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The Effectiveness of Inhibition Local Bacterial Isolate from Rhizosphere Of Oil Palm Plant On Leaf Spot Disease In Purple Eggplant (*Solanum melongena* L.)

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ABSTRACT

Longterm use of pesticides can cause side effects on the environment and human health. PGPR is an environmentally friendly solution for biological control of leaf spot disease. This research aims to obtain bacterial isolates from the rhizosphere of oil palm plants which have the potential as biological agents to control fungal leaf spot diseases in eggplant plants. The research was carried out using 10 treatments, namely giving 9 samples of different bacterial isolates and 1 control and repeated 2 times, bringing the total to 20 plant samples. The results showed that there were 4 bacterial isolates from the rhizosphere of oil palm plants that had great potential to inhibit the growth of leaf spot fungi, namely isolate R5 (99.35%), isolate R2 (98.1%), isolate R6 (94.69%) and isolate R10 (94.29%). The bacterial isolates that were able to reduce the percentage of disease attacks compared to the first week of observation to the fourth week were isolates R4, R2 and R10, with a reduction in the percentage of disease attacks respectively of 15.15%, 7.14% and 1.98%. Based on the data, it can be seen that the R2 bacterial isolate is an isolate that has the potential to inhibit fungal isolates and reduce the percentage of leaf spot disease attacks on eggplant plants.

Keywords: *rhizosphere bacteria, leaf spot disease, Solanum melongena L.*

1. INTRODUCTION

Purple eggplant is a vegetable plant belonging to the Solanaceae family. Eggplant is also very popular and liked by many people because of its delicious taste, especially when used as a vegetable or fresh vegetable. Apart from that, eggplant also contains quite high levels of nutrition, especially vitamin A and phosphorus. Every 100 grams of raw eggplant contains 26 calories, 1 gram of protein, 0.2 grams of charcoal hydrate, 25 IU of vitamin B and 5 grams of vitamin C. Apart from that, eggplant also has medicinal properties because it contains the alkaloids solanine and solasodine (Sunarjono *et al.,*2003). According to the Central Statistics Agency, the productivity of purple eggplant plants in Indonesia from 1997 to 2012 reached 518,827 tons/ha, an increase of 1.43%. National eggplant production tends to increase every year, but eggplant production in Indonesia is still low and only contributes 1% of world demand. According to (Simatupang, A. 2014), this is because the area of land for eggplant cultivation is still small and the form of cultivation is still sideline and not yet intensive. Purple eggplant production results usually influence and depend on farming inputs, fertilizer use and in terms of treating weeds, pests and diseases in eggplant plants. Some farmers stated that the production of purple eggplant plants had decreased due to attacks by pests and diseases on the leaves and fruit so that the production results were not as desired (Westphal et al., 2009)

Leaf spot disease is one of the main diseases of purple eggplant plants. This fungus infects all stages of plant life but will be dangerous if it attacks during flowering (Semangun, H. 2004).Symptoms of leaf spot disease are rarely found on fruit, only limited to the leaves. Typically, this disease is known as brown spots covered in stains that appear on the surface and in the middle they are black decorated with yellow spots surrounding the parent spot (Jusfah, 2009). This leaf spot disease can reduce photosynthetic capacity, resulting in premature leaf shedding and encouraging uneven fruit formation. After the leaves drop, followed by the death of the roots and twigs, it can result in a decrease in eggplant production of up to 50% (Anggraeni, I. 2007)

In the early stages, the symptom of leaf spot disease is contact with the agent pathogenic with a susceptible host, followed by infection into the host tissue, then the development of interactions between the pathogen and the susceptible host and eventually disease will arise (Saleh, N. 2010). According to(Suwahyono and 2001), the initial sign of leaf spot disease in purple eggplant plants is stains or spots on the surface of the leaves with clear boundaries, the shape of the spots on the leaves varies and tends to be irregular, the size of the spots gets bigger over time and will expand. until it covers all parts of the leaf. On leaf spots that have expanded, the color boundary between the middle and the edge will be clearly visible, in the middle of the spot the color is clearer and brighter than on the edges of the spot. In general, the symptoms of leaf spot are the formation of dead areas due to necrosis with varying colors ranging from yellow, brown to black.

