Ability test of endophytic fungi to suppress the development of anthracnose disease (*Colletotrichum capsici*) in chili plants (*Capsicum annum*) in the Karo Highland

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Abstract. Anthracnose is one of the important diseases in chili plants. One way to control this disease is using endophyte fungi. The aim of this research was to examine the ability of endophytic fungi to suppress the development of anthracnose disease in chili plants in the Karo Highland. This research was conducted at the Rajapayung village, Sub-District Berastagi, District Tanah Karo, North Sumatera and at Plant Pathology Laboratory, Agrotechnology Study Program, Agriculture Faculty, USU. This research used Factorial Randomized Group Design method with 2 factors, 27 treatments and 2 replications. The first factor was the application method: application to seeds (A1), application on leaves (A2), and application on fruit (A3) while the second factor was the kind of isolates endophyte fungus. The results of the isolation were *Trichoderma* sp., *Rhizoctonia* sp., *Aspergillus* sp., *Penicillium* sp., *Gliocladium* sp., *Hormiscium* sp. The results of this research showed that the lowest of percentage disease anthracnose in last observation was in the application on leaves treatment (5.36 %) and treatment with *Penicillium* sp. (0.00 %). The highest plant height was in the treatment with a application on leaves with unknown hyphae (112.08 cm). The highest production was in the application on leaves treatment (0.58 ton/ha).

1. Introduction

Red chili (*Capsicum annum* L.) is a vegetable commodity that has quite a high economic value. The need for chili increases every year as the population increases and the industry needs for chili raw materials. The production of large fresh chilies with stems in North Sumatra in 2016 amounted to 152,629 tons decreased compared to 2015 amounted to 35,206 tons (23.06%) [1].

The productivity of chili is decreasing due to pests and diseases. One of the diseases that causes chili productivity is decreasing anthracnose disease by the pathogen *Colletotrichum capsici*. This pathogen causes damage to fruit and reduces production by 20-40%. Handling this disease is usually done by spraying fungicides. However, chemical control is horrible for environment [2].

The use of biological control agents as an alternative to fungicides synthetic is increasingly being developed in line with the increasing awareness of the negative effects of synthetic fungicides. Biological control agents have several advantages, including being effective in controlling plant diseases, not having a negative impact on the environment, effective during the life span of plants and can produce compounds that are of double benefit to plants [3].
Biological control is currently being developed, one of which is the use of endophytic fungi. Endophytic fungi are fungi that live in plant tissue without showing symptoms [4]. The potential of endophytic fungi is large enough to be developed as a biological control agent, because this fungus lives in plant tissues so that it plays a direct role in inhibiting the development of pathogens in plants [5] and promotes plant growth.

The purpose of this study was to test the ability of endophytic fungi isolated from chili plants by treating the application of different endophytic fungi in suppressing the development of anthracnose disease in red chili plants in the Karo highlands.

2. Material and methods

2.1. Experimental design

This research was conducted in the Plant Pathology Laboratory of the Agrotechnology Study Program, Faculty of Agriculture, Universitas Sumatera Utara and in Raja Payung Village, Sub District Berastagi, District Tanah Karo, North Sumatra, Indonesia. Plant material used in this study is chili seeds, namely Lidya F1, planted in field with a mixture soil with compost. The basic fertilizer used are NPK (10 g/L) of a planting hole. This research used endophyte fungus that isolated from chili plants. Observation of height plants is done by measuring the height starting from one week after transplanting until fifteen weeks after transplanting. Observation the incidence and severity of disease is done by counting the incidence and severity of disease from the beginning of young chilli fruit plants. Observation of root lenght is done by measuring the length of the root from the root base to the tip of the root. Observation of yield is done by calculate the weight of fresh fruit from chilli plants. The research method uses factorial randomized block design with 2 factors and 2 replications, namely:

Factor I : how to apply endophytic isolate (A), namely :
A1: endophytic isolate application on chili
A2: application of endophytic isolates on chili leaves
A3: Application of endophytic isolates in chilli seeds

