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Use of *Trichaderma sp.* for Biological Control of Diplodia Disease in Siam Banjar Citrus

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ABSTRACT

Siam Banjar Citrus has a very bright prospect to be developed, becomes a contributor to the national fruit products with various types and varieties and has the production that does not depend on seasons. Diplodia disease in citrus still becomes a major problem because it causes loss of high yield and affecth the gain of production. The disease, caused by *Botryodiplodia theobromae pat., indicates symptoms of lesions on citrus trunk, such as narrow lines on the* trunk, and can cause plant death due to the disruption of transport of water and nutrients from the soil to the plants. The objectives of the research were to find out the effects of the interaction between Trichokompos fertilizer sp.,to determine the combination of Trichokompos fertilizer and Tricjoderma sp. Solution for Diplodia disease control. The study was conducted from january to april 2012, in Rantau Badauh sub- district. This experiment employed Randomized Blok Design (RBD) consisting of three replications and two factors , namely the dosage of *Trichokompos* fertilizer (0 kg, 10 kg and 20 kg), and the dosage of *Trichoderma* sp. (0 g/l lt EKG, 50 g/l lt EKG , 100 g/l lt EKG, 150g/l lt EKG and 200 g/l lt EKG). The observations were conducted on the presence of fungi and the measurement of bark lesions. The result showed that there was no intraction between the two treatmeants, and the significant effect of the application of *Trichoderma* sp. Against the widespread symptoms of doplodia at all observation time with the best dosage was 50 g/l ECG. The results of the regression indicated that the higher concentration and the longer time of the applicatin of *Trichoderma* sp. Would reduce the widespread of symptoms caused by diplodia in citrus.

Keywords: *Diplodia, Siam Citrus, Trichoderma sp.*

1. Introduction

Siam Banjar Citrus are oranges from Siam (*Thailand*). In their country, Siam Banjar Citrus known as som kwin wan. In the level of the *Rutaceae* family, it is not wrong if the first choice focused on *Citrus nobilis*. Furthermore, if the tangerines themselves are compared, then the Siam citrus get the top position because they are among the most cultivated varieties and the most widely spread. Therefore, it is not surprising that 60% of the citrus market is controlled by siam citrus.

This citrus commodities have a very bright prospect to be developed because the market demand for this commodity is very large. This orange is a contributor of national fruit products, with a many varieties, fairly wide distribution and production that does not depend on the season, so its become the mainstay of the fulfilment of fruit consumption in Indonesia.

In the tropics, there is a lot of disease explosion in unreported horticultural plants even though it has caused a large spoilage. The cumulative disease of *Diplodia* in the Barito Kuala District is 7,189 trees. Often farmers do not feel these losses and do not carry out controls properly. This is because lack of knowledge by staff who guide and recommend to farmers.

According to Semangun (1989) in Ramdan (2011), citrus skin disease, Diplodia, which was divided into two types of attacks, *Diplodia* "wet" and Diplodia "dry". Diplodia's disease is caused by fungus *Botryodiplodia theobromae* Pat. Which causes lesio symptoms in the orange stem in the form of narrow lanes. The advanced symptoms of this disease are in the form of a golden to black gom. The symptoms that very severe disease will cause the death of plants because the bark will be damaged which causes disruption of the transport of water and nutrients from the soil to parts of plants that need water and nutrients (Salamiah, 2008).

In South Kalimantan, *Diplodia's* disease over the past four years, from 2008 to 2011 reached 22,266 trees spread across 10 districts / cities, including the Regency of Barito Kuala reaching 5,741 trees. *Diplodia's* disease control by quarantine, fungicide, California porridge, porridge bordox, eradication, and other methods. However, these methods are not very effective until can be proven by the still-developing disease.

Some fungi have the potential as biological agents of pathogenic fungi including Trichoderma spp. *Trichoderma spp*. used as an antagonistic fungus that can inhibit the development of pathogens through the process of *microparasitism*, antibiosis, and competition (Baker and cook, 1983 in Tindaon, 2008).

