Promoting *Dolichoderus thoracicus* as an Agent to Disperse *Trichoderma* sp., a Fungus that Controls the Black Pod Disease, Central Sulawesi – Indonesia

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Abstract. In this study, we propose to use *Dolichoderus thoracicus* to act as a double agent; not only as an agent to control cocoa plant pests, but also as an agent for distributing *Trichoderma* sp., a fungus that suppresses the development of the black pod disease caused by *Phytophthora palmivora*. In the experiments, *D. thoracicus* was more attracted to honey-soil media (M5) and coconut waste pulp-soil media (M6). However, 10% sucrose-potato-soil media (M3) was the best media for growing *Trichoderma* sp. Morphological study showed that spores attached to many parts of *D. thoracicus*. The efficiency of *D. thoracicus* in distributing the spores compared to the conventional method of using a sprayer was measured. The results showed that the growth of *P. palmivora* was suppressed by 83.33%, which is not significantly different from when *Trichoderma* was dispersed through spraying (87% suppressed). It was concluded that *D. thoracicus* can act as a double agent and can be used to disperse *Trichoderma* sp.

Keywords: *Black pod disease; cocoa plantation; D. thoracicus; P. palmivora; Sulawesi; Trichoderma sp.*

1 Introduction

Just like the invasion of pests such as *C. cramerella* and *H. theobroma*, the black pod disease is a major problem for cacao plantations in Central Sulawesi, Indonesia. Altogether they cause significant losses in production of up to 90%. The black pod disease is caused by the fungus *P. palmivora*, which can be dispersed quickly by several media, including wind and water, although the cocoa black ant, *D. thoracicus*, is known to be the main contributor in its dispersal. The presence of *D. thoracicus*, however, is important since these ants can control both *C. cramerella* and *H. theobroma* [1]. In our previous study [2], it was shown that one local species of *Trichoderma* sp., based on their performance in sporulation, antagonistic ability, was effective in suppressing the development of *P. palmivora*. This finding has already been applied in the

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field; *Trichoderma* sp. spores have been applied to suppress the growth of *P. palmivora* on the cocoa pod using sprayers. Inspired by the work of Sutton & Peng [3], who succeeded in using *Botrytis cinerea* as an agent for biological control in a strawberry plantation, we attempted to use *D. thoracicus* as an agent to disperse *Trichoderma* sp. spores. The aims of this research were to find the best soil composition to grow *Trichoderm*a sp. as well as to attract *D. thoracicus*, but also to evaluate the ability of *D. thoracicus* to disperse the *Trichoderma* sp. spores, as well as their effectiveness in suppressing the development of *P. palmivora*.

2 Methods

2.1 *D. thoracicus* and *Trichoderma sp.*

The *D. thoracicus* were collected from the field using mock nests (a piece of bamboo of approximately 5 cm in diameter and 40 cm in length, filled with coconut leaf and spread with honey), which were put on a branch of a cocoa plant for about 4 weeks to grow a colony. The colony of *D. thoracicus* was then reared in the laboratory. Before use, the ants were put into a honey contained sterile jar to adapt for 24 h. The *Trichoderma* sp. used was from a collection of the Plant Pest and Disease Laboratory in Tadulako University, Palu, Indonesia. It was prepared in solid tablet form with 10.95 X 10^{20} colony-forming units (cfu), according to the method from Umrah, *et al*. [2].

2.2 Media for *Trichoderma* sp. Growing

Samples of thirty grams of sterile soil each were mixed with 10 mL of six different sterile media according to Table 1. Except for M0, all media were mixed with 10.95 x 10^{20} cfu of *Trichoderma* sp. Before use, these mixtures were then incubated at room temperature for 48 h.

Table 1 Variation of media for growing *Trichoderma* sp. before dispersion by *D. thoracicus* or sprayer.

Code	Variation of media	100 mL media : with / without 10,95 X 10^{20} cfu of *Trichoderma* sp.	Dispersed by
M0	Water	Without	*D. thoracicus*
M1	Water	With	Sprayer
M2	Water	With	*D. thoracicus*
M3	10 % sucrose - potato	With	*D. thoracicus*
M4	10% sucrose	With	*D. thoracicus*
M5	10% honey	With	*D. thoracicus*
M6	10% coconut waste pulp	With	*D. thoracicus*

2.3 Simulated Track and Preference of *D. thoracicus* for Variant of Media

A simulated track of *D. thoracicus*'s journey from soil to cocoa pod with a mock nest was prepared, as shown in Figure 1. In order to keep the ants on the track, each track was put inside a 70 cm x 40 cm x 40 cm container filled with about 1 cm height of water.

