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Full Length Article

Composted Plant Residues Improve Control Capability of *Trichoderma asperellum* against Vascular Streak Dieback Disease on Cacao

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Abstract

Plant residues and microorganisms determine the ability of compost to control soil-borne pathogens. In this study, we evaluated the compatibility of plant residues and *Trichoderma asperellum* and applied the compost of these plant residues in combination with the fungus through soil amendment to control above ground disease of vascular streak dieback on cacao. The sterilized medium containing combination of gliricidia, billy goat and rice straw and unsterilized medium of these composted plant residues supported the growth *T. asperellum*. A reduced VSD disease incidence 11.0, 24.9 and 37.5% was observed 13 weeks after treatment by composted plant residues, *T. asperellum*, and combination of *T. asperellum* and composted plant residues, respectively compared to reduction of 4.0% in positive control. In addition, we observed that combination of composted plant residue and *T. asperellum* can stabilize content of nitrogen, phosphorus, and potassium almost similar to those in negative control. This data showed the compatibility of medium containing plant residues to support *T. asperellum* growth and its application through soil amendment can improve the control capability against VSD disease.

Keywords: Above ground disease; Gliricidia; Billy goat; Rice straw; Compost; Soil amendment

Introduction

Vascular streak dieback (VSD) caused by the basidiomycetouse fungus, *Ceratobasidium theobromae* Talbot and Keane (Guest and Keane, 2007; Samuels *et al*., 2012) is an important disease in Indonesia. Basidiospores of this fungus released and dispersed by the wind during high humidity periods in rainy season infect young leaves and penetrate to colonize xylem vessels, then move into petiole and branch. This infection causes the death of shoot apex, branches, and in susceptible clone can kill a mature cacao tree (Samuels *et al*., 2012). This disease is found in 1983 and today has been distributed to all over the country (Rosmana *et al*., 2016). So far, the known methods of disease control are pruning, use of healthy plant material, and propagation of resistant cacao clones.

A new approach of control by using endophytic *Trichoderma* have been developed and this fungus shows high efficacy in controlling VSD disease on cacao seedling in the green house and on cacao side graft in the field (Rosmana *et al*., 2015, 2016). In our recent study, we investigated that *Trichoderma* is able to spread systemically in the plant after application through foliar spraying, stem infusion, and soil drenching (Rosmana *et al*., 2018). However, the last method offers the best deployment of the fungus into root, stem, and leaf tissues. In this regard, it is interesting to combine *Trichoderma* and composted plant residues for permitting multiple actions in disease control. Application of compost through soil amendment, in addition, can improve soil fertility and quality of production, also control both soil and air-borne pathogens by direct and nondirect mode of actions (Stone, 2003; Vallad *et al*., 2003; Celano *et al*., 2012; Pane *et al*., 2013). Plant residue-based composts such as paper mill residual, tomato, escarole, artichoke, pine bark, banana leaves, olive mill waste, sawdust, rice straw, and empty fruit bunchof oil palmhave been used for this purpose (Vallad *et al*., 2003; Pane *et al*., 2013; St. Martin, 2014). For soil-borne pathogens, these composts have been extensively proposed to control *Rhizoctonia*, *Sclerotinia*, *Pythium*, *Verticillium*, *Fusarium*, *Phytophthora*, and *Thielaviopsis* and this control is considered as a direct mode of action relating to microbiological and chemical characteristics of compost (Bonanomi *et al*., 2007; Pane *et al*., 2013). Resident microbial community dominated by bacteria has a role important in inhibition of pathogens, while chemically as a role of substances such as phenolic C and methoxyl C (Hadar, 2011; Zaccardelli *et al*., 2011; Pane *et al*., 2013).

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Such inhibition of fungi mentioned above would also inhibit introduced *Trichoderma* used for biocontrol, it is thus important to understand the effect of plant residues against *Trichoderma* before mixing between the two for the application.

The research describes a step toward understanding the compatibility of plant extract consisting gliricidia leaf, billy goat leaf, and rice straw with *Trichoderma asperellum* through assessing its ability to growth in sterilized and composted medium of these plant residues, as well the control and the nutrient supply ability through soil application of composted plant residues and *T. asperellum* combination in cacao seedlings infected by VSD disease. Therefore, the objective of the present research was to increase the supply of *T. asperellum* into cacao seedling by functioning the composted plant residues as culture medium for multiplication of the fungus and as source of plant nutrition in order to develop effective and efficient methods with broader implications for remediation of this disease in cacao field.