2. Literature Review

Diplodia Disease

The symptom of the presence of this fungus is a mixture. Penetration of fungus *B. theobromae* causes the plant to react by releasing a yellow substance in the form of gumosis (gum / blondok). Gumosis is released by plants as a form of reaction after an attack of pathogens in the system, gumosis is produced to localize pathogens so they do not develop wider. Gumosis that comes out from the surface of network shows the level of attacks that have been advanced. However, in the study of Salamiah (2008), it was shown that the fungus colonies could be found on the surface of plant parts without showing symptoms of illness.

Symptoms of Wet Diplodia Attack

This attack is easily known because of the release of a golden yellow blendok (gom) from a large stem. Sometimes attacks are limited to narrow lanes. After a while the affected skin peels and the wound heals. Generally, the disease will develop continuously so that the skin occurs irregular wounds that continue to expand but still superficial. The longer the fungus goes deeper into the skin it can even enter the wood. If it reaches this level, the disease usually develops more quickly. The fungus develops between the skin and wood, damaging the cambium, so the branch is immediately auctioned off and dies. The affected wood is green, blue to black.

Symptoms of Dry Diplodia Attacks

This disease is more dangerous because the initial symptoms are hard to know. The skin dries, if we cut it, the skin and wood beneath it is greenish black. The diseased skin forms small crevices from the inside and came out the original white sppora mass, then black. The diseased part generally extends rapidly, in a short time the big branches have been auctioned off. The infection is only known if the leaves have turned into yellow so that the stems/branches that sick cannot be cured again (Dirjen Hortikultura, 2002).

Diplodia's Disease Cycle

Until now the cycle of disease is not yet known clearly, *B. theobromae* is a polyphagic fungus that can attack various plants, so that the source of infection will always be there. The spread of this fungus was thought only through water, it turned out several ways, such by the air, vector attack, attachment, and splashing of water. The fungus can survive in various places; the seeds, fruit skin, plant stumps, and tree branches that are still healthy (Salamiah, 2008).

The Factors that Influence Diplodia Disease

Environmental factors that contribute a major role in influencing the beginning and development of plant diseases are temperature, humidity, light, nutrients, and soil pH. Diplodia disease likes the moist environment of plants, especially citrus gardens which is lined with living fences, so that the fence prevents irradiation of plantations. Pest attacks and mechanical damage can also help in disease progression (AAK, 1994 in Fitri, 2005).

Biological Control

Biological control can be in the form of: 1) technical culture (habitat management), its make the environment supportive for antagonistic growth, the use of host plants that are resistant, or 2) crossing plants to increase resistance to pathogens or the condition of host plants that support (preferred) antagonistic activity, 3) antagonist introduction, non-pathogenic strains, and other organisms that have the same benefits.

The use of Trichoderma spp. in biological control

Rasminah (1995) in Khaeruni (2010) states that the use of microorganisms as control agents still need to be developed. The development of microorganisms needs have knowledge of the microorganisms types, types of diseases and also mechanisms for controlling plant diseases using microorganisms. This utilization is expected to help control disease without using environmental conditions.

Factors that strengthen *Trichoderma spp*. As one of the biological control agents is the discovery of processes, among others, the emergence of the chemotropic response of *Trichoderma spp*., Influenced by the host by microparasites, excretion of cellular enzymes, and the occurrence of release in the host. (Chet, 2987 in Ramadhani, 2011).

3. Research Methods

The treatment in this research is:

1. Fertilizer dosage *Trichokompos (p*) consists of 3 levels:
   P_0 = control without treatment, P_1 = 10 kg p_2 = 20 kg
2. *Trichoderma spp*. (t) dosage consists of 5 levels:
   t_0 = control without treatment t_1 = 50 g/lt EKG, t_2 = 100 g/l lt EKG,
   t_3 = 150 g/l lt EKG, t_4 = 200 g/l lt EKG

Experimental design

This experiment is factorial experiment consists of 2 factors, *Trichokompos* (3 levels) and *Trichoderma spp.* (5 levels). This environmental design used randomized block design (RBD) with 3 repetition, so there are 45 experimental units. The combination of treatments from this experiment can be seen in Table 1.

