Colonization Ability of Biological Control Agent *Tricoderma* spp on Cocoa Pod and Seedling

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Abstract
Some of *Trichoderma* species as antagonistic fungi are usually considered soil microorganism, They colonize plant roots, some- times forming a symbiotic relationship. Three species of Trichoderma (*T. virens*, *T. harzianum* and *T. asperellum*) have been inoculated on cacao seedling and cocoa pod. *Trichoderma* species can be re-isolated from surface sterilized cacao seedling, including the stem and leaf, root, and pod then observed their colonization ability. Fungal hyphae were observed under the microscope emerging from the leaf, steam, root of seedling and pod as soon as 1 day after their isolation from surface sterilized cacao seedling and pod. All *Trichoderma* species were able to enter and make colonization. The highest percentage of colonization occurred in the *T. harzianum* by 73.3% (leaves), 46.7% (trunk) and 86.7% (roots). While colonization on the skin cocoa pod (epidermis) also has a different percentage, the highest percentage indicated in the treatment of *T. harzianum* by 63.3%. We conclude that *T. harzianum* better biological control agent base on their ability to colonize all part of seedling and pod. *Trichoderma* species into the cacao stem, leaf, root and pod allowing systemic colonization of this tissue.
Key Word: Colonization, *Tricoderma* spp, cocoa pod, seedling, biological control, challenge

Introduction

Productivity of cocoa recently have been decreasing caused by pest and disease pathogen. Some fungal pathogens attacked cacao plants cause large yield losses. One of the pathogen, *Phytophthora Palmivora*cause black pod disease of cocoa rot. The high potential losses have been caused by this black pod disease. Biological control methods that was effective and efficient with the sustainable system.

Currently many bacterial and endophyte fungi isolated from the plant and roots. Those organism that were antagonistic against soil borne pathogens and potentially could be use as biological control agents. The use of biological agents to reduce the number of inoculum pathogen, reducing the pathogen's ability to infect its host and inhibit the development of pathogens in the host. Antagonism mechanism was the ability of an organism to inhibit the growth and development of other organisms (Cook & Baker, 1989). Sriwati et al., (2009) Detected and Identified of Endophytic fungus *Trichoderma* associated in cacao plant in Aceh Sumatera. They reported that there are several species of endophytic fungi associated in cacao leaves from East Aceh.

Trichoderma is one antagonistic microorganism capable to suppressing pathogens and can be used as a biocontrol against fungal pathogens. Utilization of the fungus *Trichoderma* also has potential as a biological control for antagonism against several plant pathogens, such as *Fusarium* sp, *Rhizoctonia solani* and *Phytium* (Ramada, 2008). *Trichoderma* sp also the saprophyte microorganisms acting as organic decomposer. They can degraded the materials containing fibers, lignin and organic compounds, containing carbon and nitrogen used from organic matter (Saraswati & Sumarno, 2008). *Trichoderma* ability to produce antibiotics and parasite pathogen in host plant tissue has been studied extensively (Harman et al., 2004). There are several species of *Trichoderma* are used as biocontrol agents include *T. harzianum, T, Viridae, T. konigii,* and *T. virens* (Howell, 2003). Muarif (2013) reported that *T. virens* which high concentration were able to inhibit *P. palmivora* attacks in cacao seedling. In addition, some strains of *T. harzianum* were able to suppress the development of *Sclerotium rolfsi* on bean plants by 30-50% (Papavizas & Lewis, 1989). While research Rosmana et al., (2013) revealed that an effective and efficient way to control leaf blight disease on cacao seedlings caused by soil-borne pathogens by using *T. asperellum*. The use of *T asperellum* at a concentration of 10^6 indicates the percentage and intensity of the disease were lower than the control treatment.

One of the problem in the utilization of *Trichoderma* as biological control agents of plant diseases was low colonization ability (Nurbailis & Martin, 2009). Research on the effectiveness of some species of *Trichoderma* as biological control agents have been carried out, but on the ability of *Trichoderma* spp in colonized parts of the plant has not been known. Therefore, it is necessary to study the ability of endophytes colonization in some *Trichoderma* species on cacao seeds and pod.

Materials and methods

This research was conducted at the Laboratory of Plant Pathology Agrotechnology Department, Faculty of Agriculture Syiah Kuala University. *T. virens, T. harzianum, T. asperellum* suspension were used for inoculation to seedling and pod. Completely randomized design (CRD) non factorial consisting of 3 treatments and 6 replications have been done. Observation abstain on cacao seeds and pod.