Factor II : The type of endophytic fungi isolate (E), namely :
E0: Without endophytic fungi isolate (control)
E1: Endophytic 1 fungus isolate from chili root
E2: Endophytic 2 fungus isolates from chili root
E3: Endophytic fungus 3 isolate from chili root
E4: Endophytic 1 fungus isolate from chili stem
E5: Endophytic fungus 2 isolate from chili stem
E6: Endophytic fungus 3 isolate from chili stem
E7: Endophytic 1 fungus isolate from chili leaf
E8: Endophytic 2 fungus isolates from chili leaf

2.2. Application of Endophytic fungi

2.2.1. Application to seeds

The seeds to be used must be disinfected first with 1% chlorox solution for 2 minutes, then rinsed with sterile distilled water three times. The seeds were then immersed in a conidia suspension of endophytic fungi with a concentration of $10^5$ conidia/ml for 6 hours.

2.2.2. Application on leaves

Application on chili leaves is carried out when the plants are 10 days after transplanting. Spraying the conidia suspension of endophytic fungi with $10^5$ conidia/ml suspension sufficiently until the leaves are wet evenly. Spraying is done 5 times at intervals of once a week.
2.2.3. Application on fruit

Application to the fruit is done once, after the young fruiting plants. Spraying the conidia suspension of endophytic fungi with a concentration of $10^{5}$ conidia/ml is carried out on all fruits in the sample plants and sprayed until all the surface of the fruit is wet evenly.

3. Result and discussion

3.1. Identification result

Table 1 shows identification result isolation of fungi from healthy chili plants.

| Code | Plants Part | Name of endophytic fungi     | Divisi    |
|------|-------------|------------------------------|-----------|
| E1   | Root        | Trichoderma                  | Ascomycetes |
| E2   | Root        | *Rhizoctonia* sp.           | Ascomycetes |
| E3   | Root        | *Aspergillus niger* sp.     | Ascomycetes |
| E4   | Stem        | *Aspergillus flavus* sp.    | Ascomycetes |
| E5   | Stem        | *Gliocladium* sp.           | Ascomycetes |
| E6   | Stem        | *Penicillium* sp.           | Ascomycetes |
| E7   | Leaf        | *Hormiscium* sp.            | Ascomycetes |
| E8   | Leaf        | Unknown                      | -         |

8 endophytic fungal isolates were obtained. Furthermore, the 8 fungi isolates were screened in vitro to see their ability as biological agents. From the exploration results, 8 isolates were obtained, namely isolates E1, E2, E3 from the roots of chili plants. Isolates E4, E5, E6 was isolate from the stems of chili plants, E7 and E8 was isolated from the leaves of chili plants. Non pathogenic endophytic fungi are suspected of including fungi *Trichoderma* sp., *Rhizoctonia* sp., *Aspergillus niger*, *Aspergillus flavus*, *Gliocladium* sp., *Penicillium* sp. and *Hormicium* sp.

3.2. Disease severity (%)

The number of disease severity was measured by counting the severity of disease from the beginning of young chilli fruit plants as shown in Table 2.

| Endophytes | A1  | A2  | A3  | Mean (%) |
|------------|-----|-----|-----|----------|
| E0         | 19.89 | 14.71 | 26.80 | 20.47a  |
| E1         | 0.00  | 2.55  | 0.00  | 0.85d   |
| E2         | 27.04 | 7.42  | 0.00  | 11.49b  |
| E3         | 5.01  | 8.31  | 4.48  | 5.93bc  |
| E4         | 25.99 | 0.00  | 0.00  | 8.66bc  |
| E5         | 9.63  | 0.00  | 15.60 | 8.41bc  |
| E6         | 0.00  | 0.00  | 0.00  | 0.00e   |
| E7         | 9.43  | 0.00  | 0.00  | 3.14cd  |
| E8         | 0.00  | 15.23 | 2.28  | 5.84c   |
| Mean (%)   | 10.78 | 5.36  | 5.46  | 7.20     |

For endophytes the highest number of disease severity in chilli plants was E0 treatment as much as 20.47% and the lowest was E6 treatment as much as 0.00%. Based on the results of the study showed that the best application method is the application on the leaves compared to other applications.
Endophytic fungi have a fairly good ability to control *C. capsici*. The mechanism of action of inhibiting the growth of pathogenic fungi is that endophytic fungi secrete antimicrobial compounds against pathogenic microorganisms. This is consistent with the literature of Zhao et al [6] which states that endophytic fungi are able to produce bioactive compounds actually derived from plants, such as paclitaxel, podophyllotoxin, camptothecine, vinblastine, hypericin and diosgenin.