Table 1. Combination of factorial experimental treatments in a randomized block design

Fertilizer dosage	***Trichoderma spp*. (t) dosage with EKG solution (t)**				
Trichokompos (p)	**Control (t_0)**	**50 g (t_1)**	**100 g (t_2)**	**150 g (t_3)**	**200 g (t_4)**
Control (p_0)	t_0p_0	t_1p_0	t_2p_0	t_3p_0	t_4p_0
10 kg (p_1)	t_0p_1	t_1p_1	t_2p_1	t_3p_1	t_4p_1
20 kg (p_2)	t_0p_2	t_1p_2	t_2p_2	t_3p_2	t_4p_2

The additive linear model to analyze each variable observed is:

$$\mathbf{Y_{ij}K = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \sum_{ij}k}$$

4. Results and Discussion

Based on the results of variance analysis it is known that there is no interaction between the application of *Trichoderma spp*. With the fertilization of Trichocompos on the extent of symptom diplodia at all times of observation. The significant influence is shown by the treatment of applying Trichoderma spp. (independently) which is seen in all observations, which is in observations $X_{1\text{-}2}$ to $X_{5\text{-}6}$. While the real effect is only seen the treatment of giving *Trichocompos* (independent) occurred in the observation $X_{1\text{-}2}$. The average effect of applying *Trichoderma spp.* The extent of the symptoms of diplodia at all times of observation.

Table 2. The average effect of applying *Trichoderma spp*. (t) on the extent of symptom *diplodia* (cm_2) at all the time observations (X_{1-2} hingga X_{5-6})

Treatment (g/I EKG)	Observation									
	ΔX_{1-2}		ΔX_{2-3}		ΔX_{3-4}		ΔX_{4-5}		ΔX_{5-6}	
	Asli	Transf. Ln.x	Asli	Transf. Ln.x	Asli	Transf. Ln.x	Asli	Transf. Ln.x	Asli	Transf. Ln.x
t_0 = 0	0,00	1,61 c	1,28	2,44 d	0,367	2,73 c	1,06	3,05 c	0,24	3,01 c
t_1 = 50	-0,26	1,56 c	-2,67	1,99 c	-7,12	**1,95 a**	-8,43	**2,34 a**	-7,42	**2,48 a**
t_2 = 100	-0,63	1,47 c	-3,27	1,91 b	-2,26	2,53bc	-3,34	2,80 b	-1,53	2,91bc
t_3 = 150	-1,74	1,17 c	-4,03	1,88 a	-3,69	2,37 b	-5,92	2,60 a	-2,16	2,87 b
t_4 = 200	-2,92	**0,67 a**	-3,93	**1,79 a**	-3,11	2,46bc	-5,86	2,58 a	0,00	2,99bc

Description: The numbers with the same letters are followed, in the same column it shows not significantly different based on DMRT on level α = 0,05

