Short Communication

Antagonistic activity of *Trichoderma* sp. against pathogens in the leaves of *Allium ascalonicum* L.

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Abstract

Pathogenic fungi pose constraints and reduce shallot production. *Trichoderma* sp. is an antagonistic fungus capable of controlling pathogen growth in shallots. The aim of this study was to determine the characteristics of *Trichoderma* sp. and pathogens in shallots and to assess the antagonistic ability of *Trichoderma* sp. against shallot pathogens (*Allium ascalonicum* L.). *Trichoderma* sp. and shallot pathogens were isolated using a serial dilution agar plate method using potato dextrose agar medium with 7-day incubation. The antagonistic activity of *Trichoderma* sp. against shallot pathogens was evaluated based on the dual culture method. In this study, we successfully isolated *Trichoderma harzianum* from the shallot leaf and its root systems. Moreover, four morphologically distinctive pathogens from shallot roots and leaves were successfully isolated (l *Aspergillus* sp., *Colletotrichum* sp., *Phytium* sp. and *Penicillium* sp. *T. harzianum* was found to have the ability to inhibit 23.45% growth of *Aspergillus* sp, 26.19% growth of *Colletotrichum* sp., 75.40% growth of *Phytium* sp., and 40.38% growth of *Penicillium* sp. In conclusion, the isolated *T. harzianum* had a strong antagonistic activity against some pathogens in the shallot, but the activity was weak against some others.

Keywords: Antagonism, biocontrol agent, *Trichoderma harzianum*, *Trichoderma* sp., *Allium ascalonicum* L.

Introduction

Being the key in Indonesian culinary, and in some extent – traditional medicine, shallot has become a significant agricultural commodity. In Indonesia alone, shallot cultivation is widespread across almost all provinces, including in Aceh, with shallot production reaching more than 10,000 tons annually [1]. The government has put a lot of effort into optimizing the commodity development. Nonetheless, one of the primary challenges in shallot cultivation is the supply of good quality seeds, where accumulation of pathogens is plausible [2]. The presence of pathogens and its accumulation in shallots could reduce the productivity of its cultivation.

Common fungi that could cause diseases in shallots include *Fusarium oxysporum* (Moler disease), *Alternaria porri* (purple blotch disease), and *Colletotrichum sp.* (anthracnose) [3]. Currently, synthetic fungicides are the main modalities to control fungal infection albeit their effectiveness requires high doses [4]. Synthetic fungicides are indeed practical and effective; however, their continuous use could pose negative environmental and adversely impact the plant's health [5]. Alternatively, researchers have explored antagonistic activities of

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microorganisms for their use as bioagents. Of the microorganisms reported previously, *Trichoderma* is considered as a promising bioagents which exhibits antagonism against fungal pathogens in plants [6]. Moreover, *Trichoderma* sp. was observed to have potent activity against soil-borne pathogens, promote plant growth, and minimally affect the surrounding environment [6]. Fungi from this genus are also known to fertilizing the soil, synergistically interacting with the host, and produce enzymes that could enhance plant nutrition [7]. Several species, such as *Trichoderma reesei*, *Trichoderma viride*, and *Trichoderma harzianum*, are commonly known for their potential in controlling fungal pathogens [6,8].

In this present study, we isolated the *Trichoderma* sp. from the shallot leaves and root system. Isolating *Trichoderma* directly from the target plant intended for protection is a strategic approach aimed at enhancing the probability of discovering strains finely tuned to the host plant's environment. This method increases the likelihood of identifying *Trichoderma* variants that could effectively inhibit the specific pathogens. This approach has been used by previous studies in different plants [9, 10]. Studies from Indonesia reported the *Trichoderma* sp. isolation in some provinces (such as West Sumatra and East Java) [11,12], but those carried out in Aceh Province remains underreported. Therefore, as the novelty of this study, the isolation of *Trichoderma* sp. was carried out from the shallot collected in Aceh Province.