The isolate E6 (*Penicillium* sp.) had a good ability to inhibit disease progression. This is in accordance with the literature of Wakana et al [7] which states that *P. citrinum* has an antifungal compound such as citrinin which is indicated by the inhibition zone of 13-17 mm in the *Candida albicans* and *Aspergillus fumigatus* tests. Mycelium becomes thicker, the tip of the hyphae twisting or twisting and branching which goes along with Ma et al [8].

### 3.3. Disease incidence (%)

The number of disease severity was measured by counting the incidence of disease from the beginning of young chilli fruit plants as shown in Table 3.

| Endophytes | Application | Mean (%) |
|------------|-------------|----------|
|            | A1          | A2        | A3        |           |
| E0         | 42.78       | 34.21     | 55.67     | 44.22a    |
| E1         | 0.00        | 11.48     | 0.00      | 3.83de    |
| E2         | 52.03       | 30.83     | 0.00      | 27.62b    |
| E3         | 13.83       | 20.45     | 15.43     | 16.57b    |
| E4         | 57.79       | 0.00      | 0.00      | 19.26bc   |
| E5         | 23.71       | 0.00      | 49.02     | 24.24b    |
| E6         | 0.00        | 0.00      | 0.00      | 0.00e     |
| E7         | 28.72       | 0.00      | 0.00      | 9.57c     |
| E8         | 0.00        | 38.11     | 11.42     | 16.51bc   |
| Mean (%)   | 24.32a      | 15.01ab   | 14.61ab   | 17.98     |

The results of observations of the study showed that the highest average disease incidence was E0 treatment with as much as 9.51% and the lowest average disease incidence was E6 (*Penicillium* sp.) treatment as much as 0.00%.

Isolate E6 (*Penicillium* sp.) obtained the lowest percentage of disease incidence. In addition to removing alkaloid compounds, *Penicillium* sp. also produce chitinase and cellulase enzymes which can degrade pathogenic fungal chitin, which goes along with Nugroho et al. [9]. *Penicillium* sp. also has the ability to inhibit the growth of pathogenic fungi due to competition and expenditure of several alkaloid compounds such as agroklavine and ergometrine which have antifungal properties which goes along with Haggag and Al [10].

### 3.4. Plant height (cm)

Table 4 shows the number of plant height. The highest average plant height was A2E8 (application on leaf with unknown hyphae) as much as 112.08 cm and the lowest was A1E1 (application on fruit with *Trichoderma* sp.) treatment interaction as much as 87.33 cm.

That shows that A2E8 treatment (application on leaves with unknown hyphae) can increase plant growth, it is suspected that endophytic fungi produce hormones that can stimulate plant growth. This is consistent with the Seviani [11] statement which states that endosymbiosis testing on chili plants shows that endophytic fungi have a real effect only on canopy height and biomass dry weight. Endophytic fungi can symbiosis and penetrate the leaves through epidermal tissue. Endophytic fungi can also support plant growth, by increasing growth regulators such as cytokinins and auxins, so that plant growth is better and it is difficult to infect pathogens as going with Gao et al [12].
Table 4. Number of plant height

| Endophyte | Application |    |    |    |
|-----------|-------------|----|----|----|
|           | A1          | A2 | A3 |    |
| E0        | 103.25 bcd  | 95.88 def | 106.45a |    |
| E1        | 87.83 f     | 91.35 efg | 96.18 def |    |
| E2        | 105.18 bc   | 103.75 bcd | 102.60 cde |    |
| E3        | 104.83 bcd  | 100.80 cdef | 93.35 cdef |    |
| E4        | 98.13 cdef  | 97.35 ef | 95.88 cdef |    |
| E5        | 102.83 cde  | 102.88 cde | 98.45 ab |    |
| E6        | 105.08 bc   | 92.25 ef | 106.50 ab |    |
| E7        | 107.53 ab   | 96.45 cdef | 106.35 ab |    |
| E8        | 98.80 cdef  | 112.08 a | 92.60 def |    |

3.5. Chili production (ton/Ha)

Table 5 shows the number of chili production. The highest average fruit weight was A2 (application on leaves) treatment with an average of as much as 0.51 tons / ha and the lowest average fruit weight was A3 (application on seeds) treatment as much as 0.34 tons / ha.

