

FORMULATION OF TRICHODERMA VIRENS ORIGIN OF ACEH COCOA CONTROLLING BLACK POD DISEASE CAUSED BY PHYTOPHTORA PALMIVORA

TJUT CHAMZURNI, RINA SRIWATI, RIZKY MUARIF, BUNI AMIN, ABDUH ULIM
Agrotechnology Department, Syiah Kuala University, Darussalam, Banda Aceh, Aceh,
Indonesia

Corresponding Author: rin_aceh@yahoo.com

ABSTRACT

Trichoderma virens have been isolated from rooting cocoa in Aceh and in the laboratory have been reported capable of inhibiting the growth of *P. palmivora* in vitro. However, the formulations of *T. virens* from Aceh can be used as an alternative product for farmers in controlling black pod disease of cocoa. This research was conducted at the Laboratory of Plant Pathology College of Agriculture Syiah Kuala University and cocoa fruit samples obtained from cocoa plantations owned by farmers in Pidie Jaya District of Aceh. The purpose of the study was to obtain an application formulations strains of *T. virens* from Aceh, as one of the biological control study consisted of seven treatments, namely W (water), Pest (Mancozeb Fungicide), Det (Detergent), Det+Tv (detergent+strains of *T. virens*), P+Tv (Adhesive +strains of *T. virens*), Co+Tv (Corn Oil+strains of *T. virens*), Po+Tv (Palm oil+strains of *T. virens*) and each treatment was repeated 10 times to obtain 70 experimental units. The parameters observed incubation period, disease incidence (%), and the diameter of the spots (cm). Application results show that the strains of *T. virens* Origin Aceh effective in controlling *P. palmivora* on cocoa pods and the best formulation in controlling *P. palmivora* on cocoa pods are corn oil+*T. virens* formulation (Co +Tv) compared with other formulations are characterized by the length of the incubation period, the low incidence of *P. palmivora* disease, and the small diameter of the spots on the cocoa pods.

Keywords: Formulation, *Trichoderma virens*, Black Pod Disease, *Phytophthora palmivora*

Introduction

Theobroma cacao L.(cacao) is one of the commodities that planted for the largest scale in Indonesia. Recently, Aceh cocoa cultivation have many problem in declining quality of the cocoa fruit and cocoa production. The greatest limiting factor in reducing the cocoa pod disease was black pod disease which is caused by *Phytophthora palmivora* fungi. Interviews with several farmers in Aceh Besar and Pidie Jaya known that black pod disease is a major constraint on the cocoa plantations and the important disease that could limiting yields in Aceh (Nasir, personal communication, 2012). The disease could reduce cocoa production. Greatest damage occurs when the infection started 2 months before the pod ripe. The pods were infected in this phase can cause a total loss because pathogens can easily penetrate through the skin layers into ovules fruit in green fruit growing.

A biological control techniques to control pathogens highly preferred because it can minimize damage to the environment. The use of antagonist agents *Trichoderma* is one of the techniques in biological control against pathogens. *Trichoderma* species are commonly used as a biocontrol agent against fungal pathogen and some strains can produce antibiotics, parasite other fungi, antagonistic to many plant pathogens and compete with pest microorganisms (Harman et al., 2004). *Trichoderma* is able to produce antimicrobial compounds, as well as mycoparasite activity. The dominate competition in the growing space that can suppress the growth of pathogenic fungi (Ekowati, 2006). Species of *Trichoderma* spp. which proved to be