Materials and Methods

Preparation of Culture Medium with Plant Residues

Plant residues chosen in this work were gliricidia leaves, billy goatleaves and stems, and rice straws, these were selected according to conformity level when these plant residues made for solid medium and their activity level against microorganisms tested previously (Limbongan, 2013; I-Ketut *et al.*, 2015). For preparing of solid medium, 200 g of single plant residue, 100 g respectively of the mixture in the two, and 6.7 g respectively of the mixture in the three, added with 20 g of dextrose and 15 g of agar (Bacto agar) in one liter of distilled water. Each Petri dish of 9 cm diameter was filled with 20 mL of this medium after sterilization at 121ºC for 30 min. PDA medium was made from potato, dextrose, and agar with the same weight respectively as in preparation of above medium, for comparison.

In addition to sterilized medium, the unsterilized medium was also prepared. This medium contains the composted mixture of gliricidia, Billy goat, and rice straw in the weight ratio of 1:1:1. The composting process was done in period of one month where raw materials previously chipped with aeration by reversing these materials every 2 to 3 days, followed by a one month-curing period.

Assessment of Plant Residues Compatibility to Support *Trichoderma* Growth

Trichoderma asperellum grown on solid medium containing plant residue was measured for its colony diameter and mycelia fresh weight. The diameter of the colony was measured until five days after inoculation. The mycelia of seven days old from the solid medium were harvested by floodding of dishes with 10 mL of distilled and then mycelia and spores were scraped into sterile Erlenmeyer flasks. The contents of flask were shaken and filtered through sterile muslin cloth to separate the mycelia and spore suspensions. The suspensions were sampled to calculate the number of spores, while mycelia were weighed as fresh weight after being incubated for about 30 min on filter paper.

To evaluate that *T. asperellum* can grow and develop in unsterilized medium, 100 g of composted plant residues was filled into the transparent plastic bag and inoculated with 10^6 spores/mL of this fungus. This plastic bag was punctured using needle for permitting to get an aeration and the fungus population was observed four weeks-post inoculation.

Trial of Composted Plant Residues and *T. asperellum* on VSD Disease

Ceratobasidium theobromae, the causal agent of vascular streak dieback (VSD) diseases was isolated from cacao branches naturally infected in the field. The fragments of branches were surface sterilized with 2% sodium hypochlorites for three minutes and vigorously washed several times in sterile distilled water. The bark was removed from the surface sterilized segments which were then cut into 1 cm sections and placed onto water agar (WA) in Petri dishes. *C. theobromae* was characterized as cottony, nonsporulating white mycelium that grows slowly from the end of the sections.

For inoculation into the plant, young mycelium explant of around 3 mm in diameter taken from the end of the colony was pasted on the surface of young cacao leaf and fixed with transparent tape to avoid falling due to leaf friction or wind. Three leaves were inoculated for each seedling. After the VSD disease reaches maximum infestation as a result of primary infection by fungus and continued by secondary infection to other leaves, the treatment was done. This treatment consists of composted plant residue, *T. asperellum* 2.0 g/kg growth medium, mixture of composted residue and *T. asperellum* 2.0 g/kg growth medium. Each treatment has five seedlings and therefore, the total including negative and positive control was 25 seedlings. Growth medium of seedling consists of 0.5 kg of composted plant residues and 1.0 kg of soil and *T. asperellum* in form of powder (Rosmana *et al*., 2016) suspended in 250 mL distilled water was applied through drenching.

Disease incidence was observed every two weeks over 13 weeks and calculated using the formula of $I = A/B \times 100$, where A is the number of leaves showing VSD symptoms and B is the number of all leaves observed. While, remediation or reduction of disease was calculated according to the formula $R = (Ii - It)/Ii \times 100$

where Ii is initial incidence of VSD and It is incidence of VSD at 3, 5, 7, 9, 11, and 13 weeks post-treatment.

Assessment of Nutrition Sufficiency in Cacao Seedling

To detect nutrition sufficiency as the impact of VSD pathogen infection and plant residues and *T. asperellum* application, leaves were sampled. Third and fourth young leaves were selected for measurement of nitrogen (N), phosphorus (P), and potassium (K) content. The total N content was analyzed after dry sample digestion in concentrated sulfuric acid, distillation with 10 mol L^{-1} NaOH and 2% boric acid and titration with 0.1 mol L^{-1} HCl, following the Kjeldahl method (Bremner, 1996). The total P content was determined colorimetrically using a UV/VIS-spectrophotometer, according to the NaOBr method (Dick and Tabatabai, 1977). While the total content of K was measured after this powder was digested with 65% of HNO_3 and 95% of H_2SO_4 and then furthermore analyzed by Atomic Absorption Spectrophotometer (AAS).

