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Coffee Plants Anti-Virus of Decomposited *Trichoderma Viride* of Cow's Manure

Lelya HILDA[1*] and Syafiruddin[2]

[1.] *Insitut Agama Islam Negeri, Padang Sidimpuan, Indonesia*
[2.] *Universitas Ghraha Nusantra (UGN)*
Corresponding email: lelya.hilda@gmail.com

Abstract

It is often found that the *Hemileia vastatrix* fungus attack on coffee plants that causes the decrease of coffee production. This Experimental Design applied the *trichoderma viride* as an antagonistic fungus. It sought the effectiveness of *trichoderma viride* to suppress the growth of the fungus *hemileia vastatrix*. Technique of application of cow's manure is by combining *trichoderma viride* to increase soil nutrient. It was used for decomposition of cow dung in increasing soil nutrient values that helps coffee plants to grow rapidly. The cow dung added with bran which was useful for feeding *Trichoderma viride* to make its growth faster. The research results showed that within 20 days, there was a decrease in attacks by 50.87 percent (20.64 mm^2 on day 0 to 10.5 mm^2 on day 20). It concludes that the biological control for the fungus Hemileia vastatrix (leaf rust) on coffee plants was effective to suppress in its development. It found that the *Trichoderma viride* suppressed the growth of the fungus Hemalia vastartrix higher with longer application period (day 10^{th} to 20^{th})

Keywords: *Hemileia vastatrix*, arabica coffee, leaves; *Trichoderma viride*, cow's manure

Introduction

Many reports revealed that leaf rust was caused by the fungus *Hemileia vastatrix*. That fungus is one of the most damaging diseases of coffee *arabica L*. It is causing premature leaf fall, loss of yield and even death of the tree in severe attacks [1,2]. Talhinhas *et al* [3] elaborated the disease symptoms and signs include large yellowish-orange spore masses, powdery and rounded blotches on the lower leaf surface. It notes that since 2008 to 2013 there is a more intense of coffee rust epidemics than those of previously observed occurred in America such as Colombia, Mexico, Peru, Ecuador and some Caribbean countries [4]. These outbreaks were the worst since the disease first appeared in Central America in 1976.

The low productivity of Indonesian coffee is caused by the high disturbance of leaf rust disease; it is due to the lack of proper technical culture practices, which are included in controlling leaf rust disease. This disease is especially found in smallholder plantations covering an area of 1,241,500 ha (95.5% of the area of the coffee plant). *Arabica L.* coffee plant is one of the export and commercial plants in tropical and subtropical developing countries. Arabica coffee is the most preferred type because it has excellent taste (aroma, taste, aftertaste, sweetness, body and flavor balance), however, this annual plant has the potential to lose high production due to pests and diseases. There are three factors that influence the development of this disease, namely pathogens, hosts and plants. The response of plants to the attack of a pathogen begins with the recognition of the pathogen. This recognition can occur extracellularly when pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRRs), activating the pathogen-triggered immunity (PTI) mechanism [5,6]. In the tropic area, *Hemileiavastatrix* occurs persistently as uredospores (rust fungus spores), uredium (uredospora-producing fruiting bodies), and mycelium (hyphal collection) rust fungus.

The pathogen response in the study of Bebber et al. [7] found that the climate change had no effect on germination and appressorium formation and consequently it did not significantly favor leaf infection. On diseased leaves, it will continue to make an infection in plants. From some of the structure of the fungus, the development of leaf rust disease is greatest influenced by uredospores. Thus, Uredospora is the fungus Hemileia vastatrix, which is orange with a length of 25-35 µm and a width of 12-28 µm, shaped like a kidney and prickly in a convex part. The development of leaf rust is due by lack of regular pruning, providing poor shade and unbalanced fertilization.