effective in controlling fruit rot of cacao were *T. harzianum* and *T. Koningii* (Sukanto, 2003). In addition, Sriwati *et al* (2009) also succeeded in *Trichoderma virens* isolate Gl-20 stains from Aceh isolated from cocoa plant roots in Aceh. From the results of these studies reported that *T. virens* is able to inhibit *P. palmivora* in vitro on cocoa pods (Mufakkir, 2011) as well as cacao seeds (Muttaqin, 2011). The test results on the ability of *T. virens* antagonistic stains Gl-20 from Aceh and compare it with other *Trichoderma* species, indicating that *T. virens* Gl-20 stains from Aceh has a better potential to inhibit the development of *P. palmivora* through antibiosis test, mycoparasitict, and pre colonization conducted in the laboratory SPCL - USDA (Sustainable Crop Perennial Lab. USDA) Maryland Beltsville (Sriwati, 2012). Faisal (2012), states that the application of the suspension at a concentration of 10^7 *T. virens* can suppress the development of *P. palmivora* when inoculated on cocoa fruits in vitro. This is evidenced by the smaller diameter of the spots on fruit sprayed with a suspension of *T. virens* at a concentration of 10^7 compared with non-sprayed fruit (control). Results of other studies conducted by Nurjanani (2009) showed that the percentage of *P. palmivora* attacks on cocoa pods were sprayed with a suspension of four isolates of fungi *Trichoderma* spp. 2 weeks after the application was lower than the percentage of *P. palmivora* on cocoa pods that are not sprayed with a suspension of the fungus *Trichoderma* spp. Lab-based research on formulations with *Trichoderma* isolates indicate a significant benefit to *Trichoderma* efficacy in response to added nutrients, humectants, and vegetable oils (corn oil). Initially, these formulations (including nutrients, humectants, and oils) should be evaluated for their abilities to enhance *Trichoderma* biocontrol efficacy in cocoa pod, small scale and short term field studies.

Under this research, it is necessary to examining and testing the formulation of *T. virens* Gl-20 stains from Aceh for biological control black pod disease. USDA team has successfully applying formulation *T. oviosporum* for 2 years to control the disease in cacao plantations in Costa Rica in order to reduce the intensity of the disease and increase cocoa production (Bailey *et al*, 2012). Therefore, it is necessary to study the formulation of *T. virens* Gl-20 stains from Aceh with several oil and nutrition at the lab scale and can be used as a disease control products against *P. palmivora*.

Material and Methods

The experiment was conducted by using sampling on cocoa farmers in pidie jaya and cocoa fruit carried out in the laboratory of plant disease, agrotechnology, faculty of agriculture syiah kuala university.

Instruments used in this study include laboratory equipment such as a petri dish, measuring cups, test tubes, microscopes, analytical scales, needles, autoclave, shaker, haemocytometer, pipettes, incubator, hot plate, Cork Borrer, scapel, magnetic stirrer and handsprayer, knives, scissors fruit, plastic box, gloves, and a fruit box. While the materials used were distilled water, alcohol, heat-resistant plastic, cocoa pods, chopped corn, corn oil, palm oil, rice, corn fruit, PDA (Potato Dextrose Agar), and isolates of *T. virens* Gl-21 from Aceh.

Survey of cocoa gardens in Pidie Jaya

The survey was conducted aimed to determine potential land as a place of sampling. Samples were needed were the young cocoa pods and not infected with *p. Palmivora* uniform size for each treatment approach

Preparation of isolates of *T. virens*

Preparation of *t. Virens* isolates performed by multiplication oft. *Virens* culture using pda, twhen the isolates were grown on pda medium were transferred to corn. Preparation of *T. virens* isolates on pda and then prepares each formulation, mixed according to each treachment.

Sampling cocoa pods

Sampling was conducted in pidie jaya cacao farmers. The samples used were young cocoa pods not infected with *p. Palmivora* and the near-uniform size. The number of pieces is done for experimental sample were 10 pieces each treatment so that the number of samples were 70 pieces experiment.

Application formulation *T. virens*

This study uses experimental methods performed with treatment as in table bellow.

No	Treatment	Description
1.	W	W
2.	Pest	Pestisida
3.	Det	Deterjen
4.	Det+Tv	Deterjen+ <i>T. virens</i>
5.	P+PDB+TV	Pektin+PDB+TV
6.	Co+PDB+L+TV	Corn oil+PDB+Lecitin+ <i>T.virens</i>
7.	Po+PDB+L+TV	Palm oil+PDB+Lecitin+ <i>T. virens</i>