Based on the average area of symptom diplodia in Table 2, it can be seen in the observation X_{1-2} and X_{2-3} application of *Trichoderma spp*. With concentration 200 g 1^{-1} EKG (t_4) the wounded parts of the plant can cause a greater decrease in the area of *diplodia* symptoms compared to the application of *Trichoderma spp*. Concentration 150 g 1^{-1} EKG (t_3), 100 g 1^{-1} EKG (t_2), 50 g 1^{-1} EKG (t_1) and control (t_0). In treatment X_{1-2}, the control treatment had the highest average of symptoms of attack and was not statistically different from the treatment 50 g 1^{-1} EKG and concentration 100 g 1^{-1} EKG. Treatment with concentration 150 g 1^{-1} EKG showed a significant difference in effect with control, treatment with concentration 50 g 1^{-1} EKG and 100 g 1^{-1} EKG. The smallest attack symptoms can be seen in the treatment of applying *Trichoderma spp.* With concentration 200 g 1^{-1} EKG. Observation of X_{2-3} shows plants treated with the application of *Trichoderma spp.* With concentration 200 g 1^{-1} EKG it does not have different effect with treatment 150 g 1^{-1} EKG with the area of each wound 1,79 cm^2 and 1,88 cm^2. Both treatment have different effect in suppressing the widespread attacks of *Diplodia* disease symptoms when compared with concentrated basting treatments 100 g 1^{-1} EKG, 50 g 1^{-1} EKG and control. The area of injury caused by *Diplodia's* disease is highest seen in the control treatment with extensive 2,42 cm^2. observation X_{3-4} for widespread symptoms of *diplodia* with the application of *Trichoderma spp*. Range between 1,95 – 2,73 cm^2. The smallest wound area was seen in plants applied with *Trichoderma spp*. With concentration 50 g 1^{-1} EKG. The difference effect is not real is shown by the treatment of the application of *Trichoderma spp*. At concentration 150 g 1^{-1} EKG and 100 g 1^{-1} EKG and 200 g 1^{-1} EKG, but it has different treatment with control.

The average area of symptom *diplodia* in observation X_{4-5} range between 2,34 – 3,05 cm^2, the highest area of symptom *diplodia* did not receive treatment, The best effect of *Trichoderma spp.* is still shown by the concentration treatment 50 g 1^{-1} EKG, although it does not have different from the treatment 150 g 1^{-1} EKG and 200 g 1^{-1} EKG. This can be seen from the average area of the wound that can be suppressed by Trichoderma spp., that the concentrated 50 g 1^{-1} EKG, has been able to suppress wounds as large as 2,34 cm^2. It also in observations X_{5-6}, the best treatment is shown by plants that get the application of Trichoderma spp. With concentration 50 g 1^{-1} EKG, the area of the wound around 2,48 cm^2. This treatment indicate very significant difference with this treatment that showing a very significant difference with the treatment applied to the concentration 100 g 1^{-1} EKG (2,91 cm^2), 150 g 1^{-1} EKG (2,87 cm^2), 200 g 1^{-1} EKG (2,99 cm^2) and control (3,01 cm^2).

The effect of *Trichocompos* treatment that independently is also known to reduce the extent of diplomatic symptoms in citrus plants which are generally that more able to reduce the extent of symptoms when compared with plants not given *Trichocompost*, although the effect of fertilizer is only seen in observations X_{1-2}. Usually the effect of administering *Trichocompost* independently at a dose of 20 Kg is a treatment that has the best effect on suppressing symptoms of attacks by *Diplodia*. The treatment showed significant differences with Control plants, but did not show a significant difference with the plants that were treated with a dose of 10 Kg.

Table 6. The average *of Trichocompost* influence (p) on the extent of *diplodia symptom* (cm^2) at all the time observations (X_{1-2} to X_{5-6})

***Tricho-kompos* (kg)**	**Observation**									
	ΔX_{1-2}		**ΔX_{2-3}**		**ΔX_{3-4}**		**ΔX_{4-5}**		**ΔX_{5-6}**	
	Asli	**Transf. Ln.x**	**Asli**	**Transf. Ln.x**	**Asli**	**Transf. Ln.x**	**Asli**	**Transf. Ln.x**	**Asli**	**Transf. Ln.x**
$P_0 = 0$	-0,91	1,38 b	-2,33	2,02	-4,35	2,250	-5,67	2,570	-3,47	2,752
$P_1 = 10$	-1,09	1,31 ab	-2,55	1,98	-2,19	2,256	-4,21	2,687	-1,17	2,928
$P_2 = 20$	-1,33	**1,20 a**	-2,69	1,93	-2,94	2,449	-3,61	2,769	-1,87	2,871

Note: Numbers with the same letters are followed, in the same column it shows no significant difference based on DMRT at the level α = 0,05

Based on the results of a regression analysis that illustrates the relationship between application of *Trichoderma spp*. the different concentrations on the extent of the *diplodia* symptoms showed a real negative linear relationship only on observation X_{1-2} and X_{2-3}. A real negative linear relationship between the application of *Trichoderma spp*. With certain concentrations of the widespread decrease in *diplodia* symptoms in oranges. In this case, it can also be suspected that every increase in concentration 10 g 1^{-1} EKG *Trichoderma* spp. In once observation period, it will reduce the extent of the symptoms of diplodia by as much as 2,36 cm^2.