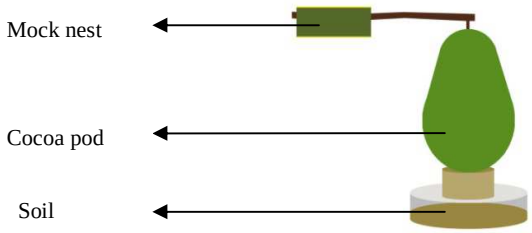

Figure 1 Simulated track – *D. thoracicus* route from soil to cocoa pod with mock nest.

Five different media (M2, M3, M4, M5, M6) were tested for the preference of *D. thoracicus* (Table 1). On each track, 600 *D. thoracicus* individuals were infested and the percentage of the ant activity in the media tested was recorded. 0.1 g of test medium was diluted with water and then cultured on Potato Dextrose Agar for 48 hours. The number of *Trichoderma* sp. in the medium was then counted according to the plating method by Cappuccino & Sherman [4].

2.4 Position and Number of *Trichoderma* sp. attached to *D. thoracicus*

Two hundred individuals of *D. thoracicus* on the track containing the medium to be tested were infested for 2 x 24 hours. Samples of *D. thoracicus* were then examined under a microscope to identify the position of the attached spores. All the ants were then washed with 100 mL of sterile water to release the spores from their body. The spores in the water were cultured in Potato Dextrose Agar for 48 hours and the number of *Trichoderma* sp. was then counted according to the plating method by Cappuccino & Sherman [4].

2.5 Effectiveness of *D. thoracicus* in Distributing *Trichoderma* sp. and Suppressing *P. palmivora*

The cocoa pods from the different treatments were each washed with 100 mL of sterile water. The spores in the water were cultured in Potato Dextrose Agar for 48 hours and the number of *Trichoderma* sp. in the water was then counted according to the plating method by Cappuccino & Sherman [4]. The cocoa

pods, which were previously contaminated with *Trichoderma* sp. according to the treatments in Table 1, were then inoculated with *P. palmivora* and incubated for 24 hours. The effectiveness of disease suppression was calculated by measuring the area of the black pod disease.

3 Result and Discussion

3.1 Preference of *D. thoracicus* to Variant of Media

As shown in Figure 2, *D. thoracicus* was able to come on all soil media tested. However, honey-soil media (M5) attracted more insects than any of the other media tested. Statistical analysis showed that there was a significant difference with the other media tested ($p < 0.05$) except for the combination of soil with 10% coconut waste pulp (M6), which also attracted *D. thoracicus* but not significantly differently from M3 or M5. Volatile chemicals from the soil media were probably the main factor in attracting *D. thoracicus*. The preference itself is influenced by many factors, including the need for energy, growth, and development [5].

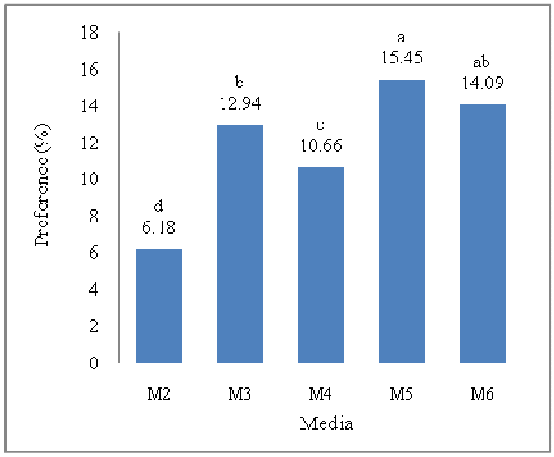

Figure 2 Preference of *D. thoracicus* to variant of media. M2: water, M3: 10% sucrose-potato-soil, M4: 10% sucrose-soil, M5: 10% honey-soil, M6: 10% coconut waste pulp-soil.

3.2 Number of *Trichoderma* sp. in the Media

10% sucrose-potato-soil medium (M3) was the best medium for growing *Trichoderma* sp., as shown in Figure 3. The number attained was 97.29 cfu and statistical analysis showed that there was a significant difference with the other

media tested ($p < 0.05$). The results also indicated that sucrose-potato is a ready-to-use element for growing *Trichoderma* sp.

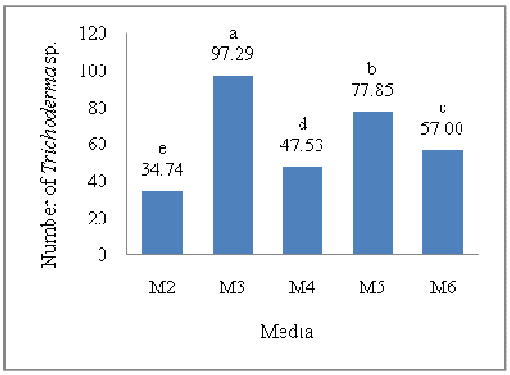

Figure 3 Number of *Trichoderma* sp. (cfu) in the variants of media. M2: water, M3: 10% sucrose-potato-soil, M4: 10% sucrose-soil, M5: 10% honey-soil, M6: 10% coconut waste pulp-soil.