Table 1. Composition of Some Species of *Trichoderma* Treatment

No	Perlakuan	Keterangan
1	Tv	*T. virens* concentration 1×10^6
2	Th	*T. harzianum* concentration 1×10^6
3	Ta	*T. asperellum* concentration 1×10^6

Pure cultures of *T. virens, T. harzianum* and *T. asperellum*has been grown on PDA media and incubated for ± 2 weeks to form spores. Cocoa seeds collected from cacao plantation Sare, Aceh Besar district. Cocoa seeds were germinated on cotton media to determine germination seed before being transferred into polybags. Seven days after germination cacao seedling were selected to obtain a good bean sprouts, then transferred to soil in the polybag media. Top soil that used has been sifted and sterilized using an autoclave at 121 ° C for 30 minutes. The sterilized soil mixed with manure in the ratio of soil and manure 2: 1 in polybag size of 1 kg. Each spore concentration 1×10^6 of *T. virens, T. harzianum* and *T. asperellum*have been sprayed on seedling and pod.

Determine the colonization of *T. virens, T. harzianum* and *T. asperellum* has been done by taking the leaves, stems and roots, sampled with a diameter of 1 cm by using cork borer, the leaves, stems and roots were taken 5 sections. These part were sterilized with NaClO (sodium hypochlorite). All samples were inoculated on PDA medium for hypha observation.

Trichoderma colonization percentage in the samples of seed and pod that have been isolated were counted and characterized by morphology characteristic of *Trichoderma* growth on the PDA. Colonies that grew identified morphologically by Barnett and Hunter (1972). *Trichoderma* colonization percentage calculated using the formula:

*Trichoderma*colonization percentage = $\frac{\textit{Trichoderma growth}}{\textit{total samples}} \times 100\%$

Results

When leaf, stem, root and pod sections of cacao seedlings colonized by *Trichoderma*, hte hyphae were observed within 24 h after surface sterilization. *Trichoderma* hyphae appeared to be emerging from the tips of leaf, steam and root (Fig 1) and from pod (Fig 2). This was a general observation for many different

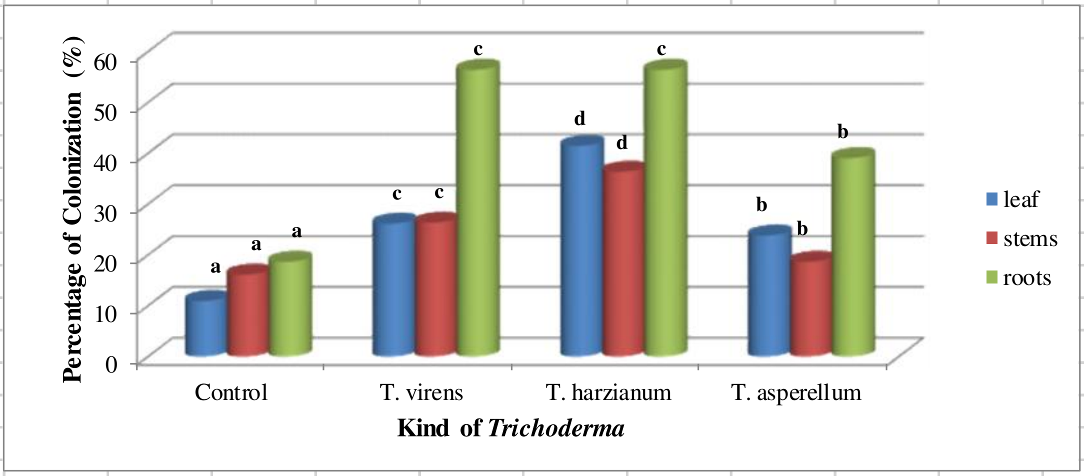

Figure 1. **Percentage of Colonization *Trichoderma* on Cacao Seedlings**
Trichoderma species was not limited to the specific strains of *Trichoderma* shown. *Trichoderma* were isolated from colonized cacao seedlings after surface sterilization to verify that they were internally colonized. Low percentage *Trichoderma* was isolated from the un-inoculated control seedlings and pod compare with inoculated treatment. All three *Trichoderma* species studied were re-isolated from leaf, steam and root. Intermediate, with emerging *Trichoderma* hyphae then were observed and isolated. *Trichoderma* hyphae most often emerged from the surface outside (epidermis) but were also observed emerged from inside of pod. The percentage of isolating the inoculated *Trichoderma* strain from seedling with emerging hyphae was higher on *T. harzanum* and *T virens* strains on this studied compere with *T. asperellum* and control. The *Trichoderma* isolated in highest percentage isolation of *Trichoderma* from each late samples were always consistent from root seedling

Tricoderma species from pod emerging hyphae were always emerged in high percentage from for *T. harzianum*. Outside skin of pod as epidermis part was higher colonization of *Trichoderma* compare with inside pod (endodermis) (Fig 2)

