

Conference Paper

Potential of Endophytic *Fusarium* sp. Against Bacterial Wilt Disease in Tomato Plants

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ABSTRACT

Ralstonia solanacearum is a pathogen that causes bacterial wilt in tomato plants (*Lycopersicum esculentum*), can decrease production until 90%. The controls generally use chemical pesticides which are harmful to the environment and humans, so it is necessary alternative controls using endophytic *Fusarium* sp. The aim of the research was to determine the effect of endophytic *Fusarium* sp. to bacterial wilt. Endophytic *Fusarium* sp. was explored on tomato plant in Tlekung, Junrejo-Batu and taken systematically randomly then brought to the laboratory. Isolation of endophytic fungi was done by washing the plants with running water, cutting 1cm then was sterilized. The sample was planted on Potato Dextrose Agar (PDA) and incubated at 37°C for 48 hours, if its growth was purified on new media. Isolation of *R. solanacearum* was done by washing the stems, cutting 0.5 cm then sterilizing. The piece is put into a test tube, added 10 ml of sterile water until the bacterial mass comes out and dissolves in sterile water. The mass of bacteria in sterile water is shaken using vortex until suspension forms. The suspension was grown on TZC agar and incubated at 28°C for 24 hours, if it grow was purified on new media. Application of endophytic *Fusarium* sp. carried out using immersing technique of the roots of tomato plant. Inoculation of *R. solanacearum* was done into sterile soil before the plant were planted. The study used completely randomized design (CRD) with control and isolate of endophytic *Fusarium* sp. as treatments with 3 repeat and conducted in the greenhouse of the Faculty of Agriculture UPN "Veteran" East Java. Observations are incubation period and disease intensity, data were analyzed using BNT 5%. The results show that endophytic *Fusarium* sp. delayed the period incubation and suppressed the intensity of bacterial wilt disease in tomato plants.

Keywords: Bacterial wilt, *Fusarium* sp., *R. Solanacearum*, Tomato Plants

Introduction

Tomato (*Lycopersicum esculentum*) is one of the most widely cultivated horticultural crops in Indonesia and belongs to the Solanaceae family. Tomato plants are widely used as processed ingredients, in industry, have good health benefits, and grow in lowlands and highlands. As the population increases, the demand for tomatoes increases as well. However, the increase in demand still has obstacles and can cause a decrease in production. One important constraint is the attack of *Ralstonia solanacearum*, which causes wilt disease and causes losses of up to 90% (Nurjanani, 2011). *R. solanacearum* has many hosts including tomatoes, chilies, eggplants, bananas, and potatoes, and can inhibit the transport of water and food in plant tissues, capable of producing enzymes that destroy plant cell walls (Istiqomah and Kusumawati, 2018).

Bacterial wilt control in the field generally uses chemical pesticides and if done continuously, it can have harmful effects on the environment and humans. Based on this, there is a need for alternative controls that are environmentally friendly. One alternative control is the use of endophytic fungal biological agents. Endophytic fungi are fungi that associate with host plants without causing disease symptoms (Pangestu et al., 2022). Some research on the utilization of endophytic fungi as biological

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agents of plant pathogens is research conducted by Hasanah et al. (2023) mentioned that the endophytic fungus *Aspergillus* sp. can reduce the intensity of bacterial wilt disease in tomato plants and research by Indarwati et al. (2022) that endophytic fungi from eggplant plants, namely *Trichoderma* sp., *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., *Chalara* sp., and *Ampuliferina* sp. can inhibit the growth of the pathogenic fungus, so that endophytic fungi can be developed as a biological disease control solution.

Material and Methods

Isolation of Fusarium sp. and applications

Sample of *Fusarium* sp. was collected from tomato plants in Tlekung, Junrejo-Batu at an altitude of 600-3000 m above sea level with an average temperature of 17-25°C and was taken randomly systematically. The plant samples were then washed under running water and cut into 1 cm. The sample pieces were surface disinfected by immersing them in 70% alcohol for 30 seconds, 5% sodium hypochlorite (NaOCl 5%) for 5 minutes, and rinsed with sterile water for 3 minutes. Two pieces of each sample were planted in Petridish containing Potato Dextrose Agar (PDA) medium for microorganism growth. Each sample was placed face down and incubated at 37°C for 48 hours. If fungi grow, then purified on the new medium. The application of the endophytic fungus *Fusarium* sp. was done using the technique of immersing the roots of a tomato plant into the fungal suspension (10^7 spores/ml) for 30 minutes.

Isolation of Ralstonia solanacearum and applications

Samples were taken from symptomatic tomato plants. Stems from the tomato plant were cut 0.5 cm and sterilized. Then the pieces were put into a test tube and 10 ml of sterile water was added, shaken with a vortex until homogeneous. Then the bacterial isolates were grown on TZC medium at 28°C for 24 hours. Then, after growing purified on new media. *R. solanacearum* isolates that grow on TZC are characterized by a convex round shape and pink in the center. Furthermore, identification was done by Gram staining and cell shape. Bacterial isolates were then inoculated into sterile soil before the plants were planted at 10^8 cfu/ml and the suspension was poured as much as 10 ml into the soil. Observations made in this study include the incubation period and disease intensity, the data were analyzed using BNT5%. Disease intensity was calculated using the formula:

$$I = \frac{\sum n \times V}{Z \times N} \times 100\%$$

- I : Disease intensity
 n : Number of affected plants at the i scale
 v : Scale of damage to i
 Z : Highest attack value
 N : Highest attack category value