Each treatment was repeated 10 times to obtain 70 experimental units. *T. virens* formulations obtained by getting the suspension at a concentration of 1×10^7 *T. virens* first. Suspension of *T. virens* was obtained by the method of dilution (1: 9). *T. virens* are derived from corn media weighed on analytical scales as 6 g and mixed with 54 ml of distilled water in a flask (10^{-1} dilution) and stirred using a hot plate. Take 1 ml of the 10^{-1} dilution and mixed with 9 ml of distilled water in a test tube (dilution 10^{-2}) and were taken 1 ml of the previous dilution and mixed with 9 mL of distilled water to obtain further dilution up to 10^{-4} dilution. After that, take 1 ml of the 10^{-4} dilution and dripped on haemocytometer for *T. virens* spores counted using a microscope. Then the number of spores calibrated by using the following formula.

$$\begin{aligned} TV &= (S1 + \dots + S5) / 5 = (a) \\ &= (S1 + \dots + S5) / 5 = (b) \\ &= ((A) + (b)) / 2 = (c) \\ &= (C) \times 25 \times 10^2 \times 10^n \end{aligned}$$

Specification:

- S1 ... S5 = number of spores in 5 box haemocytometer
- 25 = Decision total number of boxes in haemocytometer
- 10^2 = Decision box up and down on haemocytometer
- 10^n = Dilution to-n

Having obtained a suspension of *T. virens* with a concentration 1×10^7 , then the suspension was grown in PDB medium (Potato Dextrose Broth). After that, formulated into a variety of formulations and treatment, sprayed on fruit by using a sprayer tudor .

Inoculation of *P. palmivora*

Inoculation of *P. palmivora* was performed 1 day after application (DAA) suspension of *T. virens*. Inoculation performed using cocoa pods infected *P. palmivora* as a source of inoculum. Taken the middle of infected fruit (the boundary between the infected and healthy part) by using Cork borer and placed on fruit that had been previously wounded with a needle.

Observation

Observations was began at 1 day after inoculation (DAI). The parameters observed were Incubation period, spotting diameter, and the incidence of disease. Incubation period is observed every day since the symptoms and the diameter of the spots was observed by measuring the patches contained in the cocoa fruit caused by *P. palmivora* infection by using paper millimeters (mm). Observation by comparing the incidence of disease lesions on infected fruit with a total wound on the same fruit.

Result and Discussion

Incubation period

Observation of the incubation period of the disease aims to determine the effectiveness of the formulation of *T. virens* strains Gl-21 from Aceh that applied on cocoa pods in delaying the symptoms of *P. palmivora*. The observation of an incubation period of *P. palmivora* as in Figure 1.

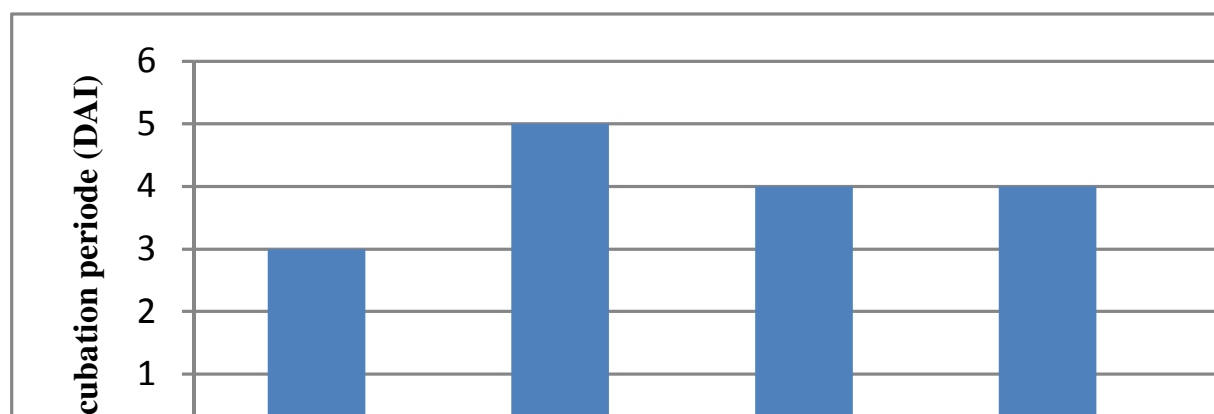


Figure 1. Incubation period of *P. palmivora* infection on cocoa pods given some formulations of *T. virens* strains Gl-21 Origin Aceh

