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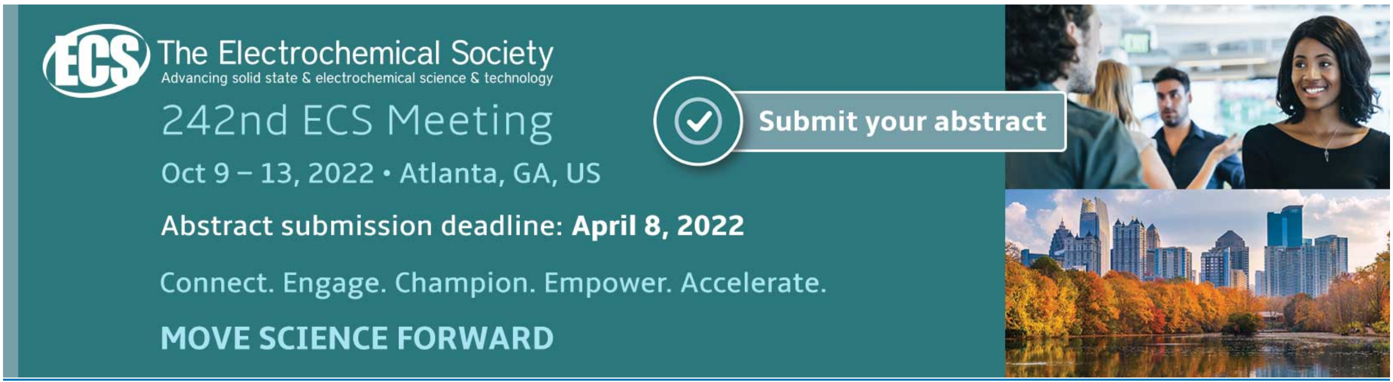

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Potential of *Paenibacillus polymyxa* bacteria and *Trichoderma* sp. as biological pesticides to control maize leaf blight (*Zea mays* L)

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Abstact. Maize Leaf Blight Disease caused by the fungus *Helmintosporium turcicum* is one of the diseases that always found on maize plantation. The disease attacks in the vegetative phase and it causes up to 27% loss of yield. Most of the farmers control the disease by using chemical pesticides that cause pollution to the environment, while this study offers control by using biological pesticides with *Paenibacilus polymyxa* Mace bacteria and *Trichoderma* sp fungus. This research aims to find out the effectiveness of *P. polymyxa* Mace and *Trichoderma* sp. and the combination of both against the disease of Leaf Blight in maize plantation. The experiment was conducted using a Completely Block Randomized Design. The treatments were control (without biopesticides), 10 ml *P. polymyxa* Mace, 10 ml *Trichoderma* sp., and combination of 10 ml *P. polymyxa* Mace and *Trichoderma* sp.. Each treatment has 5 (five) replications. The results showed that all treatments were effective in suppressing Leaf Blight Disease on maize, however, *Trichoderma* sp. gave the best influence in controlling Leaf Blight Disease on the maize.

1. Introduction

Sweet maize (*Zea mays* L) is the main commodity crop that is cultivated in various countries, especially in Indonesia because it is the main consumption material for people after rice. The very large need for maize in Indonesia is a great opportunity for farmers to cultivate this plant in various regions, apart from the great need this plant also has a high selling value so that it can support farmers' income. However, with the cultivation of maize, other factors have emerged that hinder the production of these crops.

Plant disease is the main inhibiting factor that can reduce the production of sweet maize. Diseases that attack maize plants include leaf blight caused by fungi, pathogens. Given the importance of these plant disease problems, it is necessary to take control measures. Farmers still use conventional methods for controlling pests and plant diseases by using chemical pesticides that cause negative impacts on plants and the environment. The most negative impact of the use of chemical pesticides is the accumulation of residues in the soil and in plants that are dangerous if consumed continuously by humans [1].

Based on the problems of using chemical pesticides, researchers found that using biological control agent is useful to protect plant from diseases. Biological control using biological agents can suppress the maximum population of pathogens, but the use of biological agents must also be right on target. Endophytic fungi *Trichoderma* sp. and *Paenibacillus polymyxa* bacteria are biological agents that capable of suppressing the growth of pathogens such as fungi and bacteria that we often encounter in plants and cultivated lan.. Endophytic fungi *Trichoderma* sp. and *P. polymyxa* Mace

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bacteria produce secondary metabolites which can trigger plant growth and suppress the growth of pathogenic fungi and bacteria[2].

In dealing with the increasingly large and widespread use of chemical pesticides, it is hoped that the endophytic fungi *Trichoderma* sp. and the *P. polymyxa* bacteria can play a good role and according to their function to increase plant resistance to disease attacks as well as being a biological pesticide product to replace chemical pesticides.