Hemileia vastatrix is a cause of leaf rust disease which is very detrimental to coffee plants [8]. This disease has been developing in Indonesia since 1876 and in it is developed and resulted in a decreasing in coffee production by 25% [9]. Increase in resistance of Arabica coffee to rust mushrooms is now an important priority for economical and sustainable coffee production. Among the strategies available to increase plant resistance to parasites, which are based on host defense induction [1]. Medicines for pests and weeds by reusing high quality organic fertilizer will reduce the use of chemical fertilizers to be very important in order to obtain a sustainable increase in land productivity. Organic fertilizers need to be decomposed by microbes and require an appropriate environment so that they mature quickly and have no impact negative on social, aesthetic and health aspects of living things and the environment [10].

Some research reports show the way of overcoming this problem which might be to add extracts of ginger, turmeric, clove leaves and betel; it is also giving black tea extract. Both of these studies gave quite good results, namely a decrease attacks, but the treatment in the laboratory might not be similar to the plantation location of the coffee plants. Moreover, such Biological Control Agency (APH) control is another alternative in suppressing rust attack. This biological control agent can be used because it is able to limit the growth of pathogens for a longer time, and does not leave residues and can maintain the balance of the ecosystem [11].

The presence of antagonistic fungi that are easily found in agricultural ecosystems and can be used as APH and the most common antagonistic fungus is Trichoderma spp. [12,13]. Therefore, its role in inhibiting the growth of pathogens has been widely studied. Trichoderma spp. may inhibit the growth of *Phytophthora infestans* [14], *Phytium sp*., *Diplodia sp*. [15], and several other pathogenic fungi. Antagonistic power of *Trichoderma spp*. against pathogenic fungi can differ from one another.

Agus et al. [10] show that the organic matter should be composted before it is being applied to agricultural land. The composting process shows that the microbial content of the starter microbes is quite large and is a microbial capable of decomposing organic material in manure, both made from chicken or cow manure.

This research already produced the compost from cow dung and decomposed by *Trichoderma viride* to combat the fungus *Hemileia vastatrix* in arabica coffee leaves. This research was to seek the effect of using Trichoderma viride in decomposing cow dung which is used to suppress the attacks of *Hemileia vastatrix* on Arabica Coffee leaves.

Methods and Materials

This experimental Design has two stages in treating the decomposition for combating the *Hemileia vastatrix* on the Arabica coffee leaves. The first stage was the process of decomposition of cow dung using trichoderma. The decomposition process was prepared by adding *trichoderma* around 100 grams per 10 kg mixture of cow mannure, black soil, bran and charcoal husk. A mixture of cow dung, black soil, rice bran and charcoal with a ratio of 10: 20: 1.5: 1. This material was then incubated for 5 weeks and every week, it involved an analysis of the content of Nitrogen, Phosphorus, Potassium, organic C, Fe and Zn is carried out. The soil analysis was carried out at the Research Lab of Universitas Andalas, Padang of Indonesia.

The second stage used the best results from the initial research and applied to coffee plants. Compost used in coffee plants was divided into 5 treatments, namely without the addition of compost (T0), 50 g (T1), 100 g (T2), 150 g (T3) and 200 g (T4) of compost per plant. Process of analysis was done by taking soil samples from the location of the coffee plants, precisely at the location of the provision of decomposed compost. Soil samples were taken within different periods; days 0, 10^{th} and 20^{th} with 5 replications (5 plants per sample). The sampling soil was then dissolved in soil in *aquades* 1: 10, this solution was then taken as much as 1 ml and cultured in a petri dish filled with PDA. Measurements were administered by comparing the growth area of *Hemileia* and *Trichoderma* on the cup starting from day 0, 10^{th} and 20^{th}.

Results and Discussion

The initial research was conducted to obtain the best comparison between *Trichoderma* mushrooms with a mixture of cow dung, bran, husk and soil. The design was consisted of a ratio of 10 g, 25 g, 50 g and 100 g in a 10 kg mixture. Compost analysis showed the decomposition of cow dung which was using Trichoderma with a mixture of 100 g in 10 kg. It can be seen in the following table.