One alternative for controlling leaf spot disease is to use commonly used biological agents, namely from the fungal group, such as *Trichoderma harzianum*, which can be applied in dry or wet form to seeds before planting. Giving *Trichoderma harzianum* can increase the number of roots and leaves become wider, *Trichoderma harzianum* can produce lytic enzymes and antifugal antibiotics. Apart from that, *Trichoderma* sp can also compete with pathogens and can help plant growth, and can inhibit various types of fungi (Kishore *et al.,* 2005).Another way to use biological agents is that it can be done by using types of bacteria or fungi that can become parasites or natural enemies.

Biological control of leaf spot disease is carried out by spraying a suspension of *Bacillus circulans* GRS 243 or *Serratia marcescens*. These bacteria can reduce yield losses by 62% and 75% respectively in the field (Walida et al.,2019)

Research regarding the use of biological agents has been widely carried out in the world, but in the Labuhanbatu area there is still very little research. One of the studies conducted by (Sriyanti., 2015), succeeded in isolating 10 bacterial isolates with different macroscopic and microscopic morphological characteristics. Based on this research, it is known that there are 2 bacterial isolates that can inhibit the growth of the white root fungus pathogen. The bacterial isolates that have been successfully isolated will be continued by testing them for leaf spot disease in eggplant plants. It is hoped that the results of the research will isolate bacterial isolates from the rhizosphere of oil palm plants as a potential and effective biological agent in controlling fungi that cause leaf spots.

2. MATERIAL AND METHODS

The research was carried out in the basic laboratory and experimental field of the Faculty of Science and Technology, Labuhanbatu Rantauprapat University. The research was carried out using 10 treatments, namely giving 9 samples of different bacterial isolates and 1 control. Each sample plant was repeated 2 times. So the total number of plants is 20 plant samples. The research was carried out in several stages, namely:

Isolation of Leaf Spot Pathogen

Isolation of the pathogen causing the disease was carried out by taking samples of purple eggplant leaves infected with leaf spot disease. The leaves are cut to a size of 1 cm x 1 cm right in the symptomatic area. The leaf pieces were put into an elemental flask containing a mixture of 10 ml of water and 10 ml of NaOCl and then left for 1 minute. Next, the leaf pieces are washed clean using sterile distilled water. The leaf pieces are then placed in 70% alcohol and left for one minute. The leaf pieces are then washed again using sterile distilled water and drained. The leaf cuttings were then placed in a petri dish containing PDA media, then incubated at 25^{o}C for 2 - 7 days.

Antagonist Test of Bacterial Isolates Against Leaf Spot Disease Pathogens

Each oil palm root rhizosphere bacterial isolate was streaked at a distance of 3.5 cm from the edge of the cup containing PDA medium. 5 mm long leaf spot fungus mycelium from a 7 day old culture was placed on the other side of the petri dish. The petri dishes were then incubated at 25^{o}C for 10 days. Next, the percentage of inhibitory power of bacterial isolates against pathogens is calculated using the formula by [12].

