9</sup> spores/L by watering, and (F) *M. anisopliae* application with organic fertilizer at 10<sup>9</sup> spores/200 g.

<sup>2</sup>% Mean intensity of damage with the same letters (in the same column) did not significantly differ, according to the Duncan Multiple Range Test at 5% level of significance.

4. Conclusion

We found that entomopathogenic nematodes causes 100% mortality to larval pests of cassava when applied at a concentration of 10<sup>7</sup> spores/100 ml of IJ, whereas the biological control agents fungus *M. anisopliae* causes only 10% mortality when applied at a 10<sup>7</sup> spores/ml concentration. Both types of biological control agents in organic fertilizer killed the three species of pest larvae, eliciting 100% mortality at a 10<sup>7</sup> IJ/100 ml concentration, whereas 10<sup>7</sup> spores/L of *M. anisopliae* only elicited 70% mortality after 72 h of inoculation. Mortality caused by *M. anisopliae* fungus was mainly due to epizootic conditions occurring in the population after more than 7 days of inoculation. The intensity of cassava damage caused by the white grub larvae pest *L. stigma* following application of either biological control agents (Entomopathogenic nematodes or *M. anisopliae*) was 0% to 5% at 90 d after planting. Thus, both provide protection to cassava.

5. References

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