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Potential use of Several Fungalendofit Fungus in Rubber Crops (Hevea Brasiliensis) Plant for Healrth Invesment (Colletotrichum gloeosporiodes) in Rubber Plant

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Abstract

Rubber is a key plantation commodity in Indonesia, serving as a significant source of non-oil and gas foreign exchange. However, rubber productivity remains low, largely due to inadequate cultivation technology and the impact of pests and diseases. One of the most economically significant diseases affecting rubber plants is deciduous disease caused by Colletotrichum Gloeosporioides, which spreads through spores carried by wind and rain. This study was conducted at the Sungei Putih Rubber Research Institute, North Sumatra, from February to June 2019, at an elevation of ± 25 meters above sea level. A factorial randomized block design (RBD) with three replications was employed. The treatments included three types of endophytic fungi (E1, E2, E3) derived from different isolates of PB 260 rubber clones, and four application methods (M0, M1, M2, M3). The observed parameters included latent period, disease occurrence, and disease intensity. The results indicated that the E2M2 treatment, which combined the second endophytic fungus with metabolite application, was the most effective in controlling C. Gloeosporioides deciduous disease. This combination resulted in the highest average latent period (3.67%), the lowest disease occurrence (0.03%), and the most effective reduction in disease intensity (17.67% after 12 days). The study demonstrates the potential of specific endophytic fungi and application methods in managing rubber plant diseases, offering insights for improving rubber productivity in Indonesia.

Keywords: *Endophytic Fungus, C. Gloeosporioides , Rubber Plants*

1. INTRODUCTION

Rubber plant is one of the plantation commodities which occupies an important position as a source of non-oil and gas foreign exchange for Indonesia, so it has bright prospects. Therefore efforts to increase the productivity of rubber farming continue to be carried out, especially in the field of cultivation technology. Indonesia once controlled world rubber production, but currently Indonesia's position is being pressured by two neighboring countries, Malaysia and Thailand. More than half of the rubber used today is synthetic, but several million tons of natural rubber are still produced annually, and it is still an important material for several industries including automotive and military (Felix, 2013).

Indonesia has the largest rubber area in the world, namely 3.4 million ha with an annual rubber production of 2.7 million tons. Even so, its productivity is only 1.0 tons/ha, lower than Malaysia (1.3 tons/ha) and Thailand (1.9 tons/ha). Rubber production in Indonesia, Thailand and Malaysia contributes 85% of total world production (Kemenperin, 2012).

One of the reasons for the low productivity of rubber is disease. Disease in rubber plants is one of the important disturbing factors than other disturbance problems. In general,

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rubber plant diseases are caused by fungi or pathogens. The most important diseases that attack rubber plants are white root fungus, tapping groove dryness, corynospora leaf fall, Colletotrichum and Oidium (Sajar. S., 2017).

Colletotrichum disease is a type of disease found in rubber plantations. Colletotrichum disease is caused by the fungus C. gloeosporioides with symptoms in the form of young leaves that appear limp, black in color, wrinkled, the tips die, curl up and eventually fall. Meanwhile attacks on old leaves show symptoms of brown or black spots, holes, wrinkles, and some of the tips die (Syamsafitri et.al. 2021).

Control of Colletotrichum disease is usually done by using chemical fungicides. In preventing the use of chemicals that can cause damage to the surrounding environment to control Colletotrichum disease, we can use biological control, namely by using endophytic fungi (5).

Endophytic fungi are fungi found in plant tissue systems that do not cause disease symptoms in the host plant. Endophytic fungi can produce antibacterial compounds which have the potential as biological control agents. Groups of endophytic fungi that act as biological control agents include Fusarium solani, Acremonium zeae, Verticillium sp., Phomopis cassiae, Muscodor albus, Ampelomyces sp., Periconia sp., Neotyphodium lolli, etc (5).

Endophytic fungi spend part or even the entire life cycle of their colonies inside and outside the living tissue cells of their host plants. Endophytic fungi can be found in plant tissue systems such as leaves, twigs or roots. Endophytic fungi are one of the microbial groups that play an important role in the reaction of plant resistance to pathogens such as attacks caused by C. gloeosporioides on rubber plants (Dalimunthe, 2016).

