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Addition Of Metarhizium Anisopliae In Organic Fertilizer For Enhancing White Grub's Control

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Abstract

White grub pests have been destroyed a lot of plantation of dry land. Farmers rely on synthetic insecticides to overcome the white grubs problem. Concerning the ecosystem pollution, it is necessary to improve the method of preparing M. anisopliae which is more effective on white grub-infested land to substitute synthetic insecticides. The research was conducted by mixing compost fertilizer produced by the Faculty of Agriculture UPN "Veteran" Yogyakarta with the M. anisopliae according to the treatment (6.25g / kg; 13.5g / kg; and 20.25g / kg). The pathogenicity test was carried out in a plastic container. Each container is filled with a compost mixture with compost and sand (1:10) and one white grub larvae instar 4. Each treatment consisted of 30 white grubs. The result reveals that mixing compost fertilizer in the application of M. anisopliae was effective in increasing white grubs mortality by up to 16.67%. The greater the dose of M. anisopliae will speed up the mortality time and increase the number of mortality.

Keywords: M. anisopliae, compost fertilizer, white grubs, mortality, pathogenicity

I. INTRODUCTION

White grub pests have been controlled mechanically by tilling the soil and picking up white grubs that appear during soil cultivation. Another way is to use a systemic insecticide that is sprinkled into the soil around the plantations. Although synthetic insecticides are faster, easier, and cheaper to use, their effectiveness is still lower when compared to mechanical (manual) methods (Poerwanto & Solichah, 2010). Besides that, insecticides in the soil will be difficult to decompose, reducing the biodiversity of

organisms and increase environmental pollution, especially in groundwater (Brühl., CA. & Zaller, JG., 2019).

Metarhizium anisopliae is an important fungus that is often used in biological control techniques. This fungus has been reported to be able to infect several insect orders such as Orthoptera, Coleoptera, Hemiptera, Lepidoptera, and Hymenoptera (Lee & Hou, 2003). Haryadi *et al.* (2013) reported that *M. anisopliae* was able to reduce the population of *Holotrichia Serrata* which attacks sugarcane.

According to Harjaka *et. al.* (2011) that the application of *M. anisopliae* at a dose of 10 Kg/hectare with an additional 10 Kg at four months after planting and an additional 10 Kg when the sugarcane is six months old can reduce the population of *L. stigma* larvae in sugarcane plantations and increase yields by more than 60 %. The fungus can survive in the crop for more than six months, potentially controlling *L. stigma* in the long term.

Previous research has shown that the application of compost in the land invites the presence of white grub compared to land that is not given compost (Poerwanto & Saidi, 2016). Control activities using the *M. anisopliae* have been carried out routinely and have shown good results, with white grub pest attacks decreasing from season to season (Poerwanto & Mulyanto, 2017). The obstacle that occurs is the preparation of *M. anisopliae* cannot be done every time when needed. It is necessary to plan an *M. anisopliae* preparation schedule in line with application time. Another problem is that it also requires planning the number of fungal cultures in accordance with the application plan. So that the use of this *M. anisopliae* can not be at any time when needed.

Based on this, it is necessary to improve the method of preparing *M. anisopliae* which is more effective on white grub-infested land.

II. RESEARCH METHODOLOGY

II.1 Isolation And Culture Of *M. Anisopliae*

Exploration was carried out by looking for white grubs that were infected with *M. anisopliae*. They were indicated by symptoms of dry rot grubs with green fungal mycelium on the surface of the body. Then they were placed in plastic glasses for isolation. Isolation of the fungus was carried out by cleaning infected white grubs with alcohol then taking a piece of the white grub's flesh under the cuticle and placing it on PDA medium in a petri dish, incubated for 7 days then purified until pure isolate of M. anisopliae was obtained on PDA media in a petri dish. Pure cultures of *M. anisopliae* obtained from Petri dishes were then transferred to the medium to slant and incubated until the required number of cultures was obtained.

II.2. Propagation Of *M. Anisopliae* In Solid Media Of Milled Broken Corn

As the media, corn is cleaned and washed with water so that the dirt mixed in it can be separated from the ingredients, then half-cooked, poured with warm water several times, and stirred to accelerate the cooking of the ingredients. Half-cooked corn which is marked by the soft flesh and put on the table to dry, then mixed with Chloramphenicol solution as an anti-bacterial and stirring evenly. Finally, put them in a plastic bag as much as 200 g and tightly sealed then sterilization is carried out using autoclaving. After the media cooled, the media was inoculated with 10 mL per culture bag of *M. anisopliae* in liquid

media, then incubated at 26°C for 20 days. *M. anisopliae* harvest was done when the fungal conidia have covered the entire surface of the corn medium.

II.3.Mixing Mushrooms And Compost

The compost fertilizer produced by the Faculty of Agriculture UPN "Veteran" Yogyakarta is mixed with the *M. anisopliae* fungus produced according to the treatment (6.25g / kg; 13.5g / kg; and 20.25g / kg) using a mixer so that it is evenly mixed. The results of the mixture are then incubated or stored in a transparent plastic container measuring 2 kg with a thickness of 5 mm at room temperature (26 - 28°C).

II.4. Pathogenicity Test Of *M. Anisopliae*

The pathogenicity test was carried out in a plastic container measuring 10 cm in diameter and 7 cm in height. Each container is filled with a compost mixture with compost and sand (1:10). Furthermore, in each container, 1 white grub larvae instar 4, and 10 g of carrots were inserted as feed. Carrots are replaced every 7 days. Each treatment consisted of 30 white grubs and incubated at room temperature (26 – 28°C). Observations on mortality of the white grubs larvae were carried out starting 7 days after treatment and repeated every 7 days until pupa formation.

