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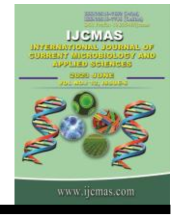

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Effectiveness of Pod coating formulation with the carrier media of *Trichoderma asperellum* in Controlling Phytophthora Pod Rot Disease

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A B S T R A C T

One of the important diseases in cocoa plants is pod rot disease caused by pathogen *Phytophthora palmivora*. Loss which caused pod rot disease varies between 26 % and 50 %.One of the biological agents which potential to control cocoa pod rot is a type of *Trichoderma asperellum*. There have been many uses of *Trichoderma* as a biofungicide, including direct spraying, mass propagation on rice media in the form of flour and with the addition of kaolin. This research aims to compare the effectiveness of using *Trichoderma asperellum* in several cocoa pod coating application techniques. Cocoa pods that had the heaviest attack intensity was on the penis (no *T. asperellum* treatment) which was 72.22% while the attack symptoms and the lowest disease attack intensity were the *T. asperellum* treatment, added kaolin and MCC, with an attack intensity of 5.56%. The weight of 100 cocoa beans in each treatment, the amount was not much different from the yield loss rate for the control, namely 17.63% and the treatment of cocoa pods sprayed with *Trichoderma* directly and pods sprayed with *T. asperellum* plus kaolin showed the amount of loss. The same yield was 13.29% and in the treatment of spraying *T. asperellum* which was added kaolin and CMC showed the lowest yield loss of 12.14%. The carrier media must contain important components to support the viability and growth of the microbes inoculated into it. This is because the carrier media functions to grow and extend the shelf life so that the carrier media must contain organic nutrients to support *Trichoderma asperellum* growth.

Keywords

Trichoderma asperellum, cocoa pod rot, Gross Domestic Product

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Introduction

Cocoa is one of the plantation product commodities that has a role as a foreign exchange earner and plays an important role in economic activities in Indonesia where the contribution of the plantation sub-sector is 3.63 percent of the total Gross Domestic Product. Cocoa is one commodity export Indonesia and Indonesia as exporter third cocoa the world's largest after Ghana and Ivory Coast. Total exports of cocoa in the last five years have fluctuated up and down, increasing from 5.40 to 7.53 percent per year, while the decline reached 5.87 percent. Cocoa production in Indonesia in 2020 was 720.666 tons (BPS, 2020).

The decline in cocoa production can be caused by several factors, including the influence of soil (land), climate, cultivation techniques and pests and diseases.

One of the important diseases in cocoa plants is pod rot disease caused by pathogen *Phytophthora palmivora*. Loss which caused disease rotten pod varies between 26 % and 50 %.

This disease can develop very quickly because the pathogen that causes this disease, namely *Phytophthora palmivora* Butl has a sporangium (zoosporangium) which can germinate directly by forming vessels sprouts whereas in a manner no direct with form zoospores. *P. palmivora* can be dormant by forming chlamydospores (Semangun, 2008).

Many attempts have been made to control the pathogens that cause pod rot disease by using synthetic fungicides. However, the continuous use of synthetic fungicides can have an impact on the environment and the emergence of pathogen resistance.

Therefore utilization of biological agents to control pod rot cocoa which caused by *Ppalmivore* is an environmentally friendly control solution. One of the biological agents which potential to control cocoa pod rot is a type of *Trichoderma*.

Sulistyowati (2008) states that the use of *Trichoderma sp* can reduce the incidence of Cocoa Pod Rot disease and is almost the same as the use of synthetic fungicides.

There have been many uses of *Trichoderma* as a biofungicide, including direct spraying, mass propagation on rice media in the form of flour and with the addition of kaolin.

The pod cloaking technique is one method which can push development level infection of pod rot cocoa disease (Asaad *et al.,* 2010). Therefore this research aims to compare the effectiveness of using *Trichoderma asperellum* in several cocoa pod coating application techniques.

Materials and Methods

Pathogen Isolation of *Phytophthora palmivora* Butler

Isolation of disease-causing fungi from symptomatic cocoa pods done by direct planting method in PDA medium. Affected plant parts are cut to a size of 1 x 1 cm, including healthy tissue. The sample pieces were then surface sterilized by means of put the piece into distilled water-alcohol 70%-aquadest and dry it. Furthermore piece the extended Cuppetr containing 4 pieces/petri of V8 Juice medium and incubated for 3 days at room temperature. After 3 days of incubation, the growing fungi were transferred to PDA medium until a pure culture of the fungus was obtained, so that its macroscopic and microscopic characters could be observed (Drenth and Sendall, 2004).