Analysis

Diameter colony and fresh weight of *Trichoderma asperellum*, VSD incidence, N, P, and K content in leaves were analyzed without any data transformation. The least significant difference was then used for evaluating significant differences between the treatment means.

Results

Compatibility of Plant Residues for *T. asperellum* Growth in Vitro

By comparing to PDA medium, colony diameter of *T. asperellum* growing in medium with single and combination of plant residue was reduced significantly in three days post-inoculation, while in five days post-inoculation, the significant reduction was just observed in medium containing the single billy goat and gliricidia (Fig. 1). Fresh weight of mycelium derived from *T. asprellum* growing in medium containing the single gliricidia and the combination of billy goat, gliricidia, and rice straw was significantly higher than that growing in PDA and medium containing other plant residue in single and in two combinations. While for the number of spores, *T. asperellum* growing in medium containing billy goat showed the highest production with 64.2 x 10^{11} spores/culture and this was significantly different with in PDA and in combination of billy goat, gliricidia and rice straw producing respectively 43.0 x 10^{11} spores/culture and 23.6 x 10^{11} spores/culture (Table 1).

Compatibility of Composted Plant Residues for *T. asperellum* Growth

Trichoderma asperellum was reisolated from inoculated compost containing the mixture of gliricidia, billy goat, and rice straw. In the uninoculated compost, *Trichoderma* was either not detected or occurred at a level that was far below that observed in the inoculated seedlings. Therefore, we are confident that the *Trichoderma* that we isolated is the one that was inoculated. Sampling of compost after four weeks post-inoculation showed that *T. asperellum* was found to reach 2.67 x 10^5cfu/g compost.

Effect of Composted Plant Residues and *T. asperellum* on Decrease of VSD Disease Incidence

Trials were conducted in the laboratory for around 6 months to estimate the potential for remediation of cacao infested by VSD with *T. asperellum,* composted residues, and its combination. In plants inoculated with pathogen without any treatment, VSD symptoms were relatively stable over 13 weeks post-treatment and the disease incidence was just decreased 4.0% at this end time of observation. On seedling treated, in the same time, with composted plant residues, *T. asperellum*, and the combination of *T. asperellum* and composted plant residues, a reduced disease incidence 11.04, 24.91 and 37.52%, respectively were observed (Fig. 2). Therefore, the highest decrease of disease incidence was found on seedling treated by the combination of *T. asperellum* and composted plant residues and this was significantly different with all treatments and control.

Effect of Composted Plant Residues and *T. asperellum* on Nutrition Supply into Plant

The nutrition content including nitrogen (N), phosphorus (P) and potassium (K) was measured from third fourth leaves of the seedling to evaluate if both *T. asperellum* and composted plant residues have a role in nutrition supply into the plant in process of VSD remediation. By comparing to negative control, VSD pathogen infection caused an increase in N content and a decrease in P content. Treatment of the plant infected with composted plant residues decreased the N content at a slight level under that in the negative control, while treatment with *T. asperellum* alone increased the N content at a level above that in the plant infected. With these two treatments, the P content was not reincreased. However, treatment by the combination of *T. asperellum* and composted plant residues decreased N content at a slight level under that in the plant infected and above that in negative control and reincreased the P content at a level approaching that in the negative control. The content of K was very low and not influenced both by infection of VSD pathogen and the treatments (Fig. 3).

Discussion

Using of plant residues as the source of nutrition is an attractive and economically important in multiplication of the beneficial microorganisms both for purpose of laboratory and field needs. Billy goat, gliricidia, and rice straw applied alone or in combination for medium preparation were well solidified when mixed with agar and without any addition of chemical substances.

