Application of Various Species of *Trichoderma* spp. in Composting Cocoa Pod Husk Contaminated *Phytophthora palmivora*

L Angraeni¹, R Sriwati², Susanna²

¹ Department of Agricultural Products Technology, Faculty of Agriculture, Teuku Umar University, Alue Peunyareng Campus, Meulaboh, West Aceh Regency
² Department of Plant Protection, Agricultural Faculty, Syiah Kuala University, Banda Aceh, Aceh, Indonesia.

Corresponding author’s email address: liaangraeni@utu.ac.id

Abstract. Cocoa is one of the most important commodities in Indonesia. Cocoa pod is usually wasted and can be the source of *Phytophthora palmivora* inoculum, causes cocoa pod rot. *Trichoderma* spp. is known in suppressing the development of *P. palmivora*. The objectives of this study were to investigate the ability of various species of *Trichoderma* spp. in composting cocoa pod husk and its ability to suppress the development of *P. palmivora* contaminated on cocoa pod husk. The chopped cocoa pod husk was mixed with manure in a ratio of 2:1. A total of 2 g/kg of *Trichoderma* spp. culture was suspended in 350 ml of distilled water. This suspension was then poured into compost material and stirred until mixed. The composting material was put into black plastic bag as composting container. This study consisted of five treatments, T0 (Control), T1 (*T. harzianum*), T2 (*T. virens*), T3 (*T. asperellum*) and T4 (*T. longibrachiatum*). The composts organoleptic was tested based on compost organoleptic criteria, including color, odor and texture. The ability of *Trichoderma* spp. in colonizing cocoa pod husk compost was detected by re-isolating *Trichoderma* spp. from compost to be grown on Corn Meal Agar (CMA) media. The ability of *Trichoderma* spp. in eliminating *P. palmivora* was detected by isolating *P. palmivora* from compost to be grown on V8 PARP media. Results showed that the application of *T. harzianum*, *T. virens* dan *T. asperellum* in composting cocoa pod husk was able to produce compost with higher organoleptic quality compare to Control and *T. longibrachiatum*. The isolation of *P. palmivora* from compost did not show the presence of *P. palmivora* survived in compost after the composting process was complete. However, re-isolation of *Trichoderma* spp. from compost also showed that *Trichoderma* spp. applied was unable to survive in the compost materials.

Keywords: Cocoa pod husk compost, *Trichoderma* spp., *Phytophthora palmivora*

1. Introduction

Cacao crop in Indonesia was estimated about 220,000 tonnes from 4,849,000 tonnes worldwide at 2017 [1]. However, annual production suffers from severe losses due to *Phytophthora* disease or usually known as black pod rot, an important fungal disease of cocoa that causes very serious losses [2]. Typical pod lesions begin as small, hard, brownish or black water-soaked spot on any part of the pod, rapidly enlarges and coverages the entire pod surface and internal tissue at any stage of pod development [3,4].
Large amount of cocoa pod husks scattered and left in the plantation after harvest can also harbour and pre-dispose the spread of *Phytophthora palmivora* inoculum [5]. The rain splash from sporulating pods also played the main role of pod infection [6]. Cultural methods such as the phytosanitary eliminating the diseased pods, have proven relatively efficient in reducing the secondary inoculum [7]. However, frequent removal of the black pods is not practiced, labour intensive and expensive [8].

Biological control has attracted greater attention as alternative to the use of chemical pesticides. Mycoparasitic fungi of the genus *Trichoderma* have been used for biocontrol of pathogens. *Trichoderma* species are free-living invasive fungi, work against fungal phytopathogens by more than one mechanism, either indirectly by competing for nutrients and space, modifying environmental conditions or promoting plant growth, plant defensive mechanisms and antibiosis; or directly through mechanisms such as mycoparasitism [9]. Studies on the potential of *Trichoderma*-based biological control against *P. palmivora* indicate promise for this approach [10].

