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Effect of Salt Content (NaCl) on the Antagonistic Power of Gliocladium sp. in Inhibiting the growth of Fusarium oxysporum f. sp. Cubense Causes Fusarium wilt in Banana Plants

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ABSTRACT

This study aims to determine the effect of salt/salinity (NaCl) levels on the growth of Gliocladium sp. (Colony area, dry weight and number of spores) and antagonistic activity of Gliocladium sp. in inhibiting Foc. The research was conducted at the Laboratory of Plant Diseases, Department of Plant Pests and Diseases, Faculty of Agriculture, University of Brawijaya. The stages in this study included counting colony area, sporulation and dry weight of Gliocladium sp. with single culture method, antagonism test between Gliocladium sp. and Foc with dual culture method. In the research stage to determine the effect of salt levels used NaCl, with concentrations of 0 gr/l, 4 gr/l, 8 gr/l, 12 gr/l and 16 gr/l. The design method used in this study was a completely randomized design (CRD). Banana plants can grow in tropical climates with a pH of 4.5-7. In banana cultivation there are several obstacles that can reduce banana production, the main obstacle is caused by the attack of the pathogen Fusarium oxysporum f. sp cubense (Foc) which causes banana plants to wilt. Efforts to control the attack of pathogenic Foc, can be done by utilizing the antagonistic fungus Gliocladium sp. The results showed that the colony area, sporulation and dry weight of Gliocladium sp. showed that overall growth increased with increasing salt content used, the results obtained were colony area, sporulation and dry weight of Gliocladium sp. The highest yield was found in the treatment with a concentration of salt content of 16 gr/l, with values of 4.184 cm2, 7.933 spores/ml and 0.132 gr respectively, while the antagonism test between Gliocladium sp. and Foc, the highest inhibition percentage of Gliocladium sp. occurred at a salt concentration of 16 gr/l of 45.556%. The research shows that the addition of NaCl to the media has a positive effect on Gliocladium sp.

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1. INTRODUCTION

Banana (Musa paradisiaca) is a fruit commodity that is widely consumed by Indonesian people. This is because bananas are easy to obtain, the price is relatively cheap, the nutritional quality is good and the way to cultivate bananas is also easy and can grow quickly. The demand for bananas by the market is quite large both locally and for export, apart from being consumed

fresh, they are also processed in other forms that have added value, for example making chips, sale and banana flour (Cahyono, 1995). All parts of the body of the banana plant can be used from the hump to the leaves. Parts of banana plants can be used as animal feed additives and cultural arts and crafts (Anonymous, 2006).

Banana plants are native plants from Indonesia with an estimated 200 to 300 types of bananas, but around 20 species that have quite high economic value. The types of bananas that are usually planted by farmers are ambon bananas, kapok, raja, moss etc. In general, in the cultivation of banana plants, farmers almost do not use planting technology so that the production obtained is still low. Banana plants can grow in tropical climates with a pH of 4.5-7.5 and with a NaCl range of 0.07% (Satuhu and Supriyadi, 1990).

In addition to environmental factors causing a decrease in banana production both in quality and quantity are pests and diseases. One of the main diseases found in bananas in Indonesia is Panama Wilt. This disease is caused by the fungus Fusarium oxysporum f. sp. cubense (Foc).

Foc fungi form conidia, branching conidiophores and crescent-shaped, small-stemmed macroconidia, often in pairs. The mycelium is mainly found inside the cells, especially in the wood vessels, also forms the mycelium which is found between the cells, namely in the skin and parenchyma tissue near the infection. Inoculum F. oxysporum f. sp. cubense consists of macroconidia, microconidia, chlamydospores and mycelia. Foc fungi can survive in the soil for several years. Pathogenic populations can persist naturally in the soil and on the roots of diseased plants. If there are sensitive plants, through the injured roots can immediately cause infection.

Foc pathogens attack stem pith tissue through injured or infected roots. The stems that are attacked will lose a lot of liquid and turn brown in color, the lower edge of the leaves becomes dark yellow (wilted), spreads to the inside quickly so that the entire leaf surface turns yellow. The petiole is broken at the base where it abuts the false stem. Sometimes the outer layer of the false stem is split, starting at the ground level. If the base of the stem is split lengthwise, you can see brown or black lines going in all directions from the base of the stem (hump) upwards, through the vascular tissue at the base and petiole. According to Natsir et al. (2003), all types of commercial bananas are susceptible to Foc and are also one of six plant diseases that are dangerous and destroy agricultural crops in the world.

Panama disease is widespread in all world banana production centers such as South America, Africa, Asia and Australia. This pathogen will soon develop or spread if susceptible banana species are planted in contaminated locations. The disease is also spread by transferring infected tillers or shoots to pathogen-free land so that the land becomes contaminated. Biological Control is an alternative solution that needs to be developed because it is relatively cheap and easy to apply, besides that it is also environmentally friendly. The use of antagonistic microbes as biological agents to control Fusarium wilt is an example of biological control.