Table 1: Fresh weight and spore number of *Trichoderma asperellum* after seven days growing in medium containing billy goat, gliricidia, rice straw, billy goat and gliricidia, billy goat and rice straw, gliricidia and rice straw, billy goat, gliricidia, and rice straw and potato dextrose agar

Medium	Fresh weight (g/culture)	Number of spores/culture
Billy goat	0.11 ± 0.025 a	$64.2 \pm 4.65 \times 10^{11}$e
Gliricidia	0.62± 0.290 b	$32.0 \pm 4.13 \times 10^{11}$c
Rice straw	0.07± 0.020 a	$10.7 \pm 0.61 \times 10^{11}$a
Billy goat + Gliricidia	0.16± 0.030 a	$38.0 \pm 1.60 \times 10^{11}$cd
Billy goat + Rice straw	0.08± 0.015 a	$22.0 \pm 1.57 \times 10^{11}$b
Gliricidia + Rice straw	0.06 ± 0.025 a	$24.9 \pm 8.83 \times 10^{11}$b
Billy goat + Gliricidia + Rice straw	0.52 ± 0.015 b	$23.6 \pm 1.90 \times 10^{11}$b
PDA	0.10 ± 0.025 a	$42.9 \pm 2.16 \times 10^{11}$d

Means in the same column followed by same letter are not significantly different according to LSD ($P \le 0.05$)

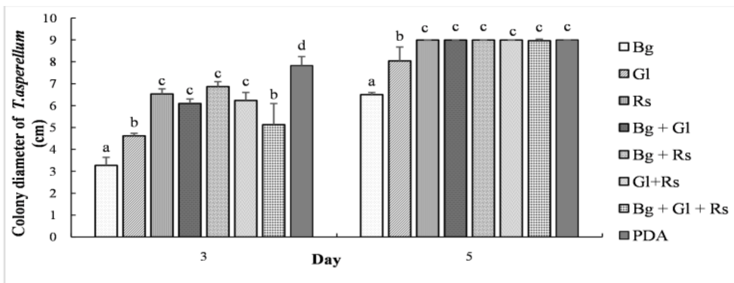

Fig. 1: Colony diameter of *Trichoderma asperellum* grown in medium containing billy goat (Bg), gliricidia (Gl), rice straw (Rs), billy goat and gliricidia (Bg+Gl), billy goat and rice straw (Bg + Rs), gliricidia and rice straw (Gl+Rs), billy goat, gliricidia, and rice straw (Bg+Gl+Rs) and potato dextrose agar (PDA) three and five days after inoculation. Means of colony diameter in the same time followed by same letter are not significantly different according to LSD ($P \le 0.05$)

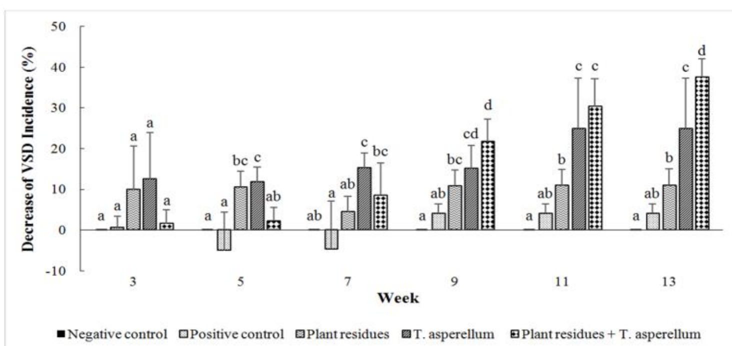

Fig. 2: Decrease percentage of vascular streak dieback incidence on cacao seedling 3, 5, 7, 9, 11, and 13 weeks after treatment with composted plant residue, *Trichoderma asperellum*, and its combination. Means of incidence in the same time followed by same letter are not significantly different according to LSD ($P \le 0.05$)

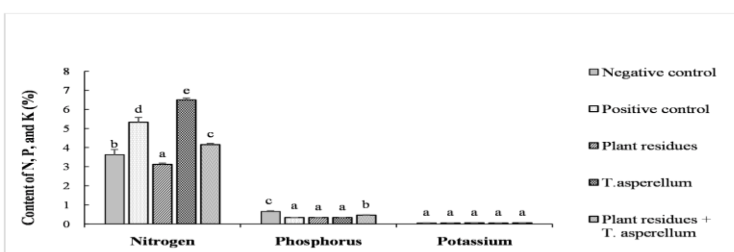

Fig. 3: Nitrogen, phosphorus, and potassium content in cacao seedling leaves 13 weeks after treatment with composted plant residues, *Trichoderma asperellum*, and its combination.Means of N, P, and K content followed by same letter are not significantly different according to LSD ($P \le 0.05$)

By comparing to potato medium, the medium containing billy goat and gliricidia alone supported more respectively spore and mycelia production, but this single content was not supported a diversity of nutrition source. When we combined between two plant residues in medium, it was not produced spore or mycelia beyond that produced by PDA medium. While, medium containing the combination of billy goat, gliricidia, and rice straw was considered reliable to support growth of *T. asperellum* because this medium produced highest mycelium weight among the medium tested and diameter colony almost equivalent to the growth in PDA medium. We think that the extensive of vegetative growth would permit fungus to more persist in the unfavorable environment and could compete with other microorganisms. Therefore, this combination of plant residues was used further for preparation of compost.