Furthermore, it is necessary to develop additional and sustainable strategies to manage the great quantities of cocoa pod waste as well as to control the pathogen survived on it. One of the cocoa pod waste utilization has been attempted by producing fertilizer or compost [11, 12]. Composting is a thermophilic, aerobic self-heating decomposition process converting organic matter into relatively stable humus-like substrates with the principal requirement is to have a very low concentration in pathogen [13]. Composts can vary according to the raw material used and according to the nature of the composting process. The aim of the study was to investigate the ability of various species of *Trichoderma* spp. in composting cocoa pod husk and its ability to suppress the development of *P. palmivora* contaminated on cocoa pod husk.

2. Methods

2.1. Study Location
The study was carried out at Plant Pathology Laboratory, Agricultural Faculty, Syiah Kuala University. Various *Trichoderma* species were also obtained from Plant Pathology Laboratory culture collection.

2.2. Treatments Arrangement
Research was done in 5 treatments.
T0: Without *Trichoderma* (Control)
T1: *Trichoderma harzianum*
T2: *Trichoderma virens*
T3: *Trichoderma asperellum*
T4: *Trichoderma longibrachiatum*

2.3. *Trichoderma* spp. multiplication
The crushed corn was soaked in water for 1 day, then drained and put into heat-resistant plastic as much as 1 kg each. Corn media was then sterilized in autoclave at 121°C for 30 minutes. *Trichoderma* spp. conidia from PDA was inoculated on corn media and incubated for 2 weeks.

2.4. Composting process
The finely chopped cocoa pod was mixed with manure in a ratio of 2: 1 with 3 kgs of total material. The matured cow manure (characterized by temperatures that have been cold, odorless and dry) was used in this study. Materials were then put into black plastic as a composting container.

2.5. *Trichoderma* spp inoculation into compost
A total of 2 g / kg of *Trichoderma* spp. culture was suspended in 350 ml of distilled water. This suspension was then splashed on the compost raw material while stirred until mixed. The compost raw material was then wrapped in black plastic so that the composting process could run properly.
2.6. **Organoleptic test of compost**

Organoleptic test for compost was carried out using compost organoleptic criteria on a numerical scale including color, odor and texture adopted from Asngad and Suparti [14] (Table 1).

| Color       | Odor                        | Texture       |
|-------------|-----------------------------|---------------|
| 1 = Extremely black | 1 = Strongly smelled of weathered litter | 1 = Very smooth |
| 2 = Black    | 2 = Smelled of weathered litter | 2 = Smooth    |
| 3 = Blackish | 3 = Mildly smelled of weathered litter | 3 = Rather smooth |
| 4 = Brown    | 4 = Odorless                | 4 = Rough     |
| 5 = Brownish | 5 = Decayed                 | 5 = Rather rough |

**Table 1. Compost organoleptic numerical scale**

2.7. **Detection of Trichoderma spp in compost**

The ability of *Trichoderma* spp. in colonizing cocoa pod husk compost could be known after the re-isolation of the compost to be grown on Corn Meal Agar (CMA). One gram of compost sample was diluted in 9 ml distilled water. Then 1 ml of suspension from $10^{-3}$ dilution was taken and sprayed on Corn Meal Agar (CMA) media. The presence of *Trichoderma* spp. survived in compost could be detected by observing microorganisms that grow on CMA media using microscope.

2.8. **Detection of Phytophthora palmivora in compost**

The ability of *Trichoderma* spp. in eliminating *P. palmivora* could be detected by the presence or absence of *P. palmivora* in the compost. Isolation of *P. palmivora* was carried out on V8 PARP. The isolation technique was the same as the isolation technique of *Trichoderma* spp. on CMA media.

2.9. **Statistical analysis**

Research used Completely Randomized Design with 5 treatments and 4 replications. Data for compost’s organoleptic were subjected to analysis of variance (ANOVA). When a significant ($P \leq 0.05$) F test was obtained for treatments, separation of means was accomplished by alphabets.