Table 1. Scale value of disease attack on tomato plants

Grade scale	Attack Level (%)
0	0
1	$0 < x \leq 20$
2	$20 < x \leq 40$
3	$40 < x \leq 60$
4	$60 < x \leq 80$
5	$80 < x \leq 100$

Results and Discussion

The results are presented in the following Table 2 and graph:

Incubation period

Table 2. The incubation period of bacterial wilt disease bacteria

Treatment	Incubation Period (days)
Control	14
<i>Fusarium</i> sp.	16

Based on Table 1, the incubation period of the *Fusarium* sp. Endophytic fungus treatment was minutes slower than the control. This is because of the application of the endophytic fungus *Fusarium* sp. Tomato plants are thought to reduce the virulence ability of *R. solanacearum* which has been inoculated in the soil. This is because the endophytic fungus *Fusarium* sp. produces secondary metabolites that can act as antioxidants and antibacterials. Endophytes become biocontrol because they form antibiotic mechanisms, mycoparasitism, competition for nutrients and space, and induce resistance in the host (de Lamo & Takken, 2020). Based on the research of Khamidi et al. (2022), the endophyte *Fusarium* sp. can extend or slow down the incubation period of the disease by up to 23 days. Non-pathogenic *Fusarium* sp. can delay disease symptoms and induce plant resistance. The inhibitory mechanisms formed are mycoparasitism, nutrient competition, and space (Aghna et al., 2019).

In addition, the incubation period is also influenced by several factors: environmental conditions, the nature of plant variety resistance to disease, and environmental conditions. Environmental conditions that greatly affect the incubation period of *R. solanacearum* bacterial wilt disease are temperature and humidity. In the implementation of the study, the greenhouse temperature was 34°C with 80% air humidity while the soil temperature was 26°C with 45% humidity. This condition is thought to slow down the incubation period so that the onset of symptoms becomes slower. According to Fajinmi and Fajinmi (2010), the temperature that supports the development of this disease is 25-35°C with 86% air humidity, but the development of the disease becomes slow when the soil temperature is less than 20°C and low humidity. While its aggressiveness decreases when the temperature is more than 35°C or below 18°C. According to Tahat and Sijam, (2010), the activity of pathogenic bacteria *R. solanacearum* to cause plant disease is influenced by temperature, rainfall, soil type, inoculum, and various other biological factors. In addition, Marwoto and Muharam (2013) mentioned that the incubation period of *R. solanacearum* in potato varieties varies from 4-24 days.

Disease intensity

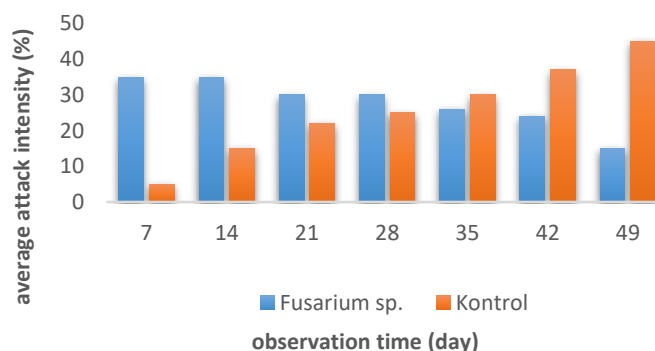


Figure 1. Histogram of bacterial wilt disease intensity on tomato plants

Based on Figure 1, the intensity of bacterial wilt attack showed that on days 7 and 14 the intensity of attack was 35%, on days 21 and 28 the intensity of attack was 30%. On day 35, the intensity of the attack was 25%, on day 42 it was 23%, on day 49, the intensity of the attack was 15%. In the control treatment, on day 7, the intensity of attack was 6%. Day 14 became 15%, and day 21, the intensity was 20%. On day 28, the intensity of the attack increased again to 25%, while on day 35 it was 30%. On day 42 it was 39% and on day 49 the intensity of the attack was 48%. Based on the data obtained, the intensity of the attack on the treatment of endophytic fungus *Fusarium* sp. The average percentage decreased. Meanwhile, in the control treatment, the average percentage of bacterial wilt attack intensity increased. The average intensity of attack in the *Fusarium* sp. endophyte treatment was 27.1%, while in the control treatment was 26.14%. It is suspected that the endophytic fungus *Fusarium* sp. can increase the resistance of tomato plants because it produces secondary metabolites that act as antioxidants. According to Marlinda et al. (2019), secondary metabolites, ethyl acetate in the endophytic fungus *Fusarium* sp. can act as antibacterials. Pathogenic *Fusarium* sp. and nonpathogenic *Fusarium* sp. have differences even though they both colonize the roots. Non-pathogenic *Fusarium* sp. colonizes the roots not enter the root vessels and only infects the epidermis and cortex while pathogenic *Fusarium* sp. colonizes the roots to the vessels and deeper tissues (de Lamo & Takken, 2020).

Conclusion

Based on the results of the study, it can be concluded that the endophytic fungus *Fusarium* sp. can inhibit the growth of the disease, with the treatment of endophytic fungus *Fusarium* sp. is 16 days while in the control the incubation period is 14 days. The treatment of endophytic fungus *Fusarium* sp. reduced the intensity of bacterial wilt attack caused by *R. solanacearum* by an average of 27.1% and the intensity of bacterial wilt attack in the control averaged 26.14%.

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