Table 1. Compost analysis results week 0 until 5

Elements	Weeks					
	0	1	2	3	4	5
N Total	0,380	0,335	1,560	1,198	1,462	1,800
P	195,963	161,963	46,687	38,939	34,067	33,039
K	1,142	1,406	1,452	1,484	1,543	1,309
C organic	5,216	13,331	20,144	48,978	22,03	18,738
C/N	13,726	15,943	32,144	40,657	27,597	17,077
Fe	44,536	68,885	66,906	82,639	88,787	71,928
Zn	8,134	8,882	8,943	12,183	13,338	9,986

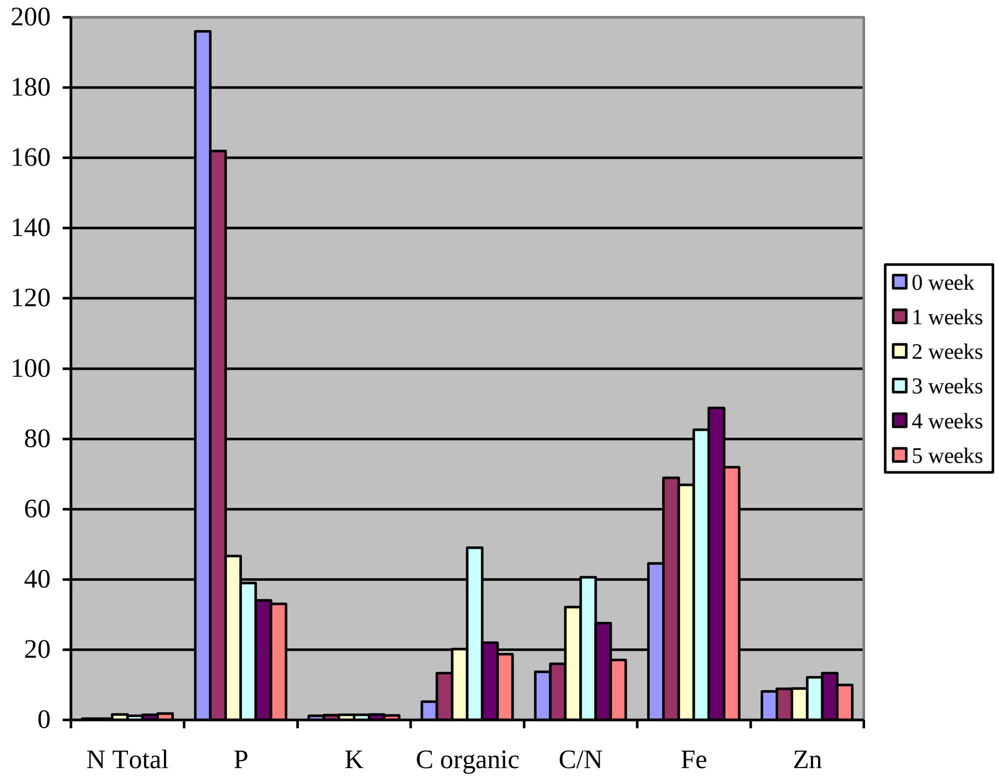

Figure 1. Compost analysis results week 0 until 5

Table 1 and Figure 1 show that there was an increase in total N up to the 5th week, whereas P decreased. The elements K, Fe and Zn increased until the 4th week and 5th week. Organic C, C/N administration compost with Trichoderma increases until 3rd week. It is similar to Indrasari and Syukur [16] report that applied the manure and nutrients in corn plants; it made the increase in all growth parameters, namely as plant height, fresh weight of trubus, dry weight of trubus, fresh weight of roots and dry weight of roots. Giving manure up to a dose of 30 tons/ha is still able to increase the fresh weight of trubus and dry weight of trubus. The results of 14 kg/ha micronutirents application resulting in higher fresh and dry weight of trubus than 28 kg/ha. The combination of 30 tons/ha manure, 14 kg/ha micronutrients and lime produce a highest fresh and dry weight of trubus. This shows that the nutrients contained in coffee plants will strengthen, fertile the plants and control pests attack. Additionally, utilization of animal manure could increase the resistance toward pests and soil fertility as it contains nutrients that required by plants such as nitrogen (N), phosphorus (P) and potassium (K) as well as micronutrients including calcium, magnesium, sulfur, sodium, iron and copper [17].