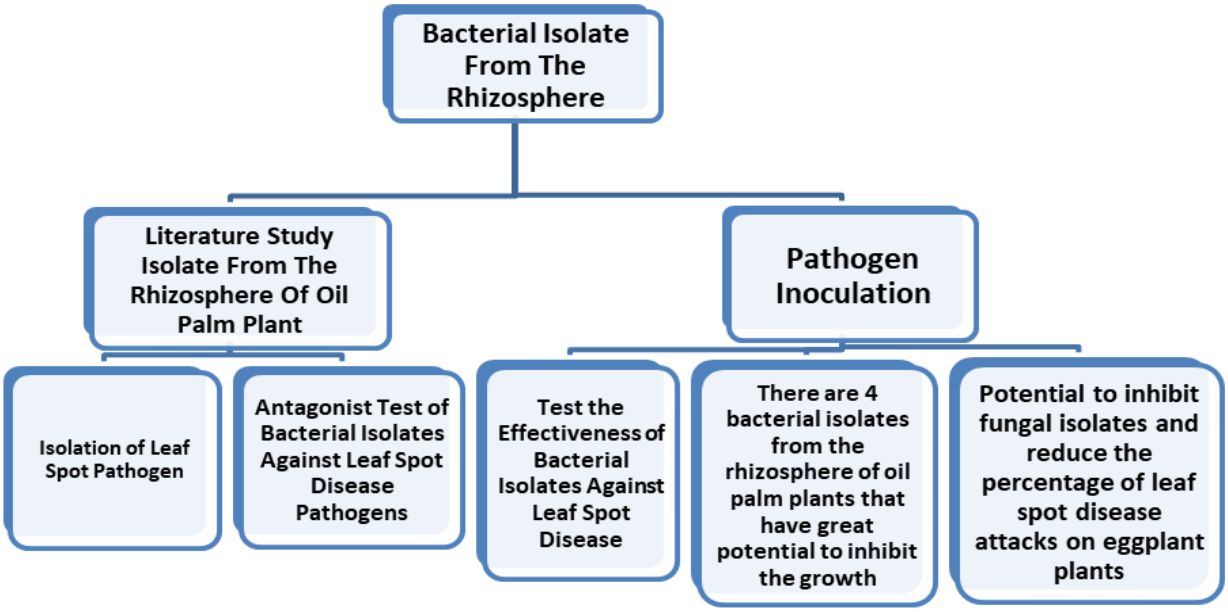
Pathogen Inoculation

The pathogen suspension was made by subculturing the leaf spot fungus first. After 7 days, the conidia were harvested by adding 20 ml of sterile water into a petri dish, then the surface of the isolate was rubbed gently using an L-shaped spatula. The conidia suspension was then filtered using filter paper. After filtering, the suspension is then inoculated onto the leaves of the eggplant plant by spraying it on 3 young leaf crowns. After the purple eggplant plants show symptoms of leaf spot disease, namely approximately 15 days after inoculation of the pathogen, the effectiveness of each bacterial isolate is then carried out.

Test the Effectiveness of Bacterial Isolates Against Leaf Spot Disease

One dose of bacterial isolate each was placed in 10 ml of NB and incubated at room temperature. After the culture was 1 day old, the bacterial density of the culture was calculated (OD around 0.5) using a spectrophotometer at a

wavelength of 600 nm. Each isolate was then sprayed onto disease-infected sample plants according to the treatment plot. Applications are carried out once a week for one month of observation. Next, calculate the percentage of disease using the formula by [13].



Figuer 1. Stages Of Research Isolate From The Rhizosphere Of Oil Palm Plant

3. RESULTS AND DISCUSSION

Based on the results of antagonist tests carried out between isolates of rhizosphere bacteria from oil palm plants against leaf spot fungi (*Cercospora* sp), it is known that there are 4 isolates that have great potential to inhibit the growth of leaf spot fungi. The percentage of ability to inhibit leaf spot fungus isolates from the four isolates was ranked as follows, namely isolate R5 at 99.35%, isolate R2 at 98.28%, isolate R6 at 98.28% and isolate R10 at 96.69%. These results are presented in Figure 1 below.

