Antagonistic screening of *Trichoderma* spp. isolated from patchouli rhizosphere

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**Abstract.** The objective of this research was to determine the antagonistic activity of *Trichoderma* spp. isolated from patchouli rhizosphere (*Pogostemon cablin* Benth.). Another objective was to perform antagonistic screening of these fungi to inhibit the growth of the wilted pathogen *Fusarium* spp. In vitro research was conducted in the Laboratory of Plant Pathology, Universitas Syiah Kuala, from January to June 2020. The study used a completely randomised design with five treatments and three replications. The antagonistic screening was carried out by using the dual culture method of *Trichoderma* spp. against *Fusarium* spp. with the medium of Potato Dextrose Agar (PDA). The result showed that five isolates of *Trichoderma* have different antagonistic percentages in inhibiting the *Fusarium*. The highest antagonistic activity was found from isolate 2 and the lowest value was shown by isolate 3.

**1. Introduction**

*Pogostemon cablin* Benth. or patchouli is the source of volatile oil often used for herbal healing products and raw material for fragrance products [1], [2]. Patchouli is found in tropical and subtropic countries such as Indonesia, the Philippines, Iran, and China. In Indonesia, patchouli is a promising export commodity. The highest production of patchouli in Indonesia is found in Aceh, scattered over four districts, namely; South Aceh, West Aceh, Gayo Lues, and Aceh Jaya.

The presence of pest organisms and the decrease in plant fertility were important factors that decrease patchouli production. Plant pest organisms that are often found in patchouli plants in the form of nematodes, insects, bacteria, viruses, and mushrooms. *Trichoderma* spp. is known to have some antagonistic mechanisms against plant pathogens, such as lytic enzymes, mycoparasitic space, and nutrient competition, to strengthen its colonisation [3].

Jiang et al [4] reported that hypha from *T. asperellum* was able to penetrate the hyphae and spore of *Phytophthora capsici* through the mycoparasitic mechanism towards the degradation of hyphae cells. The mycoparasite possesses cell wall degrading enzymes that allow the mycoparasitic fungi to degrade other fungi and extract their nutrient for its own benefit [5]. The strains of *Trichoderma* can also produce
Some antibiotics or low molecule weight compounds that can inhibit the growth of plant pathogens [6], such as 6-penthyl-pyrene [6], viridifungin [7], gliotoxin [8], and others. However, the mechanisms used by Trichoderma to suppress different pathogens differ between the species and strain, especially in its ability to produce the varied antibiotic between isolates from similar species, and isolates from different species [9]. So, for the different pathogens, the screening of Trichoderma spp. isolates that have the required antagonistic activity and mechanism characterization become a common and essential practice in its implementation as biocontrol agent [10]. Fusarium spp. is one of the common pathogens controlled by Trichoderma; therefore, the objective of the research was to determine the antagonistic activity of Trichoderma spp. isolated from patchouli rhizosphere (Pogostemon cablin Benth.) and to perform antagonistic screening of these fungi to inhibit the growth of the wilt pathogen Fusarium spp.

2. Materials and methods

2.1. Materials

Samples used in this research were patchouli root and stem, then rhizosphere in patchouli plantation that were collected from health and infected patchouli plants grown in Ranto Sabon Village, Aceh Jaya District. Other materials used were isolates of Fusarium spp. (obtained from the Laboratory of Plant Pathology Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh), Nutrient Agar (NA), Potato Dextrose Agar (PDA), distilled water (aquadest), alcohol, and spiritus (methylated spirit).

2.2. Methods

2.2.1. Fungi sampling. Samples of antagonistic fungi were collected from the patchouli plant, from the healthy and infected patchouli rhizosphere (20 cm deep from the surface). The antagonistic fungi samples were collected from the tissue of the patchouli root. They were put into plastic bags and processed within 24 hours.

2.2.2. Isolation of Trichoderma endophyte. Samples from the rhizosphere that were collected to be isolated then sterilized, while samples from the chopped roots and stems (0.5 cm) were extracted by grinding after being rinsed using NaOCl 0.5 % for 2 minutes, then rinsed using distilled water three times followed by sterile water. Distilled water was then added into the extract, and 1 ml was pipetted to be diluted with 9 ml reagent; it was then shaken at 100-150 rpm for 1 minute, then diluted to the dilution of 10\(^{-1}\). Samples were then put into PDA media and left in the dark at 25 - 27°C. The suspected Trichoderma had the specific characteristic colour of light green to dark green, hyphae that spread quickly and evenly, and a round colony shape. The growing Trichoderma in the media was then purified from other fungi and grown in the new PDA. All samples were then labelled.

2.2.3. Identification of antagonistic fungal isolate morphology. The identification of macroscopic fungi was carried out by observing the fungal morphology, such as colony shape, edge of the colony, colony surface, and colony colour. It included hyphae, spores, sporangium, conidia, and conidiophore using a microscope through the riddle method by observing the pieces of PDA (0.5 x 0.5 cm).