2. METHOD

2.1 Types of research

The research method used in this study is the dual culture method. The dual culture method was carried out to observe the direct interaction that occurs between the antagonistic agent T. harzianum and the pathogen Colletotrichum by growing the antagonistic and pathogenic molds together in one petri dish.

2.2 Research variable.

Variable observations include measurement of the increase in length of the diameter of the mushroom colony, the amount of sporulation of Gliocladium sp. and measurement of the dry weight of the fungus, the percentage of inhibition of Gliocladium sp.

2.3 Research design

This research was conducted to test the effect of salt content (NaCl) on Gliocladium sp. in the control of fusarium wilt in bananas. The treatment was arranged in a completely randomized design (CRD), with two sets of experiments and three replications. The data obtained is then analyzed using the F test with a level of 5% and if there is a significant difference, then it is done with the BNT test.

2.4 Sampling location

In this research stage using salt to determine the effect of salt levels used NaCl, with concentrations of 0 gr/l, 4 gr/l, 8 gr/l, 12 gr/l and 16 gr/l on the growth of Gliocladium sp.

2.5 Time and Place of Research.

This research was conducted from December 2008 to March 2009 at the Laboratory of Plant Diseases, Department of Plant Pests and Diseases, Faculty of Agriculture, University of Brawijaya.

2.6 Tools and materials

The tools used in this study were autoclaves, petri dishes, bunsen, ose needles, test tubes, tube racks, bunsen, pipettes, syringes, cok borrer and the materials used were NaCl, PDA, Gliocladium sp. isolates, Fusarium oxysporum f isolates. . sp. cubense, sterile distilled water, rice, alcohol.

2.7 Research procedure

The test was started with the t fungus being re-isolated on PDA media which had been mixed with streptomycin. Then incubated for 7 days with a temperature of 28° C, the results of this isolate will be used for research then the isolate will be mixed with salt and the bacteria that are tested with a mixture of NaCL. the testing was started with the t fungus being re-isolated on PDA media which had been mixed with streptomycin. Then incubated for 7 days with a temperature of 28° C, the results of this isolate will be used for research then the isolate will be mixed with salt and the bacteria that are tested with a mixture of NaCL.

2.8 Data analysis.

The data obtained was tested statistically using the F test ANOVA (5%) to see the difference in effect between treatments. If there is an effect between treatments, continue with Duncan's test at the 5% level. Correlation test is used to determine the relationship between parameters.

3. RESULTS AND DISCUSSION

3.1 Research result

3.1.1 Identification of Fusarium oxysporum f. sp. cubense and Gliocladium sp.

The results of isolation and identification showed that the morphology of Foc colonies cultured on PDA media had the characteristics of a pinkish-white central colony color, purplish white. The color of the edge of the colony is white, the color behind the center is brownish white, the color behind the edge is white and the shape of the edge is like a round, the edge spreads and the microscopic results obtained form macroconidia in the form of long, both ends blunt and short, both sharp ends consisting of three partitions.

Microconidia are oval, short and form chlamydospores in the form of intercalaries found in the middle of the hyphae. Leslie and Summerell (2006) stated that the Foc character on PDA media showed that the resulting colors were reddish white and purplish red. Jones (1999), argued that Foc cultures experienced rapid growth of 4-7 mm/day and the macroconidia of Foc were shaped like "crescents" with various sizes, short or long at slightly bent ends and generally consisting of 3 septa.

Domsch et al. (1980) added that microconidia were short, elliptical, and septa were not visible, while terminal or intercalary chlamydospores were found in hyphae, and also in conidia and Gliocladium sp. isolates. obtained from the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Brawijaya. Characteristics of the fungus (Figure 5), initially white then light green to dark green. The growth of this fungal colony is slow, developing by means of spore dispersal. From microscopic observation it is known that the conidia of Gliocladium sp. round, mycelium insulated, conidiophores branched a lot.

3.1.2 Effect of Salt Content (NaCl) on Gliocladium sp.

Salt content (NaCl) affects the growth of Gliocladium sp. For each concentration used, the salt content of Gliocladium sp. growth increased. Experiencing improvement.

Table 1. Average growth of Gliocladium sp. on the effect of salt content (NaCl)

NaCl	0g/l	4g/l	8g/l	12g/l	16g/l
Colony area average (7 days old)	2,77a	2,937a	3,659b	3,713b	4,184c
Average amount spores (spores/ml)	5,167a	5,8b	6,917c	7,267d	7,933e
Average weight dry (gr)	0.083a	0.086a	096a	0.118bc	0.132c

Note: Numbers followed by the same letter on each line were not significantly different at the LSD test level <p= 0.05%>.