5. Discussion

Based on the results of statistical analysis and regression which shows the influence and relationship of the administration of *Trichoderma spp.* In suppressing the extent of diplomatic symptoms, especially at some time of observation where the higher the concentration of *Trichoderma spp.,* The higher extent of symptoms due to diplomatic disease will be higher. Even so, concentration 50 g 1^{-1} become a better treatment that compared to the concentration of *Trichoderma spp*. are generally higher in concentration. This is related to the consideration of the efficiency of *Trichoderma spp*. Even though it is known with high concentration, it can suppress the diplodia symptoms, but with the concentration of *Trichoderma spp*. Even the low has been able to reduce the symptoms due to the disease of the *diplodia*. Thus it is expected that the farmers in the field have been able to control diplodia attacks even if only with concentration 50 g 1^{-1}.

In addition to considering the efficiency and effectiveness of the administration of *Trichoderma spp.,* The ability of *Trichoderma spp*. As well as the environmental factors that also have greatly affect development of *Trichoderma spp*. The process of *mycoparasitism* from *Trichoderma spp*. It is a complex process and consists of several stages in attacking its host. In addition, the availability of nutrients for *Trichoderma spp*. It also very important to consider. This is to prevent the occurrence of nutrient competition that is used as food by the *Trichoderma spp*.

The ability of biological agents to suppress disease events is due to their ability to produce several benefits both to plant growth and to suppress the incidence of disease in the field. The ability of Trichoderma spp. In reducing the incidence of disease directly through the mechanism of parasitism, while indirectly through space competition and nutrition.

The successfulness and effectiveness of this biological control effort also depends not only on biological control agents, but also on ways and strategies to maintain population levels and activities in accordance with plant growth and development. Maintain the quality of the biological control agent itself. Based on this, in this study it can be seen from the relationship of the regression equation between the concentration of *Trichoderma spp*. Given the widespread symptoms of *Diplodia's disease*, where the greater the concentration given, it would suppress the extent of injuries caused by *Diplodia's* disease. In addition, it is also known that the longer the administration and observations made, the smaller the injury caused by *Diplodia* disease.

6. Conclusions and Suggestions

Conclusions

1. There is no significant effect between *Trichoderma spp*. with *Trichocompost* on the extent of *Diplodia* disease attacks was observed ΔX_{1-2}, ΔX_{2-3}, ΔX_{3-4}, ΔX_{4-5}, and ΔX_{5-6}. Influence of independent that

applying *Trichoderma spp*. indicate the difference in observation ΔX_{1-2} to ΔX_{5-6}. Even though the fertilization with *Trichocompos* showed a significant effect on the extent of the symptoms of *Diplodia* on observation ΔX_{1-2}.

2. The best dosage for applying Trichoderma spp. is 50g / 1L EKG and *Trichocompost* fertilizer is 20 kg.
3. Increased concentration of *Trichoderma spp*. Given, it is known to be able to reduce the widespread symptoms of *Diplodia* disease, where at each increase in concentration of 10 $g1^{-1}$ ECG it would reduce the extent of Diplodia's symptoms by 1.46 cm^2, and the longer *Trichoderma spp.* Given, the widespread symptoms of the *Diplodia* disease attack will be suppressed by 2.36 cm^2.

Suggestion

Based on the research that has been done, it is expected that ongoing research to find out how the antagonist mechanism and the use of antibiotic compounds produced by *Trichoderma spp.,* So that more effective and efficient way for farmers, especially in suppressing widespread disease symptoms *Diplodia* in plants Siam Banjar orange in South Kalimantan.

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