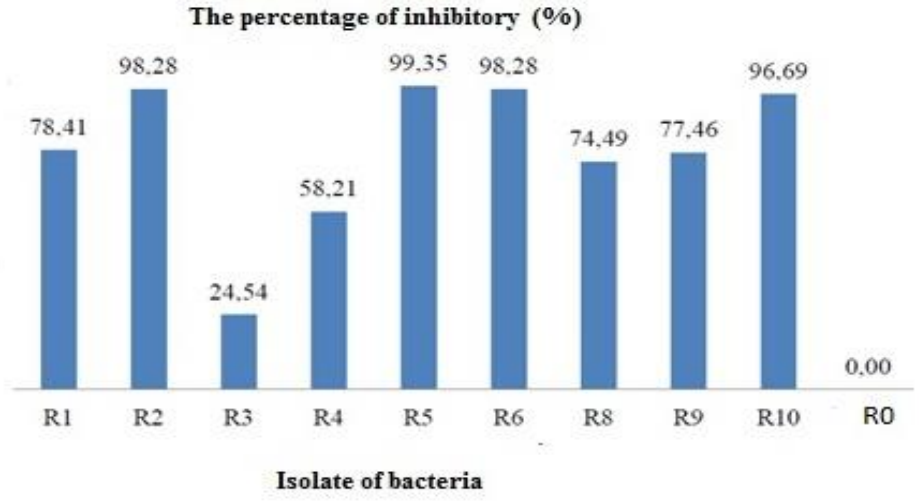


Figure 2. Percentage of Leaf Spot Fungus Inhibition

The percentage of inhibitory power of each isolate is known from the calculation of the bacterial colony area tested with the pathogen, where the bacterial isolates have a bacterial colony area that gradually increases in size from day by day and was able to inhibit and suppress the growth of fungal isolate 01 (*Cercospora* sp). This is thought to be due to the ability of bacterial isolates to produce antibiotic compounds, degrading enzymes or toxic compounds such as cyanide which can inhibit the growth of fungal isolates. The ability of each isolate to inhibit pathogens is of course different or varied, depending on the form of the compound and the number of compounds produced from each isolate.

Based on observations made on purple eggplant plants that were attacked by leaf spot disease as a result of pathogen inoculation after 15 days, it was discovered that there were yellow spots that were small in size and then gradually became larger and turned light brown to dark brown. Usually the spots grow in the middle of the leaf or on the upper edge of the leaf (Destia *et al.,*2021). This is in accordance with the statement of [8]that the initial sign of leaf spot disease (*Cercospora* sp) in purple eggplant plants is stains or spots on the surface of the leaves with clear boundaries, the shape of the spots on the leaves varies and tends to be irregular. The size of the spots increases over time and will expand to cover the entire leaf. On leaf spots that have expanded, the color boundary between the center and edge will be clearly visible, in the center of the spot the color is clearer and brighter than on the edge spotting.

Observation of the effectiveness test of bacterial isolates against leaf spot disease on infected purple eggplant plants was carried out every 7 days after the inoculation of the pathogen was declared successful. Based on Figure 2, it can be seen that most of the plants experienced an increase in disease attacks in the second week, then some experienced a slow decline in the third and fourth weeks, and some experienced an increase.

The bacterial isolates that were able to reduce the percentage of disease attacks when compared from the first week of observation to the fourth week were isolates R4, R2 and R10 with a reduction in the percentage of disease attacks respectively of 15.15%, 7.14% and 1.98%. This ability to reduce the percentage of disease is of course due to the presence of antagonistic compounds produced by these bacterial isolates to suppress the growth of pathogens.