Based on Figure 1 shown that the symptoms of *P. palmivora* on cocoa pods have been detected at 3 DAI in water treatment (W) and Palm Oil + strains of *T. virens* (Po + Tv), faster than in Fungicide treatments (Pest), Corn oil + PDB + Lecitin + *T. virens* (Co + PDB + L + TV). This result shows that on the water (W), antagonistic agents *T. virens* might be could not growth and inhibit *P. palmivora*. Therefore, pathogens are able to made infection quickly lead to symptoms of disease. According to Howell and Puckhaber (2005) Gliotoxin and antibiotics of *T. virens* reportedly produced one of the most useful as a biocontrol agent that was effective and has a very broad spectrum in controlling various types of diseases pathogen such as fungal pathogen.

This study is the first contribution of Aceh Indonesia to the development of an oil based formulation of *T. virens* conidia as a biological control tool against *P. palmivora*, the causal agent of cacao black pod disease. The objectives of this study, to develop a formulation that would increase shelf life and persistence on cacao pods of formulated conidia and to improve protection against cacao black pod disease.

Diameter spotting

Observed by measuring the diameter spots spotting any symptoms at the wound founded on the fruit. The observations of the diameter spotting symptoms of *P. palmivora* on cocoa pods shown in Figure 2.

Based on Figure 2 it was known that the treatment of corn oil formulation + strains of *T. virens* Gl-21 Origin Aceh (Co + Tv) characterized by small diameter spot compared with other formulations. In accordance with the results of Bailey et al, 2011, in the process of publication, who founded that corn oil was combined with *T. oviospolum* and sprayed on cacao plantations

in Costa Rica, reported could decrease in symptoms of fruit rot disease which was equivalent to pesticide treatment. *T. virens* can inhibit and suppress the growth of pathogenic mechanism of *P. palmivora* with various antagonists. *Trichoderma* is able to produce antimicrobial compounds, as well as mycoparasit activity and dominate the competition in the growing space that can suppress the growth of pathogenic fungi (Ekowati et al, 2006). In addition, Harman et al (2004) also declare that some *Trichoderma* species such as *T. harzianum*, *T. viride* and *T. virens* can produce xylanase enzyme that can improve plant resistance to pathogens.

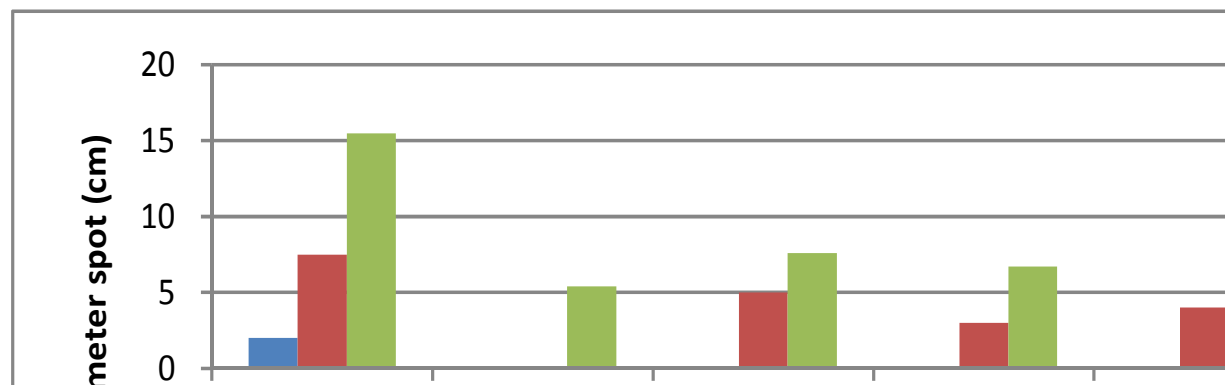


Figure 2. Diameter Spotting Symptoms of *P. palmivora* on cocoa pods given some formulations of *T. virens* strains Gl-21.

Disease incidence

Disease incidence was observed by comparing the number of wounds on cocoa fruit infected with *P. palmivora*. The observation of the disease incidence of *P. palmivora* on cocoa pods, shown in Figure 3.