Pathogenicity Test of *P. palmivora*

Healthy cocoa pods were soaked in 1% NaOCL for 2 minutes then rinsed with water sterile. Furthermore injured the pod with needle inject sterile as many as 3 points on the surface of the fruit. Then the *P. palmivora mushroom mycelium was taken* on PDA medium and then affixed to the wound on the surface of the fruit. The inoculated fruit is placed in a plastic box and covered with transparent plastic. In the box placed wet wipes to maintain humidity, then incubated at room temperature (Hapsah and Zuyasna, 2013)

Resistance Test of Cocoa Fruit Applied with Biological Agents (*T. sperellum)*

The research method was based on a completely randomized design (CRD) with four treatments consisting of 3 units and 3 replications so that there were 36 experimental units. The treatments tried were: without spraying/covering (control) (M0), spraying with *T. asperellum* directly (M1), spraying with propagation of *T. asperellum*+biokaolin(M2), spraying with *T. asperellum* + biokaolin + CMC (M3). Parameters observed included disease incidence, disease severity and amount of loss yield weight of 100 seeds dry.

Disease Severity Level

The formula used to calculate the severity of fruit rot using the equation (Sulistyowati, 2008):

$$I = \frac{\sum_{i}^{n} n_i \, x \, v_i}{Z \, x \, N} x100\%$$

Information

I = disease severity (%),

ni = number of fruit infected in each category of infection,

vi = score of each category of infection,

Z = the score of the highest infection category,

N = the number of fruits observed.

The damage scale was rated by category by Asaad *et al.,* (2010) as follows:

0 = no attack symptoms

1 = attack symptoms > 0-25%

2 = attack symptoms > 25-50%

3 = attack symptoms > 50-75%

4 = attack symptoms > 75-100%

Lost Yield

Loss of results is calculated in the following way: first determine the criteria or scoring severity using the scoring that has been made previously. Both fruits with the same severity level are grouped or mixed and then 100 seeds are taken, then weighed to obtain the dry weight of 100 seeds in each severity category. The formula for determining the percentage of yield loss using the equation:

$$KH = \frac{\sum (Bks - BKi)}{Bks}$$

Results and Discussion

Isolation of *Phytophthora palmivora*

Isolation of *Phytophthora palmivora*, a pathogen that causes pod disease on cocoa plants, was carried out by plating and microscopic identification, the following results were obtained

Trichoderma inoculation on rice media

Application of *Trichoderma* on Cocoa Pod

Application of *Phytophthora palmivora* Pathogen on cocoa pods

The application of the phytophthora palmivora pathogen was carried out using the Detached Pod method

Observation

Observations were made 7 days after the application of the pathogen and *Trichoderma* on cocoa pods and were obtained the following results:

Lost Yield

In Figure 1, a visual observation of the *Phytophthora pathogen* has the following characteristics, namely having a pure white colony color, forming a cotton-like growth pattern, the shape and direction of growth are thin-layered, growing radially and forming a chrysanthemum flower structure (Sunarti and Yoza, 2010). Microscopic observation of *P. palmivora,* namely from the hyphae form without septa, transparent and having many branches (Umaya, 2006), the shape of the ovoid sporangium like a pear (Sunarti and Yoza, 2010).

In Figure 2, multiplication of *Trichoderma asperellum* was carried out on rice media so that the concentration of *T. asperellum* met the requirements for applying the spore density of $10^{6.}$ These spore concentrations were then *given* different treatments to see the effectiveness of the *T. asperellum* antagonist treatment in controlling fruit rot disease caused by the pathogen *P. palmivora.*

Based on the observations that have been carried out on cocoa pods given various treatments of *T. asperellum*, the results showed that the *plant*cocoa infected with black pod disease *PPalmivora* has various intensity of Pod Rot Disease attacks.

Cocoa pods that had the heaviest attack intensity was on the penis (no *T. asperellum* treatment) which was 72.22% while the attack symptoms and the lowest disease attack intensity were the *T. asperellum* treatment, added kaolin and MCC, with an attack intensity of 5.56%. Images of attack intensity on each *T. asperellum* treatment can be seen in Figure 2.