2. METHOD

This research was conducted at the Sungei Putih Rubber Research Institute, Galang District, Deli Serdang Regency, North Sumatra Province with an altitude of ± 25 meters above sea level with a flat topography. This research was carried out from February to July 2019. This study used a factorial randomized block design (RBD) consisting of two factors. The factors studied were: Endophytic type factor (E) which consisted of 3 treatments, namely: E1 = Rubber clone PB 260 from isolate code KI 1 (Endophyte), E2 = Rubber clone PB 260 from isolate code KI 2 (Endophyte), E3 = Rubber clone PB 260 from isolate code KI 3 (Endophyte). The application method factor (M) consists of 4 levels, namely: M0 = Control (aquades treatment), M1 = Spore application 104, M2 = Metabolite application, M3 = Metabolite + Spore application 104. The number of treatment combinations is 3 x 4 = 12 treatments. Each treatment was repeated 3 times resulting in 36 trials.

3. RESULTS AND DISCUSSION

1. Latent Period (Days of Appearance of Disease Symptoms)

Data from observations of the latency period from the results of tests using several endophytic fungi using several application methods are presented in table 1.

Table 1: Bidirectional mean latency period (days)

Application Method	Endophyte (E)		
	E_1 (Endophyte KI_1)	E_2(Endophyte KI_2)	E_3 (Endophyte KI_3)
M_0 (aquades treatment)	2.00 a	2.00 a	2.00 a
	A	A	A
M_1 (Spore application)	3.33 b	2.00 a	2.33 ab
	B	A	AB
M_2 (Metabolite application)	2.33 ab	3.67 b	3.33 b
	A	B	B
M_3 (Metabolite + Spore)	2.00 a	2.00 a	2.00 a
	A	A	A

Note: The numbers followed by the same letters were not significantly different according to Duncan's test at the 5% level of significance. Lowercase letters are read vertically (columns) and capital letters are read horizontally (rows).

In In table 1 above the endophytic treatment of the application method treatment. In the E1 treatment, the M0 treatment was the same as the M3 and M2 treatments in inhibiting the appearance of disease symptoms, but the M1 treatment was better in inhibiting the appearance of disease symptoms. In the E2 treatment, the M0 treatment was the same as the M1 and M3 treatments in inhibiting the appearance of disease symptoms, but the M2 treatment was better in inhibiting the appearance of disease symptoms. In the E3 treatment, the M0 treatment was the same as the M1 and M3 treatments in inhibiting the appearance of disease symptoms, but the M2 treatment was better in inhibiting the appearance of disease symptoms. In the treatment of the application method of endophytic treatment. In treatment M0 treatments E1, E2 and E3 showed no difference in inhibiting the appearance of leaf fall disease symptoms. In the M1 treatment, the E2 treatment was the same as the E3 treatment in inhibiting the emergence of disease symptoms, but the E1 treatment was better in inhibiting the appearance of disease symptoms. In the M2 treatment, the E2 and E3 treatments were better at inhibiting the appearance of disease symptoms. In the M3 treatment, the E1, E2 and E3 treatments showed no difference in inhibiting the emergence of disease symptoms.

In general, it can be seen that the average latency period for each treatment combination shows differences in each treatment in inhibiting the emergence of leaf fall disease symptoms in rubber plants. Endophytic microbes are microorganisms that live in plant tissues. The relationship between endophytes and their host plants is mutually beneficial. Vurukonda, Giovanardi and Stefani (2018) stated that endophytes can act as pest and disease control agents. Endophytic fungi suppress disease by inducing plants to produce metabolites. These metabolites play a role in activating plant resistance from disease attack.

The application process was carried out on March 20 2019 in the afternoon and then incubated for 1 day. Based on the rainfall data in Appendix 4, rainfall occurred on March 20 2019 in the morning with a light rain description of 15 mm/day and on March 21 2019, with a light rain description of 15.5 mm/day with temperatures ranging from 27-30 ° C. The above data shows that climatic conditions are very suitable for the growth of the cause of leaf fall C. gloeosporioides in accordance with the statement (Situmorang *et al*, 2004) that suitable climatic conditions at the time of infection determine the occurrence of an epidemic. Environmental conditions with humidity of 96%-100% or the presence of water spots, temperature of 28-30 °C and bright or dark weather are conditions that are very suitable for the growth of disease-causing fungi in rubber plants.

2. **Disease Occurrence (Percentage of Affected Plant Leaves %)**

Data from observations of disease incidence from test results using several types of endophytes using several application methods are presented in the table 2.

Table 2: Bidirectional mean incidence of 2 DAY disease

Application Method	Endophyte (E)		
	E_1 (Endophyte KI_1)	E_2(Endophyte KI_2)	E_3 (Endophyte KI_3)
M_0 (aquades treatment)	99.98 b A	99.98 c A	93.32 c A
M_1 (Spore application)	26.68 a A	40.00 b A	20.01 a A
M_2 (Metabolite application)	40.01 a B	0.03 a A	20.02 a AB
M_3 (Metabolite + Spore)	53.33 a A	70.00 bc A	66.67 b A

Note: The numbers followed by the same letters were not significantly different according to Duncan's test at the 5% level of significance. Lowercase letters are read vertically (columns) and capital letters are read horizontally (rows).