2. Research Methods

This research was conducted for ± 4 months starting from March to June 2020, and place of research was in the Plant Protection Laboratory, Faculty of Agriculture, Mulawarman University. Field research was carried out in Teluk Dalam, Karang Tunggal Village, Tenggarong Sebrang District, Kutai Kartanegara Regency.

The study was arranged in a Completely Randomized Block Design (CRBD) consisting of 4 treatments with each treatment consisting of 5 replications, so that 20 experimental units were obtained and each treatment consisted of 4 samples for observation. The research treatments were without treatment or control. (P0), Spraying *P. polymyxa* Mace 10 ml / L of water (P1), Spraying *Trichoderma* sp. 10 ml / L water (P2), Spraying *P. polymyxa* Mace and *Trichoderma* sp. 10 ml / L water (P3).

2.1. Propagation of P. polymyxa Mace isolate

Paenibacillus polymyxa Mace was obtained from the UPT Proteksi of the Samarinda Agricultural Training Center, in liquid form that is ready to be applied.

2.2. Propagation of Trichoderma sp. isolate

Isolate of *Trichoderma* sp. was propagated by using Potato Dextrose Agar, then incubated in room temperature. Observations were made every day.

2.3. Land preparation

The land used for planting was 15 x 10 meters which has been cleared of weeds. The land then was divided into 25 blocks with a size of 2.5 x 1.5 meters per block. Maize was planted at a spacing of 15 x 60 cm with one seed of sweet maize Exsotic Pertiwi variety per one planting hole.

2.4. Helminthosporium turcicum fungus inoculation

Inoculation of *H. turcicum* was carried out when the plants were 14 days after inoculation (DAI) and 21 DAI by diluting 10 ml of *H. turcicum* isolate, then dissolved in 1 liter of water.

2.5. Inoculation of Paenibacillus polymyxa Mace.

Inoculation of bacteria *Paenibacillus polymyxa Mace.* By spraying plants at the age of 7 DAI and carried out every 7 days interval. Spraying is carried out on all parts of the plant with a concentration of 10 ml per liter of water.

2.6. Parameters

The intensity of attack was observed 4 - 8 WAI by comparing it with the scale category table for leaf blight damage (Table 1). The attack intensity is calculated using the attack scale formula and table according to the following [3]:

$$IS = \sum \frac{(n \times v)}{N \times Z} \times 100\%$$

where:
IS: Attack Intensity (%)
n: number of plant parts affected (strands)
v: scale value of the affected leaf
N: The number of leaves observed

Z: The highest scale of the attack category

Table 1. Scale categories of leaf blight damage

The intensity of the attack	Scale
No attack	0
Light damage 1 - 10% per Plant	1
Moderate damage 10 - 25% per Plant	2
Slightly heavy damage 25 - 50% per plant	3
Heavy damage 50 - 75% per Plant	4
Very heavy damage 75 - 100% per Plant	5

3. Results and Discussion

3.1. Land description

The location is in the Teluk Dalam Area, an area of ±18 ha, located in Karang Tunggal Village, Tenggarong Seberang District, Kutai Kartanegara Regency (Figure1). Karang Tunggal Village is a lowland with an altitude of 17 meters above sea level.

Figure 1. The land for planting.

The soil texture in Karang Tunggal Village is in the form of lampungan, most of which are yellow in color and have a slope of 45-60˚. The average daily temperature in Karang Tunggal village is 30 ℃ and the rainfall is 1,783mm.

3.2. Fungi that cause maize leaf blight

Leaf blight is caused by the fungus *H. turcicum* which can survive on live maize crops, several types of grasses including sorghum, on diseased maize plant debris, and maize kernels. The conidia were spread by the wind in the air. The conidium germinates and the germ vessels enter into infection through stomata or by penetrating directly, which is preceded by the formation of an apresorium.

3.2.1. Hellminthosporium turcicum. Leaf blight caused by *H. turcicum* is the main disease after downy mildew. This pathogen has airborne properties so that it is easy to spread from one plant to another. Based on the results of microscopic observations and according to the fungi identification book [4], this study identified that the fungus was *H. turcicum*. As seen in (Figure 2 B.) conidia is an elongated cylindrical shape with a blunt tip and is insulated in the middle and has a short conidiophore.

Figure 2. A: Leaf blight attack; B: *H. turcicum* microscopic fungus;
C: *H. turcicum* isolate

The classification of the pathogenic fungus *H. Turcicum* according to [5] is as follows:
Kingdom : Fungi
Division : Amastigomycota;
Class : Deuteromycetes;
Order : Hyphales;
Family : Dematiaceae;
Genus : *Helminthosporium;*
Species : *Helminthosporium turcicum*

3.2.2. The fungus Trichoderma sp. Based on the results of microscopic observations and according to the fungi identification book [4], this study identified that the fungus is *Trichoderma* sp. Colony of *Trichoderma* sp. has a whitish-green pigment, the structure of the fungus surface is not slippery, there are many peripheral parts, and are circular in shape like a mosquito repellent (Figure 3). The classification of *Trichoderma* sp. is as follows: Kingdom: Fungi; Phylum: Ascomycota; Class: Euascomycetes; Order: Hyporeales; Family: Hypocreaceae; Genus: Trichoderma; Species: *Trichoderma* sp.[6].