Methods

Materials

Chemicals used in this study were ethanol 70%, potato dextrose agar (PDA), distilled water, and NaOCl. All chemicals were analytical grade and procured from Merck (Selangor, Malaysia). Shallots (*Allium ascalonicum* L.) suspected of being infected by pathogens were collected as specimens for isolation. The infection was indicated by the discoloration of the leaf (**Figure 1**). The specimen was collected from Pidie Regency, Aceh, Indonesia (location coordinates: 5.340965°N and 95.989231°E). Approximately 100 g of soil from the surrounding area where the shallot specimen was located (at a depth of 5–10 cm) was sampled to isolate the *Trichoderma* sp. Shallot and soil samples were stored in separate plastic containers and transported to the laboratory for fungal isolation.

Figure 1. Geotagged images of the sampling location (A). Photographed images of shallot were used to isolate the pathogens with indication of having a disease (B).

Isolation of fungal pathogens from shallots

The isolation followed the protocol suggested previously [13]. Initially, the leaves were clean washed using distilled water and cut into 1 cm^2, before soaked in ethanol 70% for 1 min. Subsequently, the leaves were immersed in NaOCl 2.5% for 3 min and rinsed with sterile distilled water for 60 seconds. Thereafter, the sample was dried on sterile filter paper before transferred to PDA medium and incubated at room temperature for 5–7 days. Colonized fungi on the PDA medium were further purified using an inoculating loop and transferred to new Petri dishes containing fresh PDA medium until a single colony was obtained and subsequently incubated at room temperature for another five days. The isolated fungi were identified based on their microscopic and macroscopic characteristics, such as color, colony morphology, spores, or conidia. The observed characteristics were compared with those of standard references reported previously by Barnett and Hunter in 1972 [14]. Herein, four pathogen isolates were found and labeled as PT1, PT2, PT3, and PT4, respectively.

Isolation of *Trichoderma* sp.

Trichoderma fungi were isolated using a dilution method following the suggestion from a previous study [15]. One gram of previously collected soil sample was inserted into a reaction tube containing 10 mL of distilled water for homogenization. Dilution was performed up to a 10^{-3} level, then 1 mL was pipetted and spread onto PDA medium in a Petri dish before incubated at room temperature (27–28°C) for 3–7 days. Purification was performed on the grown colonies through an inoculating loop and transferred to fresh PDA Petri dishes until a single colony was obtained (incubation at room temperature for five days). Macroscopic and microscopic characteristics of the colony were observed, particularly on the hyphae, spores, sporangia, conidia, and conidiophores, and further compared with those of standard references [14].

Antagonistic activity test

A dual culture approach was employed to investigate the antagonistic activity by placing *Trichoderma* isolate and a particular pathogenic fungus in the same PDA-containing Petri dish. By using a sterile cork borer (diameter = 5 mm), the media grown with conidia and mycelium of either *Trichoderma* sp. or the pathogenic fungi were transported to the fresh PDA-containing Petri dish intended for the antagonistic activity test. The *Trichoderma* isolate or the pathogen isolates were arranged opposite to each other with a distance of 4 cm. Thereafter, incubation was carried out at 28°C for six days, where the observation was performed on day 7. As for the control, the same protocol was run with the absence of the *Trichoderma* sp. isolate on the opposite side of the pathogen isolate. The inhibition rate (P) was determined by $P = ((r1–r2)/r1) \times 100\%$.

Where r1 represents the distance at which the pathogen grows to the opposite direction of the *Trichoderma* isolate. As for the r2, it represents the distance of which the pathogen grows toward the *Trichoderma* isolate. The test was carried out in triplicate, where the average inhibition rate was presented. Inhibition rate ranges of 0–39%, 40–69%, and 70–100% indicate low, moderate, and high antagonistic activity, respectively [16].