In table 2 above the endophytic treatment of the application method treatment. In the E1 treatment, the M1, M2 and M3 treatments were better at inhibiting the spread of the disease than the M0 treatment. In the E2 treatment, the M2 treatment was better than the M1 treatment, while the M2 and M1 treatments were better than the M0 treatment in inhibiting the spread of the disease. In the E3 treatment, the M1 and M2 treatments were better than the M3 treatment, while the M1, M2 and M3 treatments were better than the M0 treatment in inhibiting the spread of the disease.

Look at the treatment of the application method of endophytic treatment. In the M0, M1 and M3 treatments we can see that the E1, E2 and E3 treatments had no difference in inhibiting the spread of the disease. In the M2 treatment, we can see that the E2 treatment was the most effective in inhibiting the spread of the disease.

The average disease incidence in each treatment combination showed that the highest disease incidence was in the E1M0 and E2MO treatment combinations with an average value of 99.98%. While the lowest value is found in the E2M2 treatment with an average value of 0.03%.

From the explanation above, observations of disease incidence show an average value that varies between 0.03% - 99.98% according to Semangun's statement (1991) that C. gloeosporioides leaf fall disease on rubber plants varies and even reaches 100% depending on weather conditions and the susceptibility of the rubber clones.[10] Dan Woelan (2006) stated that the BPM 24 clone had very little resistance to Colletotrichum.

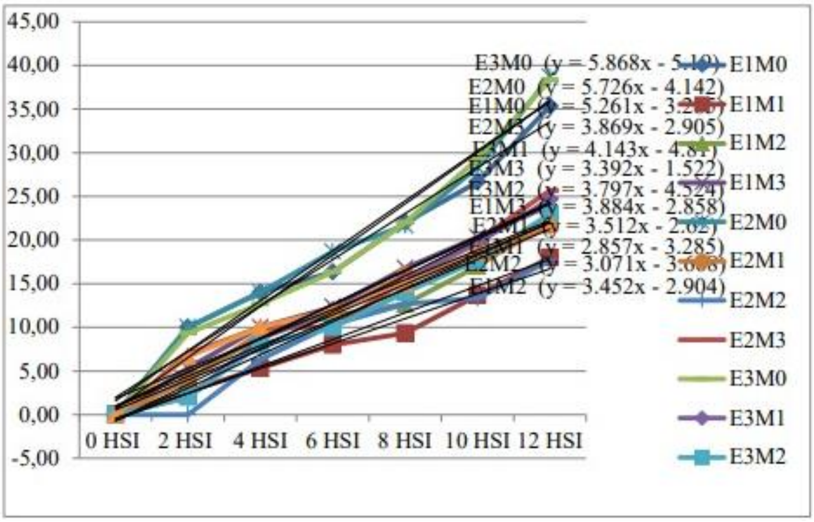
3. **Intensity of Disease (Severity of Disease %)**

Data from observations of disease intensity from test results using several types of endophytes using several application methods are presented in table 3.

Table 3: Mean Intensity of C. gloeosporioides Disease (%) on Rubber Plant Leaves 2, 4, 6, 8, 10, 12 DAY

Treatment	Mean					
	2(day)	4(day)	6(day)	8(day)	10(day)	12(day)
Endophyte (E)						
E1	5.50	9.58	12.00	15.17	19.42	24.92
E2	5.25	9.92	12.67	16.42	19.75	25.92
E3	5.00	9.83	12.58	16.58	21.33	27.00
Application Method (M)						
M_0	9.78 b	13.56 b	17.11 b	21.89 c	28.11 c	37.44 b
M_1	2.89 a	7.78 a	10.56 a	13.56ab	16.89 a	21.44 a
M_2	2.00 a	7.78 a	10.56 a	13.11 a	16.00 a	21.00 a
M_3	6.33 b	10.00 a	11.44 a	15.67 b	19.67 b	23.89 a
Interaction (E*M)						
E1M0	10.00	14.00	16.33	22.00	26.67	35.33
E1M1	2.67	5.33	8.00	9.33	13.67	18.00
E1M2	4.00	9.00	11.33	12.67	17.00	22.33
E1M3	5.33	10.00	12.33	16.67	20.33	24.00
E2M0	10.00	14.00	18.67	21.67	28.33	38.67
E2M1	4.00	9.33	11.67	16.00	17.33	21.67
E2M2	0.00	6.33	10.33	12.67	13.33	17.67
E2M3	7.00	10.00	10.00	15.33	20.00	25.67
E3M0	9.33	12.67	16.33	22.00	29.33	38.33
E3M1	2.00	8.67	12.00	15.33	19.67	24.67
E3M2	2.00	8.00	10.00	14.00	17.67	23.00
E3M3	6.67	10.00	12.00	15.00	18.67	22.00