3. **Results and Discussion**

3.1. **Organoleptic test of compost**

The success of composting was known from compost quality organoleptically tested. The compost quality is included color, odor and texture. Result showed that the application of various *Trichoderma* spp. in composting had a very significant effect on the organoleptic of compost (Table 2).

| Treatment | Color   | Odor             | Texture |
|-----------|---------|------------------|---------|
| T0        | 3.88 a  | 3.45 a           | 4.18 a  |
| T1        | 2.50 b  | 2.53 b           | 2.43 b  |
| T2        | 2.70 b  | 2.50 b           | 2.38 b  |
| T3        | 2.48 b  | 2.48 b           | 2.43 b  |
| T4        | 4.18 a  | 3.03 a           | 4.00 a  |

*Note: Means with the same letter in the same column are not significantly different according to LSD at $P < 0.05%$*

Organoleptic tests of compost showed that the application of *T. harzianum, T. virens* and *T. asperellum* (T1, T2 and T3) were able to decompose the cocoa pod husk quite well compared to Control and *T. longibrachiatum* (T0 and T4) (Table 2). The mature compost was characterized by changes in color, odor and texture of compost in accordance with the criteria for mature compost. Visually, the application of *T. harzianum, T. virens* and *T. asperellum* produced a slightly blackish-colored compost, smelled like weathered litter, crumb-textured and the aggregates had fused like soil.
(indistinguishable from its raw materials). In contrast, compost in Control and *T. longibrachiatum* had light brown, mildly smelled and rough texture, the organic fraction was still clearly visible (Figure 1).

![Figure 1. The visual appearance of cocoa pod husk compost decomposed by various species of *Trichoderma*](image)

*T. harzianum*, *T. virens* and *T. asperellum* showed the better decomposition process compared to Control and *T. longibrachiatum*. This condition was probably caused by the ability of *T. harzianum*, *T. virens* and *T. asperellum* to produce the type of cellulase compounds that matched to decompose the cellulose in cocoa pod husk so that they could be degraded quickly. Several microorganisms have ability to produce cellulose degrading enzyme derivatives in nature extensively, such as *Trichoderma* spp., *Penicillium* spp., and *Aspergillus* spp. [15]. *Trichoderma* spp. have been widely used as bio-decomposers [16,17] and organic matter builder, along with its abilities in produced polysaccharide degrading enzymes [18]. The addition of *Trichoderma* species was found to be viable in improving the composting efficiency of the compost [19].

Apparently, the physical qualities of compost are closely related to each other. Changes in the three physical characteristics of compost occur simultaneously. As the compost turns blackish brown, it also becomes odorless and the texture changes like soil. This process occurs when the organic material has been completely degraded. Changes in compost color into darkens and less offensive odor can be observed as degree of compost maturity. In another hand, stability is often determined using indices of microbial activity [20,21].

3.2. **Detection of Trichoderma in compost**

*Trichoderma* spp. which was inoculated on compost would show its ability to survive, be able to multiply or even die in the compost. The presence or absence of microorganisms that have characteristics similar to *Trichoderma* from compost re-isolation on Corn Meal Agar is presented in Table 3.

| Treatment | Replication I | Replication II | Replication III | Replication IV | Percentage |
|-----------|---------------|----------------|-----------------|---------------|------------|
| T0        | -             | -              | -               | -             | 0 %        |
| T1        | -             | -              | -               | -             | 0 %        |
| T2        | -             | -              | -               | -             | 0 %        |
| T3        | +             | -              | -               | -             | 25 %       |
| T4        | -             | -              | -               | -             | 0 %        |

Table 3. Detection of *Trichoderma* spp. in the compost on *Corn Meal Agar*
Result showed that *Trichoderma* applied to the compost material was unable to survive in the compost. When inoculated, *Trichoderma* was capable to develop and decompose the compost which is characterized by changes in organoleptic quality including color, odor and texture. But time after time, *Trichoderma* population was difficult to survive and multiply its colonies in compost. This was probably due to the fact that compost contained various types of microorganisms that had adapted and colonized the compost, both on the cocoa pod husk and on the manure that caused *Trichoderma* population in compost depressed.