Table 5. Number of chili production (ton/ha)

| Endophytes | Application | Production (ton/ha) |
|------------|-------------|---------------------|
|            | A1          | A2          | A3            |                |
| E0         | 0.35        | 0.37        | 0.29          | 0.34           |
| E1         | 0.39        | 0.41        | 0.31          | 0.37           |
| E2         | 0.25        | 0.76        | 0.74          | 0.58           |
| E3         | 0.33        | 0.79        | 0.15          | 0.43           |
| E4         | 0.35        | 0.41        | 0.38          | 0.38           |
| E5         | 0.44        | 1.03        | 0.24          | 0.57           |
| E6         | 1.06        | 0.82        | 0.38          | 0.75           |
| E7         | 0.72        | 0.38        | 0.24          | 0.45           |
| E8         | 0.70        | 0.29        | 0.29          | 0.43           |

Production (ton/ha): 0.51a 0.58a 0.34b 0.48

Endophytic fungi produce decomposing enzymes that can decompose organic matter, thus releasing nutrients that are bound in complex compounds to become available, especially the elements N, P, and S. The availability of these nutrients increases plant growth. Increased nitrogen content in plants can affect photosynthesis so that photosynthates can increase which affects the production of chili formed which goes along with Lestari and Indrayati [13]. The mechanism of endophytic fungi in increasing plant growth and production is by dissolving phosphate and nitrogen fixation which goes along with [14].

3.6. Root height (cm)

Table 6 shows the number of root height. The highest average root length was E6 treatment as much as 43.32 cm and the lowest average was E5 treatment 34.80 cm.

The results showed that the treatment of E6 (Penicillium sp.) which was applied could increase the length of plant roots. It is thought that endophytic fungi produce hormones that can stimulate plant growth. Endophytic fungi produce compounds and live in symbiotic mutualism with their host plants. The result goes along with the Vasudevan et al [15] statement which states that the presence of endophytic fungi in the chili plant tissue can cause the number of hair roots, branching roots and roots.
lateral increase. Lateral roots can expand the absorption area of nutrients by plants so that nutritional needs are more quickly met so that they are better able to increase plant growth.

Table 6. Number of root height

| Endophytes | Application | Mean (cm) |
|------------|-------------|-----------|
|            | A1          | A2        | A3          |
| E0         | 40.90       | 40.75     | 36.03       | 39.23a     |
| E1         | 35.93       | 35.88     | 32.88       | 34.89ab    |
| E2         | 32.15       | 33.25     | 41.85       | 35.75a     |
| E3         | 38.15       | 51.38     | 35.50       | 41.68a     |
| E4         | 33.33       | 43.25     | 36.65       | 37.74a     |
| E5         | 36.45       | 35.00     | 32.95       | 34.80ab    |
| E6         | 42.08       | 47.55     | 40.33       | 43.32a     |
| E7         | 38.15       | 44.10     | 35.68       | 39.31a     |
| E8         | 38.45       | 30.65     | 38.78       | 35.96a     |

Mean (cm) | 37.29       | 40.20     | 36.74       | 38.07      |

4. Conclusions
Endophytic fungi significantly influence the development of anthracnose in chili. How to apply and administer endophytic fungi significantly affected the development of the disease compared to control treatments. The application of endophytic fungi significantly affected the weight of healthy fruits (gr) by 56.88% compared to controls. Endophytic fungi significantly affected root length by 18.08% compared to controls and other treatments. The best application is application of fruit and the best endophytic fungi is Penicillium sp.

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