Results

Characteristics of the isolated fungal pathogens

Characteristics of the fungal pathogens isolated from the shallot leaf, as observed macroscopically and microscopically, are presented in **Figure 2**. PT1 had a spherical shape colony appeared with a dark color on the upper side and a mixed color of black and white on the downside. Conidia and conidiophore of PT1 were observable in round and cylindrical shapes, respectively. Colonies of PT2 had a relatively uniform size and smooth-like appearance, where the color was white on the upper side and light pink on the downside. Microscopically, PT2 had hyaline hyphae and lengthy conidia. Meanwhile, PT3 colonies resembled fine cotton with greyish and white colors on the upper side and downside, respectively. The septate appearance of the hyphae was observed microscopically along with a round shape of conidia. Colonies having Fibrous and non-uniform appearance were observed in PT4 isolate, where its upper side and downside had colors of light green and whitish green, respectively. Microscopic observation suggested that PT4 had chain-like

spherical conidia, conidiophore with upright appearance, and unique brush-like patterns of phialide. Matching these characteristics with that of the reference literature, we found that PT1, PT2, PT3, and PT4 were identified as *Aspergillus* sp., *Colletotrichum* sp., *Phytium* sp., and *Penicillium* sp., respectively. The hyphae growths of each isolate are presented in **Figure 3A**. The isolates, ranked from the fastest to the slowest growth, are in the following order: PT1 > PT4 > PT2 > PT3.