Composted plant residues also supported the development of *T. asperellum*, this is showed by the growth of this fungus after four weeks of culture and by fungus reisolation in the root, stem, and leaf tissues after its application together with composted plant residues on cacao seedling through soil amendment (data not presented here). In fact, many studies indicate that agricultural waste-based compost can suppress soil-borne fungal pathogens (Bonanomi *et al*., 2007; Pane *et al*., 2013). Essay with composted billy goat showed also that liquid form of this compost can inhibit *Phytophthora palmivora* growth in vitro and reduce Phytophthora pod rot incidence in the field (Limbongan, 2013). The suppressiveness of compost depends on the ecological relationships between organic carbon molecular and microbial structure. Nutritional microniches in compost may have profound effects on the community functions, including those linked to the suppressiveness (Pane *et al*., 2013). In the case of *Trichoderma*, it has probably special structure determining the particular condition associated with the resistance to composted plant residues.

Previous study demonstrates that application of compost through soil amendment can control above ground diseases. Arabidopsis and tomato grew in soil amended with composted paper mill residuals (PMR) exhibit reduced symptoms of bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (Vallad *et al*., 2003). Similar reduction observed with snap bean anthracnose caused by *Colletotrichum lindemutianum* (Stone, 2003). This disease reduction is due to induction of plant defenses, similar to systemic acquired resistance (Vallad *et al*., 2003). In our research by using soil from field amended with composted forms of gliricidia, billy goat, and rice straw mixture exhibited also a decrease of cacao VSD disease incidence in cacao seedling. Our result in VSD reduction was just 11%, this rather was due to probably the impact related to nutrition, not by resistant induction.

Impact of *T. asperellum* in controlling VSD disease in cacao has been proven according to trial in the laboratory and in a limited scale in the field (Rosmana *et al*., 2015, 2016). The *Trichoderma* application through soil for VSD control was done for the first time and it was evident that *Trichoderma* applied alone can reduce as well the incidence of VSD disease. When this *Trichoderma* is applied through the soil, the fungus can reach, penetrate and colonize roots and then rise to colonize stems and leaves. For this raison, the mechanism of control hypothesis of *T. asperellum* against VSD pathogen beside due to induction of resistant through interaction this fungus and roots (Harman, 2011; Hermosa *et al*., 2013; Rosmana *et al*., 2016), as well due to direct competition with VSD pathogen through the occurrence of *Trichoderma* in stem and leaves.

Synergism between *T. asperellum* and plant residues in reducing of VSD incidence was observed. This indicated that multiple actions could occur to prevent the development of VSD disease including induction of resistant, competition between Trichoderma and VSD pathogen, parasitism of *Trichoderma*, and improvement of plant nutrition.

In the context of plant and pathogen relationship, it has been known that infection by pathogen cause N content of plants is increased beyond sufficient levels, while K content is decreased (Huber and Haneklaus, 2007; Spann and Schuman, 2009). In addition to this increase of N content, we observed as well the decrease of P content after infection by VSD, but K content was not influenced. *Trichoderma* application increased the N content to very high level in seedling infected by VSD pathogen, but not P and K content. In this regard, it is probably that sensitivity of cacao to VSD due to high content of N is countered by other mechanisms of defense made by *Trichoderma*. High content of N can increase severity of obligate parasite infection such as VSD pathogen (Dordas, 2008). While when *Trichoderma* was applied with composted plant residue, the increase P and the decrease of N was observed, therefore N, P, and K content in the infected plant was almost the same as those in the negative control. This condition supported apparently high decrease of VSD incidencein the infected plant.

Conclusion

We conclude that the presence of compatibility between composted plant residues consisting of gliricidia, billy goat, and rice straw and *T. asperellum* permit control better VSD disease in cacao and this could potentially be used to remediate cacao infected by VSD disease in the field through soil amendment. The important of composted plant residues both for multiplication of microorganism and for the source of plant nutrition was also observed. However, the soil offield origin used in this research was K deficient and composted plant residues and *T. asperellum* are not capable to supply sufficiently K content, therefore it is needed to develop a formula of K rich plant residues.

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