The results of the experiment showed that the administration of NaCl to Gliocladium sp. affect the shape of the growth of the colony area. After seven days of incubation period, Gliocladium sp. can grow on PDA media with varying salt levels. The growth of the colony area of Gliocladium sp. Experiencing a change or increase to the limit of the concentration of salt used. the number of Gliocladium sp. spores. with variations in salt content also increased. The results of the BNT test (Table 1) showed that the treatment of variations in salt content had a very significant effect on increasing the number of Gliocladium sp. spores. The number of Gliocladium sp. spores.

The results of the Analysis of Variance test (Table 1) showed that the treatment of variations in salt content had a significant effect on increasing the dry weight of Gliocladium sp. mycelia. The dry weight of mycelia from the treatment with salt levels of 0 gr/l (control), 4 gr/l, and 8 gr/l was not significantly different between the three, but significantly different from the treatments with salt levels of 12 gr/l and 16 gr/l, while dry weight of mycelia in the treatment of salt content of 12 gr/l and 16 gr/l was not significantly different from each other.

3.1.3 Effect of salt content (NaCl) on the antagonist Gliocladium sp. In inhibiting Foc.

The results of the experiments showed that the inhibition of Gliocladium sp. to Foc, the resulting inhibition increased with increasing salt content. After seven days of incubation, the inhibition of Gliocladium sp. At salt levels of 0 gr/l and 4 gr/l, 31.111% and 34.827% were not significantly different, as well as between salt levels of 8 gr/l and 12 gr/l, while the inhibition at salt levels of 16 gr/l and also the inhibition highest is significantly different from the others.

According to Papavizas (1985) Gliocladium sp. capable of producing gliotoxin, antibiotics and enzymes (exoglucanase, endoglucanase, cellobiase and chitinase). These compounds play an important role in inhibiting the growth of Fusarium sp. capable of producing lycomarasmin and fusaric acid toxins.

3.2 Discussion

The results of the Analysis of Variance test (Appendix 1) show that the variation in salt content has a significant effect on the inhibition value (%) of Gliocladium sp. against Foc. The inhibition value of the 0 g/L salt content (control) was not significantly different from 4 g/L, but significantly different from the other salt content treatments (8, 12, and 16 g/L). In the treatment of salt content 8 and 12 g/L there was no significant difference between the two, and for the treatment of salt content of 8 g/L it was significantly different from the treatment of salt content of 16 g/L, while the treatment of salt content of 12 g/L was not significantly different from the treatment 16g/l.

Banana plants can grow in tropical climates with a pH of 4.5-7.5 and with a NaCl range of 0.07% (Satuhu and Supriyadi, 1990). In addition to environmental factors causing a decrease in banana production both in quality and quantity are pests and diseases. One of the main diseases found in bananas in Indonesia is Panama Wilt. This disease is caused by the fungus Fusarium oxysporum f. sp. cubense (Foc). According to Natsir et al. (2003), all types of commercial bananas are susceptible to Foc and are also one of six plant diseases that are dangerous and destroy agricultural crops in the world.

Panama disease is widespread in all world banana production centers such as South America, Africa, Asia and Australia. Hassan and Shahzhad (2004) stated that NaCl can be used to control Gibberella fujikuroi and F. oxysporum which cause root rot in asparagus. Hedge and Karande 1978 (in Hassan and Shahzhad; According to Papavizas (1985) Gliocladium sp. is capable of producing gliotoxins, antibiotics and enzymes (exoglucanase, endoglucanase, cellobiase and chitinase). These compounds play an important role in inhibiting the growth of Fusarium sp. which is capable of producing lycomarasmin and fusaric acid toxins Hassan and Shahzhad (2004) stated that NaCl can be used to control Gibberella fujikuroi and F. oxysporum which cause root rot in Asparagus. Hedge and Karande 1978 (in Hassan and Shahzhad.

4. CONCLUSION

From the results of the research that has been done, it is concluded that the addition of salt content to the Gliocladium sp. effect on the growth of Gliocladium sp., from a salt concentration of 0 – 16 g/l the growth of Gliocladium sp. Experiencing additions or improvements. Colony area, dry weight and sporulation were highest at 16 g/l salt content, with values of 4.184 cm2 colony area, 7.933 spores/ml sporulation and 0.132 g dry weight and in the antagonist test showed that the inhibitory power of Gliocladium sp. the highest was found at the salt content of 16 gr/l the average inhibition produced was 45,556%.

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This research is an initial study of the effect of salt levels on the antagonism of Gliocladium sp. in inhibiting Foc that causes wilt in banana plants on a laboratory scale. For this reason, it is necessary to carry out further research on the effect of salt levels on the antagonism of Gliocladium sp in the field.

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