Microscopic observation showed that at T3 found *T. asperellum* colony which was covered by bacteria (Figure 2). Bacteria appeared to dominate the growth space so *T. asperellum* could not develop. Microbial colonies grown from the re-isolation of compost on Corn Meal Agar were mostly bacteria. It was suspected that this bacterium was carried in the basic material of compost. This condition made it difficult to prove the survival ability of *Trichoderma* in the compost. In addition, the dose of *Trichoderma* given might be too low so *Trichoderma* was unable to survive in compost properly. The application of antagonist agent should be in adequate amount to provide the initial growth of the antagonist agent to survived in the substrate.

*Trichoderma harzianum* and *T. asperellum* were the most effective antagonists against *Fusarium solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* by produced and secreted b-1,3-glucanase, NAGase, chitinase, acid phosphatase, acid proteases and alginate lyase to degraded the cell wall of the pathogen [22]. *Trichoderma* spp. are also known to produce a number of antibiotics such as trichodernin, trichodermol and herzianolide [23].

### 3.3. Detection of Phytophthora in compost

The isolation of *Phytophthora palmivora* from compost was carried out to determine the ability and effectiveness of various *Trichoderma* species in suppressing *P. palmivora* contaminated on the cocoa. Fungal colonies obtained from compost isolation on V8 were grouped based on the similarity of its colony forms. From the whole treatment, 10 different forms of fungal colonies were obtained (Table 4). The entire fungal colonies were observed under the microscope. It aimed to observe the morphology of fungi that was similar to *P. palmivora*.

**Table 4. Species of fungi detected in compost after isolation on V8**

| Treatment | Species of fungi |
|-----------|------------------|
|           | A | B | C | D | E | F | G | H | I | J |
| T0        | + | + | + | + |   |   |   |   |   | + |

![Figure 2. Microorganism of compost re-isolation grown on Corn Meal Agar](image-url)
Result showed that *P. palmivora* could not be detected in the mature compost isolated in V8. Microscopic observation did not show the morphology of microorganisms that similar to *P. palmivora* (Figure 3). Several factors might be responsible for the suppressiveness of *P. palmivora* in the compost. It might be due to high temperatures during composting that disinfect the pathogen on plant debris. Another factor might be due to the high diversity of microflora including *Trichoderma* spp. present in the compost that could inhibit the growth of *P. palmivora*. The physical and chemical factors of composts often alter microflora structures, which can reduce the populations of plant pathogens [24]. Muryati et al. [25] reported that the addition of manure can suppress *P. palmivora* propagules in the soil. Manure as organic matter may increase the level of antagonistic microorganism, followed by the increasing of microflora competition, that tend to limit the growth of *Phytophthora* spp.

These increased microbial activities may contribute to the suppressive activity of compost-amended soil. The main mechanisms for compost microorganism to suppress *P. megakarya* are microbiostasis/ fungistasis, parasitism, predation, and an antagonism induced by specific, or non-specific microbial metabolites such as antibiotics and cell wall-degrading enzymes [26]. Studies reported that antagonistic fungus inhibited pathogens by producing antibiotics, such as lytic enzymes, volatile and non-volatile compounds and other toxic substances [27]. Both hyperparasitism and antibiosis results in a measurable decline of pathogen’s propagules. In another hand, although less visually dramatic, competition for organic compounds, for colonization sites and for nutrients, are probably the most common suppressiveness mechanisms [28].

The use of organic matter inputs like cocoa pod husk-based compost found can not only improve soil fertility, but also disinfects reservoirs of *P. megakarya* on CPH discards and survival site; and reduce the disease severity by direct effects on inoculums level in the soil [11]. Similarly, other studies also reported the effectiveness of compost in controlling plant disease cause by *P. nicotianae* [29] and *P. capsici* [30].