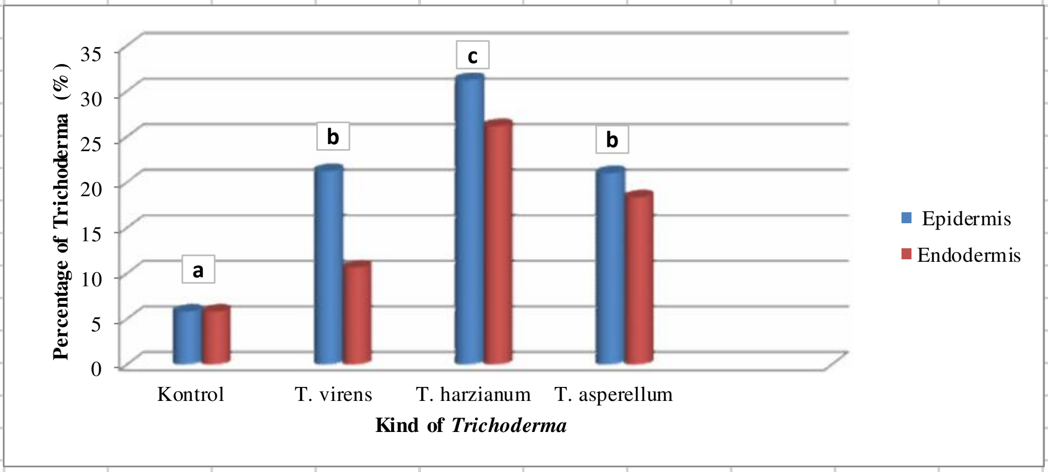

Figure2. **Percentage of Colonization *Trichoderma* on Cacao Pods**

Discussion

The high capability of colonization *T. harzianum* showed that *T. harzianum* most effective for use as biological agents. Although Sriwati at al 2015 reported that *T. virens* Aceh isolate has good potential as biological control for black pod disease on laboratories experiment but the works of this fungi have more a less than *T. harzianum*. Thrane et al., (2002) stated that *T. harzianum* produce enzymes such as cellulase, glucanase and chitinase, could speed of growth and antagonistic to plant pathogens. Among some *Trichoderma* species have been identified, the most common type used as biocontrol soil borne pathogen is *T. harzianum* (Sunarwati and Yoza, 2010). The colonies of *T. harzianum* in the root system and its consequences seen ability to suppress the growth of soil borne fungal pathogens (Cook in Ekowati, 2000). From parts of plants that have been isolated, it is known that the highest percentage of colonization found on the roots of *T. harzianum* (56.53) and T. virens (56.53%). The fungus *T. harzianum* and *T. virens* have the same capabilities at the root colonization in the 1 x 10^6. This was because the rhizosphere around the root there are many nutrients that can be used as food ingredients for *Trichoderma* growth. *T. harzianum* capable of forming colonization of roots that can extend and improve primary root growth of tomato plants (Herlina et al. 2004). According to Harman et al., (2004), *Trichoderma* spp as saprofit have good live in the rhizosphere of plants. In addition, Yedidia et al., (1999) also states that some strains of *Trichoderma* able to colonize and can be endophytic in plant root tissues that causes increased activity of the compound in the resilience of the plant roots.

On the outer skin of cocoa pod, the most high percentage of colonization indicated in the treatment of *T. harzianum* by 31.3%, while the inside part of cocoa pod showed no significant difference in each treatment. The highest percentage in in inside part in *T. harzianum* treatment with an average percentage of 26.24%. Naturally, *Trichoderma* present in the cocoa fruit tissue with a very small amount. This is proven by the *Trichoderma* in the control treatment an average of 5.8%. The addition of *Trichoderma* suspension at a certain concentration can increase the population of *Trichoderma* in the tissues of plants and fruit. Sriwati (2014) states that the provision of *Trichoderma* suspension at a concentration of 1 x 107 can suppress the growth of *P. palmivora* cause fruit rot disease of cocoa.

Conclusion

All *Trichoderma* species were able to enter and make colonization. The ability of third colonization *Trichoderma* species on cacao seeds have different percentages. Highest percentage of colonization occurred in the *T. harzianum*. While colonization on the skin cocoa pod (epidermis) also has a different percentage, the highest percentage indicated in the treatment of *T. harzianum*. We conclude that *T. harzianum* better biological control agent base on their ability to colonize all part of seedling and pod.

Acknowledgements

This work was supported by a grant from the Director General of higher Education, Department of National Education Indonesia. We thank to Bryan Bailey at Laboratory Sustainable Perennial Crop Lab. SPCL USDA-ARS Beltsville, MD, for fungal identification work.

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