The results of composting using *Trichoderma sp* as decomposer material provide great results, meaning that in accordance with SNI number 19-7030-2004, about the quality of compost produced. The amount of macro and micronutrient content of compost produced is in accordance with the standards. Therefore, this compost is good for plants.

The application of compost in coffee plants has been proven to be able to suppress the growth of leaf rust fungus. The result of analysis conducted in the laboratory from the results of culture of soil solutions taken by the field can be seen in the following table.

Table 2. Average Growth of Leaf Rust (*Hemileia vastatrix*) on PDA media in petri dish.

Treatment	Average growth of leaf rust on PDA media in petri dish (mm^2)		
	0 Day	10 Days	20 Days
T0	14.67	58	60.3
T1	39.2	24.2	18.03
T2	22.64	20.46	11.56
T3	22.64	17. 16	10.5
T4	20.64	13.17	8.3

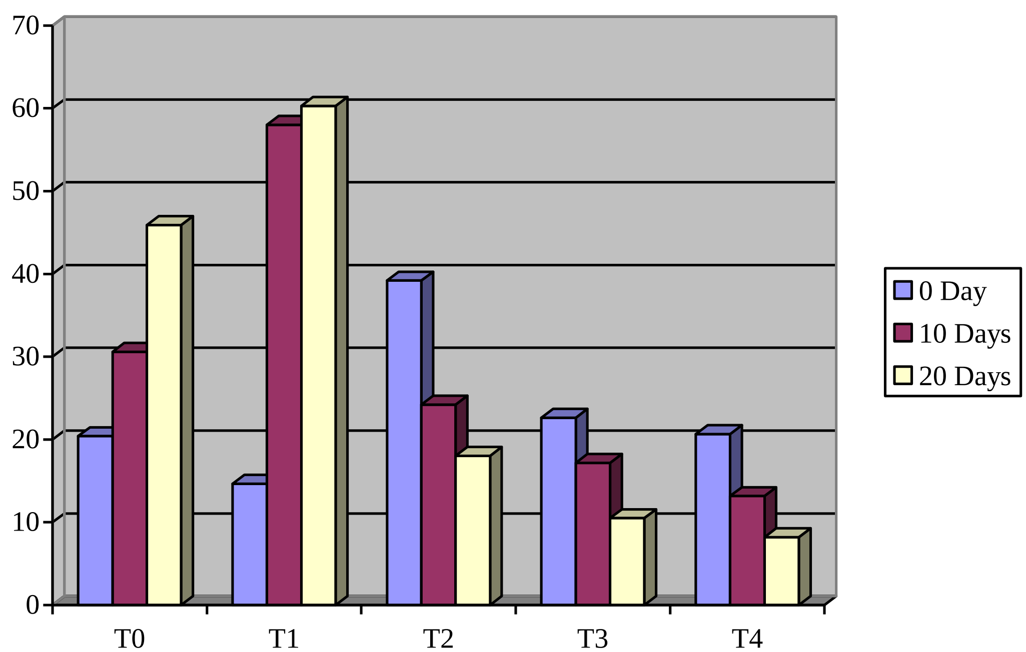

Figure 2. Average Growth of Leaf Rust (*Hemileia vastatrix*) on PDA media in petri dish.

On the 10th day after the application, it can be seen that the growth of leaf rust fungus is getting smaller; it is due to being suppressed by trichoderma growth. The ability of trichoderma to suppress the growth of other fungi is due to competition in looking for food, oxygen, hyphae are able to attack other fungal hyphae. The more percentage of compost (*Trichoderma sp*) applied has a positive effect such as the growth of leaf rust fungus is more quickly depressed. On the 20th day after planting the growth area of rust fungus gets smaller, with different values for each given treatment. The difference is caused by so many things including plant age, plant density, light intensity, soil fertility and plant diameter.