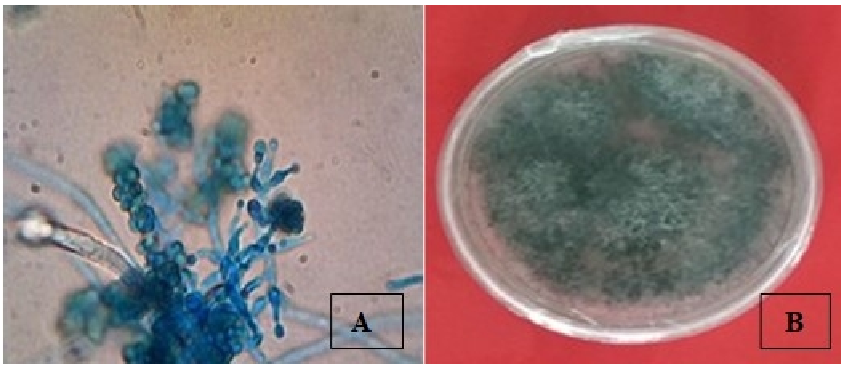

Figure 3. A: Microscopic fungus of *Trichoderma* sp .; B: Isolate of *Trichoderma* sp .

3.2.3. Paenibacillus polymyxa Mace. *P. polymyxa* mace bacteria have a role as plant resistance booster where these bacteria can control several types of pathogens. *P. polymyxa* bacteria are included in the category of non-pathogenic bacteria which are useful in the health and environment. These bacteria are able to produce antibiotics in the form of polymyxin, able to bind nitrogen elements and contain the gibberellin regulating hormone.

The classification of pathogenic fungi *Trichoderma* sp. are as follow [7]:

Kingdom : Bacteria;
Division : Firmicutes;
Class : Bacili;
Order : Bacillales;
Family : Paenibacillaceae;
Genus : *Paenibacillus;*
Species : *Paenibacillus polymyxa*

3.3. Leaf blight disease intensity

The results of observations of the intensity of leaf blight in the treatment is shown in Table 2. The disease intensity began to be seen at the fourth week of observation where the disease had increased every week until the eighth week of observation.

Table 2. The intensity of leaf blight attack on various treatments

Treatment	The intensity of the attack (%)				
	4WAI	5WAI	6WAI	7WAI	8WAI
Control (P0)	7.26c	11.36c	18.00c	21.87c	23.70c
P. polymyxa (P1)	5.08b	8.64b	14.69b	16.42b	18.81b
Trichoderma sp. (P2)	2.51a	3.22a	5.78a	7.54a	12.50a
P. polymyxa and *Trichoderma* sp (P3)	4.50b	7.56b	14.03b	15.98b	18.30b

Note: Data is transformed into Arc Sin-1√x and numbers followed by the same letter in the same column, means that they are not significantly different in the 5% LSD test

Based on the results of variance in the treatment table, it shows that treatment 1 is not significantly different from treatment 3, but shows differences in control and treatment on the intensity of leaf blight from the fourth to the eighth week of observation.

In observing the intensity of leaf blight attacks in the fourth week, it was found that the highest average occurred in the control treatment (P0), which was 23.70%, while the lowest attack was in treatment 2 (P2) of 12.50%, this is in accordance with the statement [8] which states that hybrid maize varieties have plant resistance of 23-43%. The control treatment was not given any treatment so that the intensity of the resulting disease was much higher than the other treatments. In treatment 2 (P2) given the fungus *Trichoderma* sp. disease attack is lower than other treatments.

This happened because of the growth of *Trichoderma* sp. and *P. polymyxa* mace is much faster than fungi that cause blight and *Trichoderma* sp is known to be able to adapt well to cropping conditions. The same thing was noted [9, 10, 11] that *Trichoderma* sp. can grow well in plants so that the suppression of disease becomes greater. It is also able to trigger plant resistance in suppressing pathogen development [9].

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4. Conclusion

The research can be concluded that the biological agents *Paenibacillus polymyxa* Mace and *Trichoderma* sp. are able to control maize leaf blight caused by *Hellminthosporium Turcicum*. *Trichoderma* sp. was the most effective biological agent in suppressing the development of maize leaf blight, followed by the biological agent *P. polymyxa* Mace which was not different from the combination treatment between *P. polymyxa* mace and *Trichoderma* sp.

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