III. RESULTS AND DISCUSSION

Mortality of white grub larvae was firstly observed at one week after the application of 20.25 g *M. anisopliae* per Kg compost (3.33 % ± 3.33). This phenomenon was not observed at other doses of application. Application of 6.75 and 13.5 *M. anisopliae* per Kg compost was not able to kill the larvae (Figure 1.). Mortality in dose 13.5 g/Kg appeared at three weeks after application, and after six weeks after application for the dose of 6.75 g/Kg. Application of 6.75 g/Kg *M. anisopliae* induced 3.33% mortality up to ten weeks after application. The increase in mortality was found in dose 13.5 and 20.25 g/Kg. Increased mortality was found in six and ten weeks for a dose of 13.5 g/Kg, and in six and seven weeks for a dose of 20.25 g/Kg.

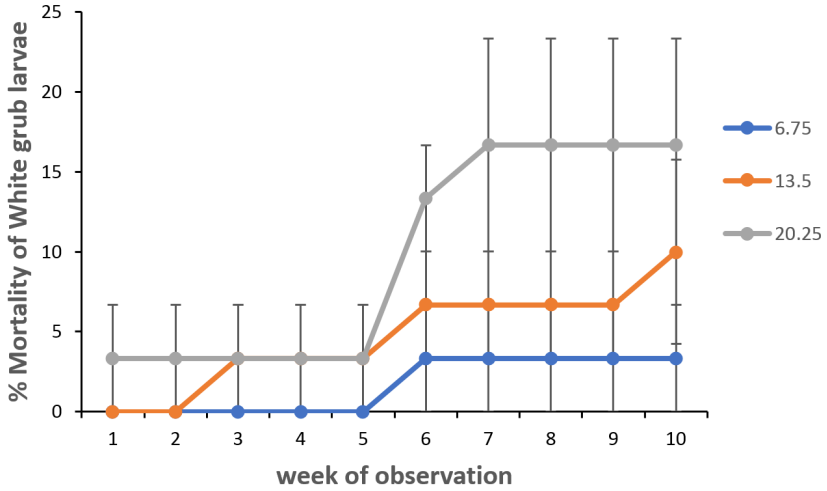

Figure 1. Average (±SE) of % mortality of white grub larvae in the application of 6.75, 13.5, and 20.25 g *M. anisopliae* per Kg compost.

A significant difference between application doses in causing mortality was not found up to four weeks after application (Table 1.). At six weeks after application, the highest percentage of mortality was found in a dose of 20.25 g/Kg (13.33 ± 3.33). It was significantly different ($P < 0,001$) to the dose of 6.75 g/Kg (6.67 ± 3.33), but not significantly different from dose 13.5 g/Kg (6.67 ± 3.33). this phenomenon was consistent up to ten weeks after application.

Table 1. Average (±SE) of % mortality of white grub larvae in 2, 4, 6, 8, 10 weeks after application of 6.75, 13.5, and 20.25 g *M. anisopliae* per Kg compost

M. anisopliae (g/Kg)	Week of observation				
	2	4	6	8	10
6.75	0.00 ± 0.00 a	0.00 ± 0.00 a	3.33 ± 3.33 a	3.33 ± 3.33 a	3.33 ± 3.33 a
13.50	0.00 ± 0.00 a	3.33 ± 3.33 a	6.67 ± 3.33 ab	6.67± 3.33 ab	10.00 ± 5.77 ab
20.25	3.33 ± 3.33 a	3.33 ± 3.33 a	13.33 ± 3.33 b	16.67 ± 6.67 b	16.67 ± 6.67 b

Virulence is one of the determinants of the success of *M. anisopliae* infection against host insects and virulence is closely related to the number of effective conidia capable of infecting the host. The number of effective conidia is influenced by the environmental conditions of the fungus habitat in the soil, especially the content of organic materials. One source of organic matter that is closely related to the epizootic development of entomo-pathogenic fungi in the soil is organic matter. Compost fertilizer is able to increase the population of useful microorganisms in the soil, including entomo-pathogenic fungi (Fuchslueger *et al*., 2014; Ojo *et al*., 2015). Compost acts as a source of nutrition for biota in the soil, including biota that makes entomopathogenic fungi as an alternative source of nutrition, especially when the availability of organic matter in the soil is running low (Namasivayam *et al*., 2015). In addition, to increase soil fertility as a result of the increasing population and activity of decomposing bacteria, the application of compost fertilizer is effective in reducing the predation activity of entomo-pathogenic fungi carried out by certain bacterial species under conditions of limited nutrients in the soil (Edesi *et al.*, 2013) so that the role of entomo-pathogenic fungi as a factor of insect pest mortality becomes more optimal. Mixing *M. anisopliae* with manure or compost is able to increase the mortality of *L. stigma* (white grubs) larvae by 5 - 12.9% (Indrayani *et al.,* 2019). As the implication, based on this study, *M. anisopliae* should be added to the organic matter that is used for fertilizing.

IV. CONCLUSION

Mixing compost fertilizer and entomo-pathogenic fungi (*M. anisopliae*) was effective in increasing white grubs mortality with the species of *L. stigma* by up to 16.67%. The greater the dose of *M. anisopliae* will speed up the mortality time and increase the number of mortality.

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