Antagonistic power efficiency of Trichoderma spp. differences in certain pathogenic fungi can be caused by the speed of growth, levels and kinds of chemical compounds and enzymes produced by each species [18]. High growth rate can determine the activity of antagonistic fungi against pathogenic fungi. Trichoderma spp. has the speed of plants that outperform leafy mushrooms, do they can master the competition of space and nutrition.

Trichoderma spp. also has the ability to release antibiotic compounds that can inhibit the growth of leaf fungi. Chemical compounds and enzymes produced by Trichoderma sp also can inhibit the development of pathogens because it functions as an antifungal. Most Trichoderma spp produce compounds that are volatile and nonvolatile which can inhibit the pathogenic fungal colonization. Antibiotic compounds produced by Trichoderma spp including harzianic acid, alamethicins, tricholin, peptaibols, 6-penthyl-αpyrone, massoilactobe, viridian, gliovirin, glycoprenins, heptelidic acid, trichodermin, dermadin and others [19,15]. The production of antibiotic compounds in each Trichoderma sp correlates with its antagonistic ability, there is the production of the same type of antibiotic but with different levels in several species of Trichoderma fungus. This is one of the causes of the different antagonistic power possessed by each species of Trichoderma fungus.

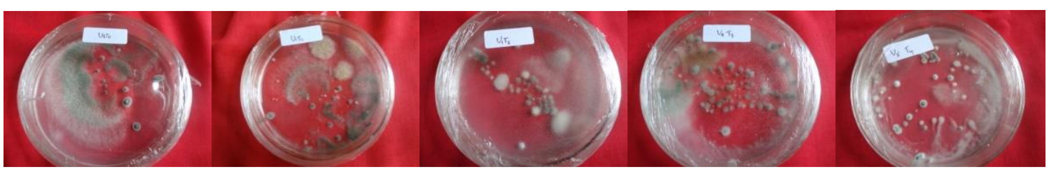

Figure 3. Observation of *Trichoderma viridae* growth in suppressing the growth of *Hemalia* vastartrix on day-0 after application.

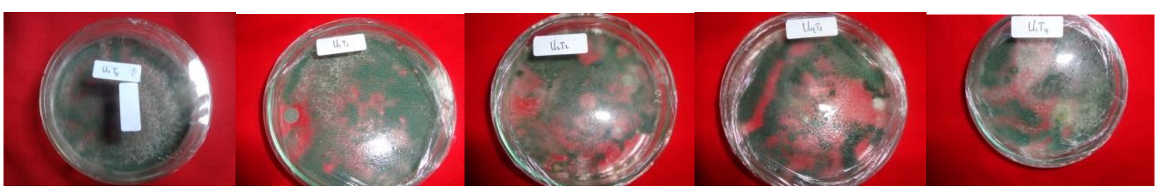

Figure 4. Observation of *Trichoderma viridae* growth in suppressing the growth of *Hemalia* vastartrix on day-10 after application.

Figure 5. Observation of *Trichoderma viridae* growth in suppressing the growth of *Hemalia* vastartrix on day-20 after application.

In figures 3, 4 and % shows that Trichoderma viridae suppressed the growth of the fungus Hemalia vastartrix higher with longer application period (day 10th to 20th). Hence, on the 20th day, all the mushrooms have been suppressed, so that the Trichoderma which is composed on manure will suppress the growth of the fungus *Hemalia vastartrix*. The researcher, Cristancho et al [20] states the most damaging coffee diseases causing 30% crop loss when left unmanaged.

Conclusion

Data analysis draws two conclusions;

(1).The use of *Trochoderma* fungi as decomposers, antifungal or antagonistic fungi or biopesticides or biological control agents was found suitable; it becomes an alternative material in organic changes to be simplified, so that the formation of macro and micro elements from decomposed materials (in this case, cow dung) becomes appropriate to the need land (SNI).

(2). It concludes that the biological control for the fungus Hemileia vastatrix (leaf rust) on coffee plants was effective to suppress in its development. It found that the *Trichoderma viride* suppressed the growth of the fungus Hemalia vastartrix higher with longer application period (day 10th to 20th)

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