Biodiversity of Endophytic Fungi from Lowland Tomato Plants and Their Potential as Biological Control Agents for Anthracnose Disease in Chili Plants at Green House

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Abstract. Anthracnose disease is one of important diseases of fruit crops that need to be controlled, influence of chili production in Indonesia, and cause significant yield losses of economic importance. Control of the disease until now using fungicides that have negative impact for the environment and the results have not been satisfactory. Based on that, alternative methods of control are studied. The control method is the use of endophytic fungi that isolated from tomato roots in the lowlands. The purpose of this study was to obtain candidates for endophytic fungi that could potentially be biological agents for controlling anthracnose disease. The stages of the research are antagonism test using dual culture method, identification, application suspension of endophytic fungi using root immersion method. Endophytic fungi be isolated from root lowland tomato plants (Jombang and Kediri) and was cultured on Potato Dextrose Agar (PDA) media to get pure culture so identified based on book Illustrated Genera of Imperfect Fungi by H. L. Barnett, Barry B. Hunter (1998). Chilli plants were inoculated with pathogens (Colletotrichum sp.) using spray techniques on leaves then endophytic fungi was applied. The identification results get Penicillium sp., Aspergillus sp., Fusarium sp., antagonistic test get 7 candidate isolates were able to inhibit the growth of Colletotrichum sp., and application suspension of endophytic fungi showed that J8 had highest potential to suppress anthracnose disease in chili plants at greenhouse (51.94%).

Keywords: endophytic fungi, Colletotrichum sp., anthracnose disease

1. Introduction
Anthracnose is a major disease that causes low productivity of chili plant in Indonesia. Anthracnose from Colletotrichum sp. as the main cause of damage to the chili, and in East Java decrease of product until 100% thus affecting the increase in chili prices [1, 14]. Anthracnose caused Colletotrichum capsici that is facultative parasitic, have asexual form or anamorph and sexual form or teleomorph. The cause of the disease spreads through the splash of water and the distance of dispersal will be further if accompanied by a gust of wind. Intercultural disease has spread widely in chili growing areas where conditions are very humid or areas with high rainfall, the fungus develops very rapidly when the humidity is high that is more than 80% RH with a temperature of 320C. Until now, control of it using
seeds teratment, crop rotation, use of fungicides, technical culture, use of disease-free planting materials, but the results are not satisfactory.

Based on this, it is possible to study the potential of endophytic fungi from lowland agricultural especially tomatoes plants as biological control agents for the disease. Chili is grown in various tropical and subtropical countries including Indonesia [19]. The price of chili in Indonesia is fluctuate because production of chili is fluctuate. The problem occure because there is anthracnose disease caused by Colletotrichum sp. that is one the most important causes of yield losses in chili around 10-80% [17, 18]. Control of the disease until now using pesticide especially fungicide, [16] and [15] said that there are more than 60 kinds of pesticide used intensively, negative impact for environment, and unsatisfactory results. Based on that need environmentally friendly control strategies such as biological control using endophytic fungi.

Endophytic fungi are microbes that live in plant tissues and are symbiotic mutualism with plants, produce certain compounds, can improve plant resistance to pathogens and pests [14]. Research about use of endophytic fungi as biological control agents for pathogen control has been done, [9] can isolate endophytic fungi and control three pathogens in cacao, [8] can aplicate endophytic fungi complex to Colletotrihum sp. complex in olive tree; [7] can aplicate of Trichoderma sp. as endophytic fungi to Colletotrichum capsici in chili.

The purpose of this study was to obtain candidates for endophytic fungi that could potentially be biological agents for controlling anthracnose disease.

2. Methods and Material

2.1. Isolation and identification endophytic fungi
Endophytic fungi were isolated from roots of healthy tomato plants from Jombang and Kediri. The roots of the tomato plant are washed then surface sterilized by soaking in alcohol 70% (2 minutes), soaked in NaOCl 1% (1 minutes) then rinsed three times with sterile aquades. The sample was cut into pieces about 0.5-1 cm and planted on Potato Dextrose Agar (PDA) media and incubated at 23±20C until the endophytic fungi grows. If culture grows was purified on PDA media and stored at 4oC [1].