From the overall fungal morphology observed microscopically, there were two types of fungi that exhibit morphological characteristics similar to *Aspergillus* sp. and *Fusarium* spp. It could be seen from *Aspergillus* sp. that had branched hyphae with spores at the ends, while *Fusarium* spp. recognizable by its crescent moon shape conidia. *Aspergillus* sp. and *Fusarium* spp. was the species...
of fungus that mostly found in the compost isolation on V8. On the other hand, the other fungi observed under a microscope were unknown species. Aspergillus sp. and Fusarium spp. were probably carried in cow manure mixed with cocoa pod husk. Aryantha & Guest [31] had isolated microorganism in manure, and the result showed that the inhibitory microbes contained in manure was Trichoderma sp. Gliocladium penicillioides, Fusarium sp. Streptomyces sp. Pseudomonas sp. dan Bacillus sp.

4. Conclusion
T. harzianum, T. virens and T. asperellum (T1, T2 and T3) were able to decompose the cocoa pod husk quite well compared to Control and T. longibrachiatum (T0 and T4) (Table 2), characterized by blackish-colored compost, smelled like weathered litter and crumb-textured in accordance with the criteria for mature compost. However, Trichoderma was unable to survive in the compost because of the application dose was low and compost contained various types of microorganisms that had adapted and colonized the compost, that caused Trichoderma population in compost depressed. P. palmivora could not be detected in the mature compost. The high diversity of microflora including Trichoderma spp. present in the compost could inhibit the growth of P. palmivora.

Acknowledgements
The author would like to thank Plant Pathology Laboratory, Agriculture Faculty, Syiah Kuala University for providing Trichoderma spp. culture and Ms Yusmaini for the laboratory assistant.