3.3 Position and Number of *Trichoderma* sp. Attached to *D. thoracicus*

During the experiment, *D. thoracicus* traveled all the time from the mock nest to the soil through the cocoa pod. This activity caused the *Trichoderma* sp. spore in the soil to attach to the *D. thoracicus* body, including legs, antennas, head, thorax and abdomen (Figure 4). As the insects were always traveling, the spores were taken accidently to other places, including the cocoa pod.

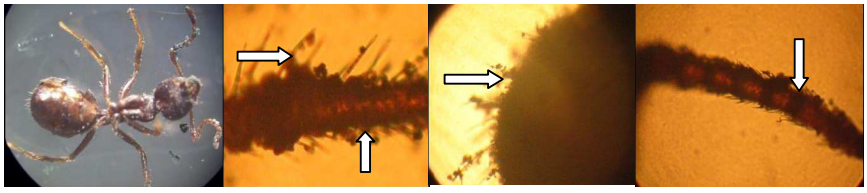

Figure 4 Position of *Trichoderma* sp. attached to *D. thoracicus* (arrows).

The number of *Trichoderma* sp. spores attached to the *D. thoracicus* body varied among the media tested. 10% sucrose-potato-soil media (M3) gave the highest number of spores attached to *D. thoracicus* with 87.43 cfu (Figure 5). This number was significantly different from the other media tested ($p < 0.05$). The number of spores attached was related to the number of *Trichoderma* sp. spores in the media, the highest of which which came from 10% sucrose-potato-soil media (M3).

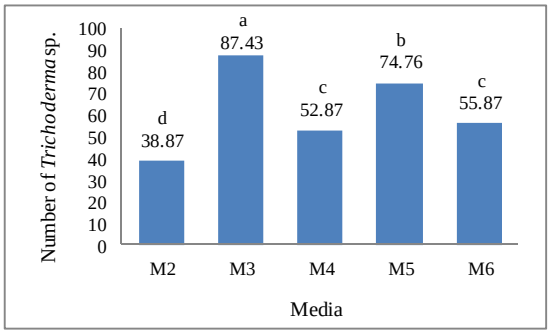

Figure 5 Number of *Trichoderma* sp. (cfu) attached to *D. thoracicus* for variant of media. M2: water, M3: 10% sucrose-potato-soil, M4: 10% sucrose-soil, M5: 10% honey-soil, M6: 10% coconut waste pulp-soil.

3.4 Number of *Trichoderma* sp. in Cocoa Pod

During travelling from mock nest to media, spores attached to the *D. thoracicus* body fell off and were deposited on the cocoa pod. The number of *Trichoderma* sp. spores on the cocoa pod using a spraying method (M1), which was 18.00 cfu, was not significantly different from the number of *Trichoderma* sp. spores on the cocoa pod that were deposited by *D. thoracicus* from the 10% sucrose-potato-soil media (M3), which was 15.77 cfu. However, this number was significantly different from that of the other media, as shown in Figure 6.

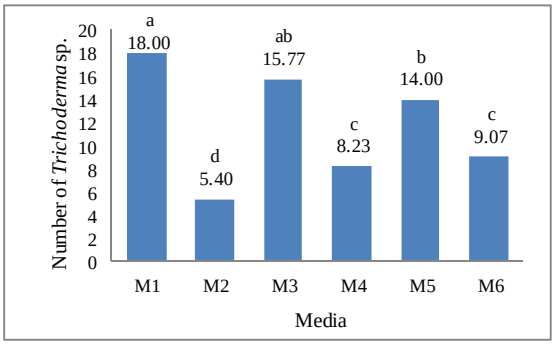

Figure 6 Number of *Trichoderma* sp. (cfu) from variant of media deposited in cocoa pod. M1: water with *Trichoderma* sp. dispersed by sprayer, M2: water with *Trichoderma* sp. dispersed by *D. thoracicus*, M3: 10% sucrose-potato-soil with *Trichoderma* sp. dispersed by *D. thoracicus*, M4: 10% sucrose-soil with *Trichoderma* sp. dispersed by *D. thoracicus*, M5: 10% honey-soil with *Trichoderma* sp. dispersed by *D. thoracicus*, M6: 10% coconut waste pulp-soil with *Trichoderma* sp. dispersed by *D. thoracicus*.