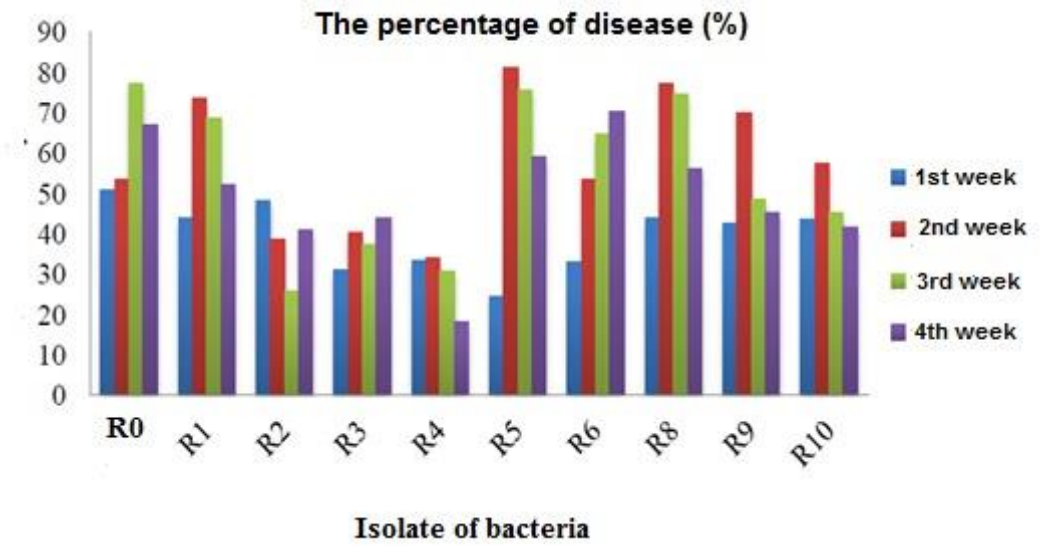


Figure 3. Percentage of Leaf Spot Disease during 1 month of observation

According to Harahap *et al.,* (2021), rhizosphere bacteria are very beneficial because they can reduce the number of bacteria, fungi, nematodes and viruses that are pathogenic in plants. The decrease in plant disease attacks is thought to be caused by bacteria being able to suppress the growth of phypathogenic microorganisms by producing siderophores, glucanase, chitinase, antibiotics and cyanide. Apart from that, bacteria can also produce antibiotics, including pyrolnitrin, pyocyanin, pseudomonic acid, phloroglucinol and phenazine. Siderophores are also produced outside cells which have a very strong binding capacity for iron (III) and act as inhibitors of pathogen growth, plant growth factors, and as antibiotics (Sudarma 2015)

(Deni, 2012), states that the antagonist test is a test of ability fungi and isolates compete for food nutrients, competition for space to survive each other. The antagonist test aims to determine the ability of isolates to suppress the growth and development of pathogens and to see the inhibitory power of the isolates used by looking at their ability to inhibit the development of pathogens by various mechanisms, including competition for space and nutrients, as well as antibiosis with produce certain antibiotics. According to (Soesanto L. 2008). antagonistic bacteria have high competitive ability as one of their antagonistic mechanisms. Competition is carried out for nutrients and sites of infection. Competition for iron (III) ions with other soil-borne microbes can suppress pathogen infections. In general, the direct mechanism of rhizobacteria can influence the balance in regulating plant growth, increase plant nutrition and stimulate resistance to plant diseases, while indirectly, it is related to its function as a biocontrol such as producing antibiotics, chelating the availability of Fe in the rhizosphere, synthesizing extracellular enzymes that can hydrolyze fungal cell walls and niche competition in the rhizosphere (Glick, 2007)

According to Viveros (2010), the effectiveness of rhizobacteria as antagonistic agents is determined by their ability to produce siderophore compounds and hydrogen cyanide or secrete various extracellular enzymes (chitinase, protease and cellulase). The extra cellular enzymes chitinase and cellulase secreted by rhizobacteria isolates are able to degrade the cell walls of infecting pathogens so that the development of the pathogen is disrupted.

4. CONCLUSION

1. There are 4 bacterial isolates from the rhizosphere of oil palm plants that have great potential to inhibit the growth of leaf spot fungi, namely isolate R5 (99.35%), isolate R2 (98.1%), isolate R6 (94.69%) and isolate R10 (94.29%).
2. Bacterial isolates that were able to reduce the percentage of disease attacks compared to the first week of observation to the fourth week were isolates R4, R2 and R10, with a percentage reduction in disease attacks of 15.15%, 7.14% and 1.98%. respectively.
3. R2 bacterial isolate is an isolate that has the potential to inhibit fungal isolates and reduce the percentage of leaf spot disease attacks on purple eggplant.

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