In table 3 above, it can be seen that the treatments E1, E2 and E3 at 2 – 12 HSI had no effect on inhibiting disease intensity. We can see this in the results of the analysis of variance in appendix 11 – 16. The application method can be seen at 2 HSI M1 treatment and M2 can control disease intensity than treatment M0. At 4 and 6 HSI it can be seen that the M1, M2 and M3 treatments can control the intensity of the disease compared to the M0 treatment. At 8 HSI it can be seen that the M2 treatment was very good at controlling the intensity of the disease. At 10 HSI it can be seen that M1 and M2 treatments can control the intensity of the disease. And at 12 HSI treatment M1, M2 and M3 could control disease intensity better than treatment M0. The interaction of the two treatments had no effect on controlling the intensity of the attack, which can be seen in attachments 11-16.



Ficture 1. Disease Intensity Rate (%)

From the graph it can be seen that the intensity of disease attacks has increased at each observation time, starting from the 2nd HSI observation to the last 12th HSI observation. The best combination of treatments in inhibiting the intensity of C. gloeosporioides disease was found in the E2M2 treatment with a value of 17.67%. This proves that endophytic fungi can inhibit the intensity of C. gloeosporioides disease. Shehata *et al* (2008) stated that one of the properties of antagonistic microbes is their faster growth compared to pathogens and or the production of antibiotic compounds that can inhibit the growth of pathogens. And Alam (2021) reported that endophytic fungi produce alkaloids and mycotoxins that enable plant resistance to disease. The mechanism of endophytic fungi in controlling C. gloeosporioides disease is by means of endophytic fungi forming hooks around the pathogenic hyphae before penetration, or sometimes entering directly. These antimicrobial compounds fight pathogenic microorganisms by damaging cell walls, interfering with microbial cell metabolism, inhibiting microbial cell synthesis, interfering with the permeability of microbial cell membranes, inhibiting the synthesis of protein and nucleic acid in microbial cells.

From the graph above it can also be seen that the intensity of C. gloeosporioides disease attacks has variations ranging from 0.00% to 38.67%, the intensity of C. gloeosporioides disease attacks on rubber plants in entres gardens continues to increase at each time of observation. C. gloeosporioides produces spores at temperatures of 10-400C, besides that ultra violet light will have an effect on activating spores, spore development can occur at humidity around 90% and temperatures around 15-350C, although the relative humidity of this fungus is around 90%. Colletotrichum spores can also survive in the temperature range above 350C, this condition supports disease development in rubber plants. Semangun (1991) stated that C. gloeosporioides leaf fall disease on rubber plants varied and even reached 100% depending on weather conditions and the susceptibility of the rubber clones. Woelan (2006) stated that the BPM 24 clone had very little resistance to Colletotrichum.

Provision of chicken manure has a very significant effect on the growth of mustard pakcoy plants with a verticulture system. This is because chicken manure contains complete macro and micro nutrients even in small amounts. In accordance with

Velusamy, Sathya & Maheswari, M. (2017) who said that poultry manure (chicken) is useful in the mineralization process which will completely release nutrients (N, P, K, Ca, Mg, S and micro nutrients), and can increase soil nutrient content. In addition, chicken manure can also improve the physical and chemical properties of the soil, improve soil structure, make the soil lighter to cultivate, increase water resistance, improve soil permeability, and increase cation exchange capacity, so that it can bind high cations. That there was a very significant effect on plant height because chicken manure contains complete nutrients to loosen the soil. This results in optimal plant growth and chicken manure can increase water absorption so that the plant's need for water is fulfilled.

4. CONCLUSIONS AND SUGGESTIONS

There are differences in the potential of endophytic fungi to control C. gloeosporioides disease in the latency period. The treatment combination that is the best in inhibiting the emergence of C. gloeosporioides disease symptoms is the E2M2 treatment with a value of 3.67%, on the parameter of disease incidence, the treatment combination is the best in inhibiting the spread of the disease is E2M2 with a value of 0.03% and on the parameter of disease intensity the best combination of treatments in inhibiting disease intensity during 12 HSI is E2M2 with a value of 17.67%. There is an interaction between endophytic fungi and application methods in controlling C. gloeosporioides disease on the parameter of the latent period.

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