2.2.4. Selection of antagonistic fungal isolate. Screening of antagonistic activity of biocontrol agent was done using the dual culture method. The dual culture was conducted by symmetrically putting a piece of Fusarium spp. and biocontrol agent (Trichoderma spp.) into a petri dish (d = 9 cm), as shown in Figure 1. They were then incubated at 25-27°C for 7 x 24 hours. This was repeated three times for each treatment.
The antagonistic activity in terms of percentage inhibition was calculated using the following formula:

\[ P = \left( \frac{r_1 - r_2}{r_1} \right) \times 100\% \]

Note:
- \( P \) = antagonistic activity (%);
- \( r_1 \) = radius of pathogen mycellium moving away from antagonist fungi;
- \( r_2 \) = radius of pathogen mycellium approaching antagonist fungi

2.2.5. Statistical analysis. The percentage of inhibition results were the analysed using one-way ANOVA, and further tested using LSD (0.05).

3. Results and discussion
Samples collected from root, stem, and rhizosphere of healthy and infected patchouli plants were isolated in five Petri dishes respectively and were purified with 5 x 3 repetitions; at the end, this resulted in 15 isolates from healthy patchouli and 15 isolates from infected patchouli. From these, five isolates were selected as morphologically different based on their growth rate, spore colour, shape, and colony pigment secretion on media of PDA. After screening their antagonistic activity against Fusarium spp., five isolates of this fungi were obtained (after five days of incubation), showing the different antagonistic activity against Fusarium spp. as shown in Figure 2.

![Figure 2. Dual culture of Trichoderma spp. against Fusarium spp. on day 5 after inoculation.](image)

Note:
- Blue arrow: mycelium of Fusarium spp. that approaches the antagonist Trichoderma spp.
- Yellow arrow: mycelium of Trichoderma spp. that suppresses the pathogen Fusarium spp.
Based on one way ANOVA test, it was obtained that the different isolates of *Trichoderma* provided the antagonist activity effect against *Fusarium* spp. (p < 0.05). Based on further LSD testing (0.05), dual culture for antagonistic screening (5 x 24 hours) showed that *Trichoderma* isolates, namely; Isolate 1, Isolate 2, Isolate 3, Isolate 4, and Isolate 5 have different activity levels against *Fusarium* spp. The highest antagonistic activity value was found form isolate 2 (44.28%). However, this value was statistically not different from isolate 1 (40.47%) and isolate 4 (38.34%). While the lowest antagonistic activity value shown by isolate 3 (18.92%). However, this value was statistically not different from isolate 4 (38.34%) and isolate 5 (20.16%), as shown in Table 1.

| Isolate | Average Inhibition Percentage (%) |
|---------|-----------------------------------|
| 1       | 40.47<sup>a</sup>                |
| 2       | 44.28<sup>b</sup>                |
| 3       | 18.92<sup>a</sup>                |
| 4       | 38.34<sup>ab</sup>               |
| 5       | 20.16<sup>a</sup>                |

The difference in antagonistic activity among the five isolates of *Trichoderma* was due to the difference in morphological and physiological characteristics of each isolate. Several species and strains of *Trichoderma* had been reported as a biocontrol agent against pathogens, but some were more efficient than others in inhibiting different pathogens [11], [12], [13].

The differences in the efficiency of antagonistic activity among *Trichoderma* spp. against certain pathogenic fungi are affected by several factors, such as growth rate, amount and variation of the chemical compound, and enzymes produced by each species [14].

Growth rate levels can determine the antagonistic activity against pathogenic fungi. Based on this research, we found that the growth performance of five *Trichoderma* isolates was better than *Fusarium* spp. So, in the case of nutrient and space competition, *Trichoderma* was more competitive and can obtain more benefit. The observation was conducted for five days (Figure 2). On the fifth day, the radius of *Trichoderma* mycelium was higher than those of *Fusarium* spp., so it was clear that 3 of the *Trichoderma* isolates in this study were effective to be used as biocontrol agents against *Fusarium* spp. [15, 16]

*Trichoderma* is also known to produce antibiotic compounds that can inhibit the *Fusarium* spp., such as harzianic, alamethicins, tricholin, peptaibols, 6-phenyl-4-pyrene, massoia lactone, vyrindine, glyovirine, glyosperinines, heptelic acid, trichodermine, dermadine and others. Other compounds and enzymes produced by *Trichoderma* also have antifungal properties. Some *Trichoderma* species or strains also produce volatile and non-volatile compounds that can suppress pathogen fungi colonisation [15], [16], [17].

4. Conclusions
The result showed that five isolates of *Trichoderma* have different antagonist percentages in inhibiting the *Fusarium*. The highest antagonistic activity was isolate 1 and isolate 2, but they were not different from isolate 4, while the lowest was found in isolate 3 and isolate 5.

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