3.5 Effectiveness of *D. thoracicus* in Distributing *Trichoderma* sp. and Suppressing *P. palmivora*

Since the number of *Trichoderma* sp. spores on the cocoa pods was not significantly different between experiment M1 and M3, similar results were also achieved by *Trichoderma* sp. in suppressing the growth of *P. palmivora*. Spraying *Trichoderma* sp. spores (M1) gave 87.01% in *P. palmivora* growth suppression, which was not significantly different from when *Trichoderma* sp. spores were taken by *D. thoracicus* from 10% sucrose-potato-soil media (M3), which gave 85.18% suppression. As a result, the spread of the black pod disease on the cocoa pod was reduced as well, as shown in Figure 7. There was no significant difference between M1 and M3 whether *Trichoderma* sp. spores were dispersed by sprayer or by *D. thoracicus* from 10% sucrose-potato-soil media, respectively (Figure 8).

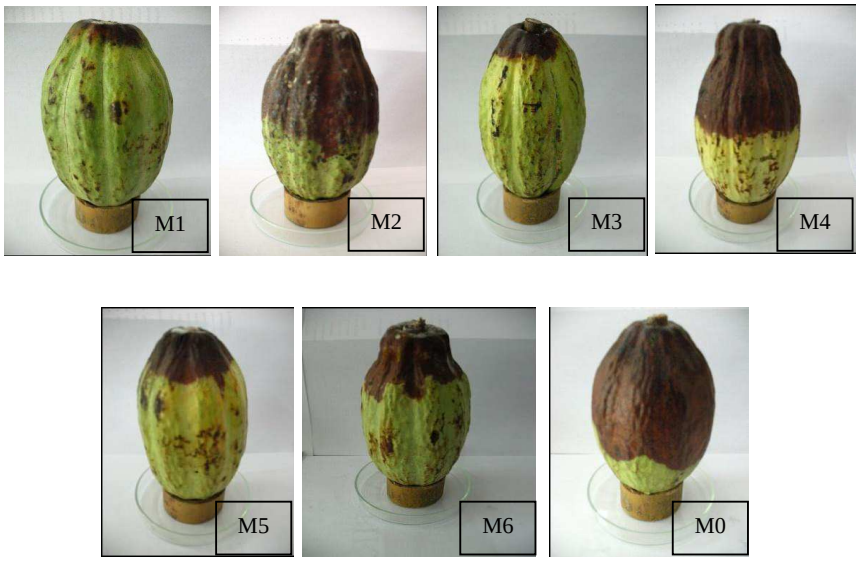

Figure 7 Growth of the black pod disease in cocoa pods. M1: water with *Trichoderma* sp. dispersed by sprayer, M2: water with *Trichoderma* sp. dispersed by *D. thoracicus*, M3: 10% sucrose-potato-soil with *Trichoderma* sp. dispersed by *D. thoracicus*, M4: 10% sucrose-soil with *Trichoderma* sp. dispersed by *D. thoracicus*, M5: 10% honey-soil with *Trichoderma* sp. dispersed by *D. thoracicus*, M6: 10% coconut waste pulp-soil with *Trichoderma* sp. dispersed by *D. thoracicus*, M0: water without *Trichoderma* sp. dispersed by *D. thoracicus*.

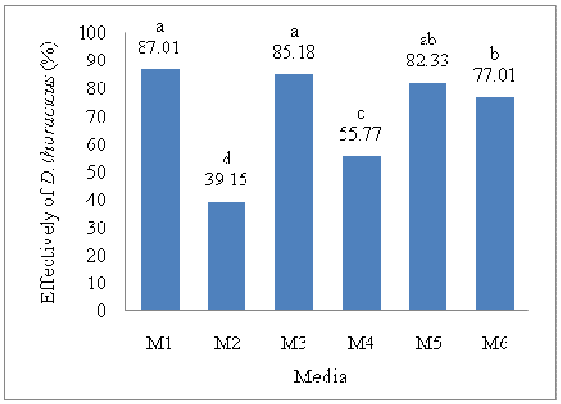

Figure 8 Effectiveness of *D. thoracicus* in distributing *Trichoderma* sp. and suppressing the growth of *P. palmivora.* M1: water with *Trichoderma* sp. dispersed by sprayer, M2: water with *Trichoderma* sp. disperse by *D. thoracicus*, M3: 10% sucrose-potato-soil with *Trichoderma* sp. disperse by *D. thoracicus*, M4: 10% sucrose-soil with *Trichoderma* sp. disperse by *D. thoracicus*, M5: 10% honey-soil with *Trichoderma* sp. dispersed by *D. thoracicus*, M6: 10% coconut waste pulp-soil with *Trichoderma* sp. dispersed by *D.thoracicus*.

4 Conclusion

10% sucrose-potato-soil media (M3) was the best media for growing *Trichoderma* sp. *Trichoderma* sp. spores attached to the *D. thoracicus* body and were dropped onto the cocoa pod, therefore *D. thoracicus* can be used as an agent to disperse *Trichoderma* sp. There was no significant difference in effectiveness with the more commonly used spraying methods.

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