Identification using the reference book Barnett and Hunter (1998) [2].

2.2. Isolate Colletotrichum capsici
The research using Colletotrichum sp. C.capsici is a collection of Dr.Arika Purnawati. Isolate was grown on PDA media and incubated at 250C for 7 days. Culture of the fungi will grow at 7 days then If grows purified on PDA media and at the age of 7 days used for research.

2.3. Identification of Endophytic Fungi
Identification of endophytic fungi is done by macroscopic and microscopic observations. Macroscopic identification to determine the color use comparison Munsell Soil Color Chart and shape of the colony, microscopic identification to determine hyphae, spore/conidia and sporangiophore use microscop binokuler type Olympus CX 33. Identification was carried out to the genus stage using book Illustrated Genera of Imperfect Fungi by H. L. Barnett, Barry B. Hunter (1998) [2].

2.4. Antagonistic Test of Endophytic Fungi and Colletotrichum sp.
This test was carried out to see the percentage of inhibition of endophytic fungi to Colletotrichum sp. fungi on PDA medium using dual culture method. Pure pathogenic cultures and endophytic fungi used were 7 days old. They taken use cork borer with diameter 0.5 cm into pieces and inoculated on Petri dish that already contains PDA medium. Position of the pieces is opposite directions within 3 cm between the two isolates. After that it was incubated at 23±20C for 7 days and continued with observation. Observation is the percentage of inhibition by measuring the radius of the pathogenic fungi, was calculated using the formula by [5]:

2
\[ P = \frac{R1 - R2}{R1} \times 100\% \]

P = Percentage inhibition (%)
R1 = radius of Colletotrichum sp. towards the edge
R2 = radius of Colletotrichum sp. toward endophytes

2.5. Isolation and identification endophytic fungi

Application was done by root soaking with suspension of endophytic fungi (106 spore/ml) for 30 minutes then planted in polybag containing sterile soil and composted with ratio 1:1 and was maintained until the chilli plants were 2 month after planting [4]. The pathogen suspension (106 spores/ml) was inoculated to chilli plants at the age of 35 HST by spraying the leaves of the plant using 5 ml suspension (106 spores/ml) per plant. Observation are incubation period and intensity of the disease using formula [6]:

\[ I = \frac{\sum (n \times v)}{(N \times Z)} \times 100\% \]

I = intensity of disease attack
n = Number of leaves attacked
v = spotting score that appears
N = Number of leaves observed
Z = Highest score category value

The value of the attack category (score) is follows:
Score 0 = healthy plant tissue
Score 1 = spotting area 1% to 20%
Score 2 = spotting area 21% to 40%
Score 3 = spotting area 41% to 60%
Score 4 = spotting area 61% to 80%
Score 5 = spotting area 81% to 100%

3. Result and Discussion

3.1. Identification endophytic fungi

Isolation endophytic fungi get 10 isolates and result identification (Table 1)

| Isolates code | Macroscopic colony | Microscopic colony | Fungi          |
|---------------|--------------------|--------------------|----------------|
| F1, F6, F7, F8 | irregular          | Green, spherical   | Penicillium sp.|
| F2, F3        | irregular          | light green, spherical | Aspergillus sp.|
| F4, F5, F9, F10 | irregular, white | crescent, moon, insulated | Fusarium sp. |

Images of macroscopic and microscopic of endophytic fungi (Table 2)
Table 2. Macroscopis and microscopis endophytic fungi

| Isolates     | Macroscopis | Microscopis |
|--------------|-------------|-------------|
| F1, F6, F7, F8 | ![Image](p.400x) |  ![Image](p.400x) |
| F2, F3       | ![Image](p.400x) |  ![Image](p.400x) |
| F4, F5, F9, F10 | ![Image](p.400x) |  ![Image](p.400x) |