Some types of *Trichoderma* spp are capable parasitic fungi that are pathogenic in the sense of potentially creating metabolites gliotoxin and viridin as antibiotics. Several known species can produce enzymes ß-1,3-glucanase and chitinase which can causing exolysis of hyphal threads the host. *T* virens has antifungal activity, properties antifugalnya produce compounds reproduction of enzymes called enzymatic then work to dissolve the cell wall pathogen. Gliotoxin and viridiol antibiotics which can inhibit various types of fungi, compete with disease pathogens and can help plant growth including cocoa plants which inhibited the phytopathogen *P. palmivora* (Nurhayati, 2011).

Pod rot disease attack cocoa plants from the young pod (nipple) to the ready fruit harvested. If the affected pod is young pod, then the cocoa pods will not get developed or had decayed before becoming mature fruit, whereas if the attacked is fruit that has matured or is almost ripe, then the fruit can still be harvested but the quality of the seeds is not Good. According to the Agricultural Technology Research Institute Lampung (2008) fruit rot disease *P. Palmivora* can attack all stages of pod, both nipples as well as large fruit and even ripe fruit. In this study the fruits infected with *P. palmivora* were mature (almost ripe) fruits.

So that from observations on fruits that have been infected with these pathogens, it can be seen that the seeds inside the fruit remain intact, only the pulp is brown and thin (Figure 9). After weighing the weight of 100 cocoa beans in each treatment, the amount was not much different from the yield loss rate for the control, namely 17.63% and the treatment of cocoa pods sprayed with *Trichoderma* directly and pods sprayed with *T. asperellum* plus kaolin showed the amount of loss. the same yield was 13.29% and in the treatment of spraying *T. asperellum* which was added kaolin and CMC showed the lowest yield loss of 12.14%. The small yield loss on the treated cocoa pods was due to the addition of kaolin which acts as a microbial carrier medium. Carrier media functions to grow, package, and extend the shelf life of biological agents.

The carrier media must contain important components in the form of organic nutrients that support the viability and growth of the microbes inoculated into it. Composition of biological fungicides based on carrier media must contain organic nutrients in the form of nitrogen, organic carbon, phosphorus, potassium and other nutrients. These elements can be processed by microbes into inorganic materials that can be utilized by plants. The manufacture of biological fertilizers must consider the carrier media that is composed.

Table.1 Intensity of *P. palmivora* attack on cocoa pods

Treatment of *Trichoderma asperellum*	Attack intensity (%)
Control	72.22 [a]
Spraying with *T. asperellum* directly	25 [a]
Spraying with *T. asperellum propagation* on rice media	22.22 [a]
Spraying *T. asperellum* with the addition of biokaolin + CMC	5.56 [b]

Numbers followed by unequal lowercase letters are significantly different according to the BNJ test results at the 5% level

Table.2 Weight of cocoa beans in each category/scale of pod disease attack

Treatment	Weight of cocoa beans (100 beans)	Yield Loss (%)
Po	285	17.63
P1	300	13.29
P2	300	13.29
P3	304	12.14

Fig.1 a. Macroscopic Observation b. Microscopic Observation

Fig.2 Inoculation of *Trichoderma* on Rice Media

Fig.3 *Trichoderma* application on cocoa pods

Fig.4 Application of *P. palmivora* on cocoa pods

Fig.5 Control (Po)

Fig.6 Application of *Trichoderma* on Rice Media (P1)

Fig.7 Application of *Trichoderma* on Rice + Kaolin (P2) Media

Fig.8 Application of *Trichoderma* on Rice + Kaolin + CMC (P3) Media

Fig.9 a. Pod attacked by *P. palmivora* b. Healthy cocoa pods

The carrier media must contain important components to support the viability and growth of the microbes inoculated into it. This is because the carrier media functions to grow and extend the shelf life so that the carrier media must contain organic nutrients to support mushroom growth. The addition of this carrier media also has an effect on maintaining the population of biological agents and the addition of CMC serves to attach the carrier media to the pod (pod seed coating) so that it is very efficient in protecting the fruit from attack by pathogens that cause pod rot by *Phytophthora palmivora*.

The pod coating method with *T. asperellum* added with kaolin and CMC was the most effective for controlling cocoa pod disease with a disease severity rate of 5.56% and yield loss of 12.14% compared to direct spraying of *T. asperellum* or just adding carrier media.

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