References
[1] ICCO 2019 Production of cocoa beans ICCO Quarterly Bulletin of Cocoa Statistics 45(3)
[2] Deberdt P, Mfegue CV, Tondje PR, Bon MC, Ducamp M, Hurard C, Begoude BAD, Ndoumbe-Nkeng M, Hebbar K and Cilas C 2008 Impact of environmental factors, chemical fungicide and biological control on cacao pod production dynamics and black pod disease (Phytophthora megakarya) in Cameroon Biological Control 44 149-159
[3] Adegbola MOK 1981 Cocoa diseases of West Africa Proceedings of the 7th International Cocoa Research Conference, Cameroon pp 243–250
[4] Guest D 2007 Black pod: diverse pathogens with a global impact on cocoa yield Phytopathology 97 1650–1653
[5] Yen JD, Waters EK and Hamilton AJ 2010 Cocoa pod borer (Conopomorpha cramerella Snellen) in Papua New Guinea: biosecurity models for New Ireland and the autonomous region of Bougainville Risk Anal 30(2) 293–309
[6] Maddison AC and Ward MR 1981 The international black pod project: 1979 review Proceedings of the 7th International Cocoa Research Conference Cameroon pp 261–266
[7] Ndoumbe-Nkeng M, Cilas C, Nyemb E, Nyasse S, Biyeysse D, Flori A and Sache I 2004 Impact of removing diseased pods on cocoa black pod caused by Phytophthora megakarya and on cocoa production in Cameroon. Crop Prot. 23 415-424
[8] Hanada RE, Pomella AWV, Soberanis W, Loguercio LL and Pereira JO 2009 Biocontrol potential of Trichoderma martiale against the black-pod disease (Phytophthora palmivora) of cacao. Biol Control 50 143–149
[9] Reino JL, Guerrero RF, Hermández-Galáns R and Coolado IG 2008 Secondary metabolites from species of the biocontrol agent Trichoderma Phytochem Rev 7 89–123
[10] Adebola MO and Amadi JE 2012 Studies on Penicillium digitatum, Botryodiplodia theobromae, Alternaria tenuis and Trichoderma harzianum for bicontrol of Phytophthora palmivora cocoa black pod disease pathogen American-Eurasian Journal of Agronomy 5(2) 30-34
[11] Doungous O, Minyaka E, Longue EAM and Ndengafac NJ 2018 Potentials of cocoa pod husk-based compost on Phytophthora pod rot disease suppression, soil fertility, and Theobroma cacao L. growth Environmental Science and Pollution Research
[12] Fidelis C and Rao BKR 2017 Enriched cocoa pod composts and their fertilizing effects on hybrid cocoa seedlings Int J Recycl Org Waste Agricult
[13] de Bertoldi M, Vallini G and Pera A. 1983 The biology of composting: a review Waste Management & Research 1 157-176
[14] Asngad A and Suparti 2005 Development model for making organic fertilizer with inoculation (a case study on rubbish at TPA Mojosongo Surakarta). Jurnal Penelitian Sains & Teknologi 6(2) 101-113
[15] Gautam SP, Bundela PS, Pandey AK, Jamaludin, Awasthi MK and Sarsaiya S 2012 Diversity of cellulolytic microbes and the biodegradation of municipal solid waste by a potential strain. International Journal of Microbiology
[16] Bari MA, Begum MF and Sarker KK 2007 Mode of action of Trichoderma spp. on organic solid waste for bioconversion. Plant Environmental Development 1: 61-66
[17] Rahman A, Begum MF, Rahman M, Bari MA, Illias GNM and Alam MF 2011 Isolation and identification of Trichoderma species from different habitats and their use for bioconversion of solid waste Turkey Journal of Biology 35: 183-194
[18] Kullnig-Gradinger CM, Szakacs G and Kubicek CP 2002 Phylogeny and evolution of the genus Trichoderma: a multiple approach. Mycological Research 106: 757-767
[19] Lopez LLM, Reyes RG and Alvindia G 2015 Evaluation of two species of Trichoderma as compost activator and bio-control agents Journal of Agricultural Technology 11(2) 525-537
[20] Wu L, Ma LQ and Martinez GA 2000 Comparison of methods for evaluating stability and maturity of biosolids compost. Journal of Environmental Quality 29(2) 424–429
[21] Ge B, McCartney D and Zeb J 2006 Compost environmental protection standards in Canada. Journal of Environmental Engineering and Science 5(3) 221–234
[22] Qualhato TF, Lopes FAC, Steindorff AS, Branda’o RS, Jesuino RSA and Ulhoa CJ. 2013 Mycoparasitism studies of Trichoderma species against three phytopathogenic fungi: evaluation of antagonism and hydrolytic enzyme production Biotechnol Lett
[23] Kucuk C and Kivanc M 2004 In Vitro Antifungal Activity of Strains of Trichoderma harzianum Turk J Biol 28 111-115
[24] Atiyeh RM, Edwards CA, Subler S and Metzger JD 2001 Pig manure vermicompost as a component of a horticultural bedding plant medium: effects on physicochemical properties and plant growth Bioresource Technol. 78 11-20
[25] Muryati, Octriana L, Emilda D, Santoso PJ and Sunarwati D 2009 Effect of organic fertilizers on Susceptibility of Potted Durian Seedlings to Phytophthora diseases Journal of Fruit and Ornamental Plant Research 17(1) 67-77
[26] Garbeva P, van Veen JA and van Elsas JD 2004 Microbial populations by plant and soil type and implications for disease suppressiveness Annual Review of Phytopathology 42 243-270
[27] Reddy BN, Saritha KV and Hindumathi A 2014 In vitro Screening for Antagonistic Potential of Seven Species of Trichoderma against Different Plant Pathogenic Fungi Research Journal of Biology 2 29-36
[28] Raviv M 2008 The use of compost in growing media as suppressive agent against soil-borne diseases Acta Hort. 779
[29] Ntougias s, Papadopoulou KK, Zervakis GI, Kavroulakis N and Ehaliotis C 2008 Suppression of soil-borne pathogens of tomato by composts derived from agro-industrial wastes abundant in Mediterranean regions Biol Fertil Soils 441081–1090
[30] Sang MK, Kim JG and Kim KD 2010 Biocontrol activity and induction of systemic resistance in pepper by compost water extracts against Phytophthora capsica Phytopathology 100 774-783
[31] Aryantha INP and Guest DI 2006 Mycoparasitic and antagonistic inhibition on Phytophthora cinnamomii rands by microbial agents isolated from manure compost Plant Pathology Journal 5(3) 291-298