Isolation results obtained 3 endophytic fungi (Table 1, 2) this shows the dominance of all them, because endophytic fungi found are Penicillium sp., Aspergillus sp., Fusarium sp. They can be isolated because the three endophytic fungi are indigenous in the root tissue of tomato plants in lowland and this is proved by Koch's Postulate. They are cosmopolitan fungi and the research conducted by [1] was successful isolation Aspergillus sp. and Fusarium sp. as indigenous endophytic fungi from roots and stems of highland tomato plants, both of them can decrease the wilt disease cause R. solanacearum in tomato. Other research conducted by [20] was successful isolation Penicillium sp., Aspergillus sp., Fusarium sp. as indigenous endophytic fungi from root, stem, leaf of chili, they as biological control agents for wilt fusarium cause Fusarium oxysporum in chili. The existence of the dominance of these fungal genera because they ability to produce conidia in large numbers. Besides that, this fungi grows fast so that in isolation media it can easily grow and defeat the growth of other genus fungi. While, the diversity of endophytic fungi is influenced by the type of host plant, environmental factors, climate and season [13].

Genus Aspergillus, Penicillium, Fusarium have higher percentage of attendance compared to other genera because the fungi are cosmopolitan [7]. The existence of the dominance of these fungal genera because they ability to produce conidia in large numbers. Besides that, this fungi grows fast so that in isolation media it can easily grow and defeat the growth of other genus fungi. While, the diversity of endophytic fungi is influenced by the type of host plant, environmental factors, climate and season [13].
3.2. Antagonistic test of endophytic fungi and Colletotrichum sp.

Result of antagonistic test (Figure 1)

Based on Fig 1, the highest inhibition was formed by J3 (43.47%), followed by J4 and J6 (43.42%), J7 (41.56%), J10 (41.42%), J8 (33.48%), J9 (30.03%). This inhibition is formed because endophytic fungi produce secondary metabolites. This is consistent with the statement of Manurung et al. (2014) that endophytic fungi Penicillium sp. and Aspergillus sp. produce alkaloids, agroklavine and ergometrine. Fusarium sp. produce toxic antibiotics that diffuse with growth media so pathogen growth is inhibited [8, 9].

3.3. Application of endophytic fungi

Application of endophytic fungi to chilli plants was carried out using 7 isolates which formed percentage inhibition more than 30% in the laboratory or in vitro.

Result of application (Fig 2)

Based on Fig 2, the highest suppress disease intensity are J8 (51.94%) followed by J4 and J6 (49.61%), J3 (44.56%), J7 (35.46%), and J10 (10.83%). Their ability to suppress disease intensity because they produce secondary metabolites. This is in accordance with previous research which states that antagonistic endophytic fungi have a high activity in producing compounds that can be used to control pathogens. The compounds released by endophytic microbes are secondary metabolites which are bioactive compounds and can function to kill pathogens [10, 11, 12]. According to [12], endophytic fungi also induce plant resistance to produce peroxidase enzymes so plants are resistant to pathogens, and Rajeswari et al., (2014) state that endophytic fungi improve the resistance of the host plants to adversity by secretion of various bioactive metabolites of unique nature which includes alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, and xanthone.

are endophytic fungi that able to suppress anthracnose disease with pathogenic C. capsici up to 18.32% and 16.37% compared control (26.29%). This is because they produce antibiotics or compound that can inhibit the growth of plant pathogens. This is in accordance with previous research which states
that antagonistic endophytic fungi have a high activity in producing compounds that can be used to control pathogens. The compounds released by endophytic microbes are secondary metabolites which are bioactive compounds and can function to kill pathogens [10, 11,]. According to [12], endophytic fungi also induce plant resistance to produce peroxidase enzymes so plants are resistant to pathogens.

4. Conclusion

Conclusion of the research:

1. Isolation result there are 3 genus of endophytic fungi are Penicillium sp., Aspergillus sp., Fusarium sp.
2. The highest inhibition was formed by J3 (43.47%).
3. The highest suppress disease intensity are J8 (51.94%).

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