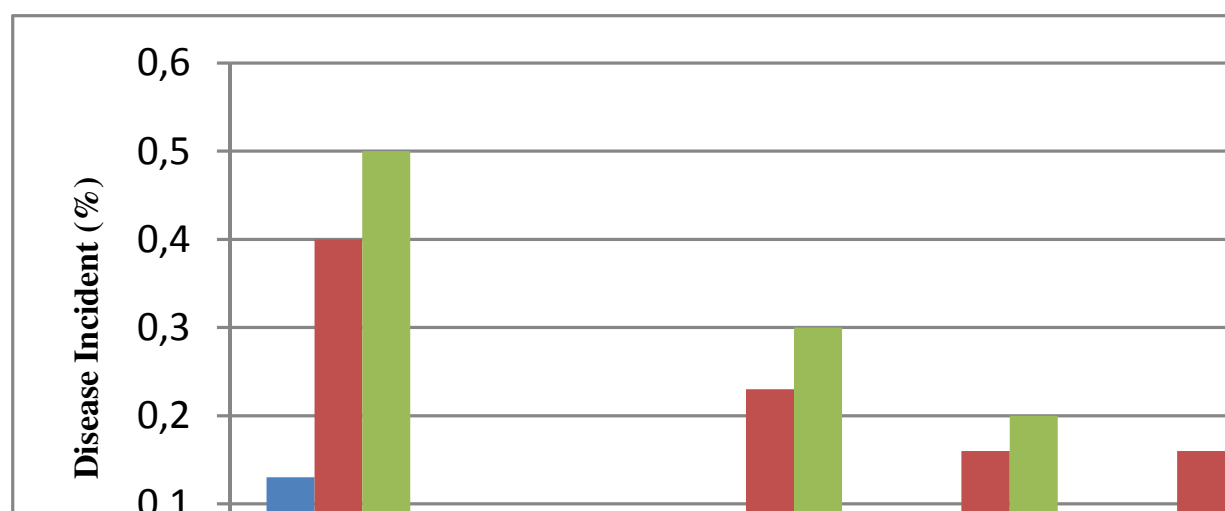


Figure 3. Disease incidence of *P. palmivora* on cocoa pods were given some formulations of *T. virens* strains Gl-21 Origin Aceh

Based on Figure 3, all treatment with *T. virens* Gl-21 origin Aceh in some formulations could reduce the incidence of *P. palmivora* disease compared with the treatment of water (W) and detergent (Det). This result suggested that the *T. virens* Gl-21 Origin of Aceh on oil formulation can inhibit the growth and development of *P. palmivora* on cocoa pods than minimize the incidence of disease. Gultom (2008), states that the more population of antagonistic fungus will cause the suppression of the growth of pathogens. In addition, Aneja et al (2006) specifically states that endophytic fungi can inhibit pathogen infection in plant tissue by two mechanisms: directly through antibiosis, competition, and mycoparasite and also indirectly by increasing plant resistance. To our knowledge, reverse to emulsions (of the water-

in-oil type) are the only type of oil-based formulations of *Trichoderma* spp. conidia known to have been used so far (Wijesinghe et al., 2011). Mbarga et al. (2014) recently reported that their *Trichoderma* conidia formulated as an oil dispersion is therefore very interesting. Especially so, since it offers one important advantage compared with reverse emulsions: it is better adapted to side level knapsack (SLK) sprayers, which are the sprayers most commonly used by small-scale farmers. The conidial formulation of *T. asperellum* as a soybean oil-based oil dispersion mixes readily with water and the conidia remain in stable suspension for a long time. Therefore more information on spraying the formulation of *Trichoderma* based on oil should be determined in the future.

Conclusions

From the research, it can be concluded:

1. Application *T. virens* strains GI-21 Origin Aceh effective in controlling *P. palmivora* on cocoa pods.
2. Best formulation in controlling *P. palmivora* on cocoa pods are formulation corn oil + *T. virens* strains GI-21 Origin Aceh (Co + Tv) compared with other formulations, characterized by the length of the incubation period, the low of disease incidence, and the small diameter of the spots on the cocoa pods.

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