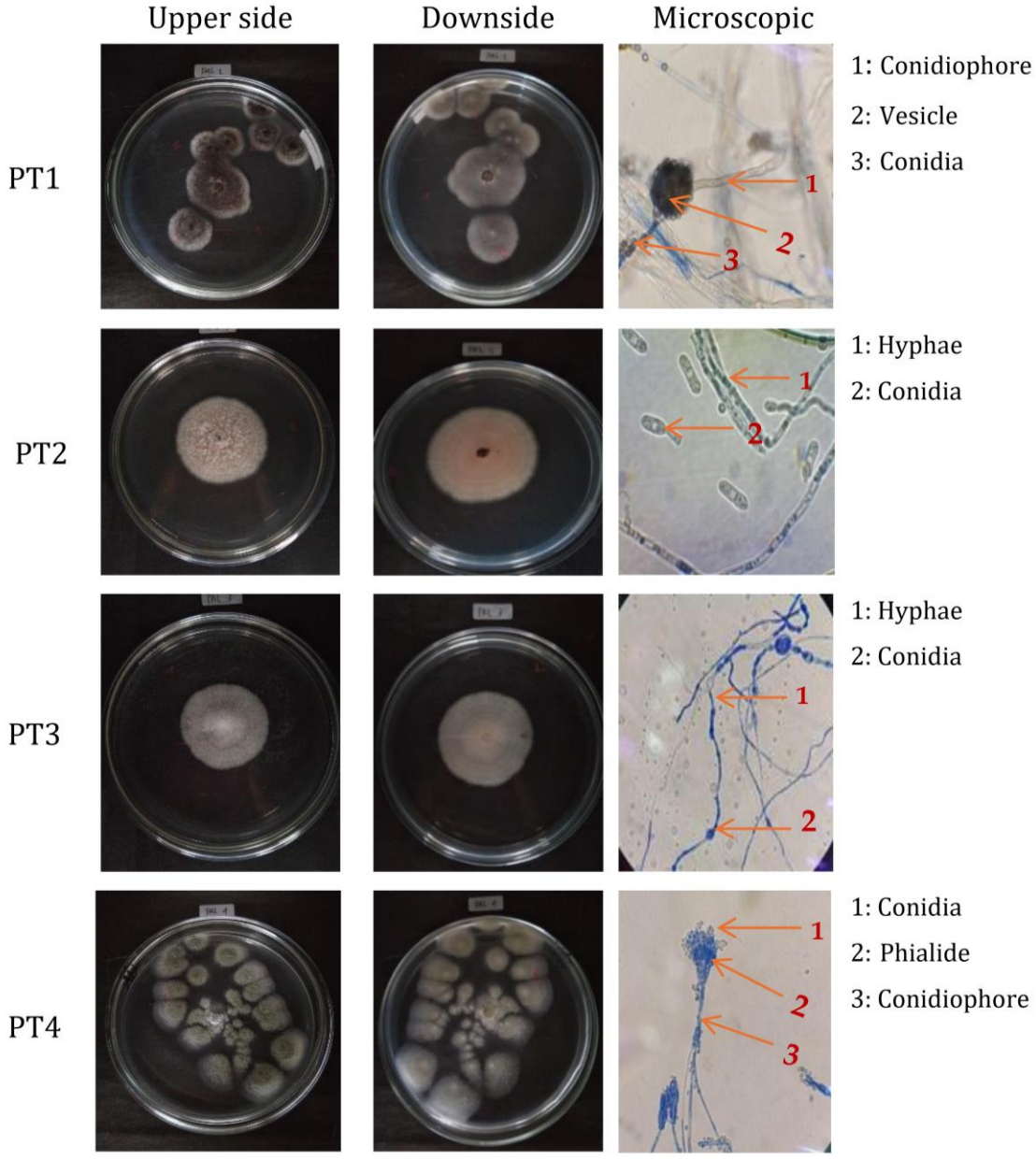

Figure 2. Macroscopic and microscopic morphologies of the shallot pathogens (PT1–4).

Characteristics of the isolated *Trichoderma* sp.

Observation on the morphological characteristics of *Trichoderma* isolate revealed that the colony appeared in a mixed color of dark green and white (**Figure 3B**). Its hyphae had round and fibrous shapes, spreading all over the Petri dish. Furthermore, microscopic observation suggested that its conidiophore appeared in an upright position with round shaped conidia and short phyalid (**Figure 3B**). These characteristics suggest that the isolate is *T. harzianum*. The hyphae reached 59.89 mm on day 6 and kept increasing to 68.74 mm on day 7, exceeding that of pathogen isolates (**Figure 3A**).

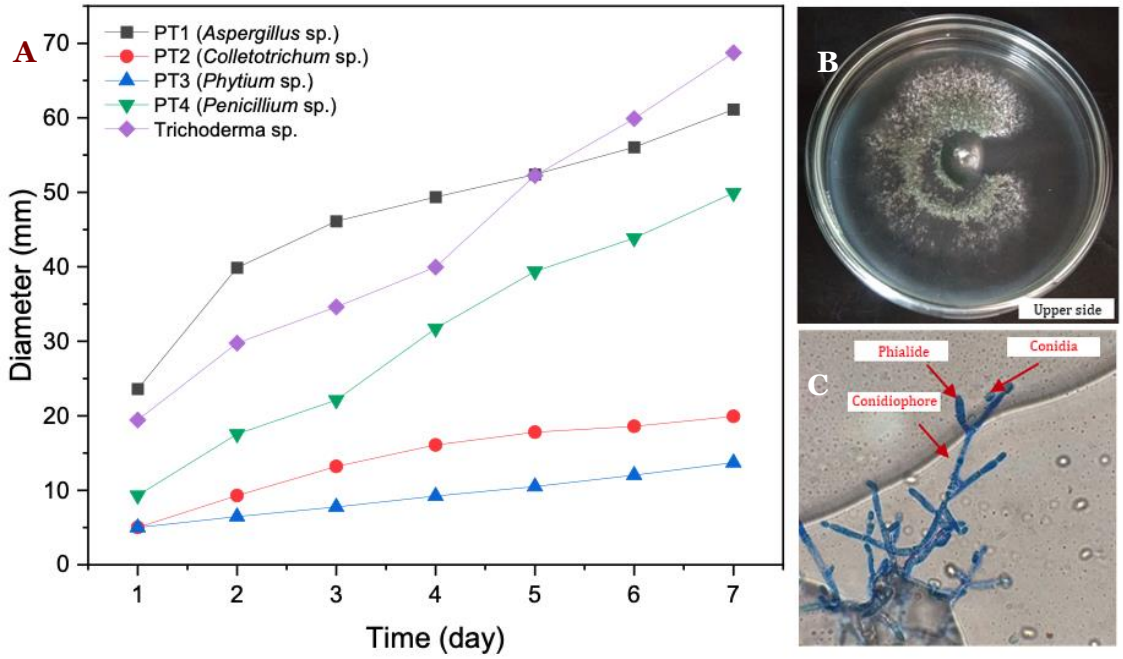

Figure 3. Characteristics of the isolated *Trichoderma* sp. and isolated pathogens from shallot roots. (A) Hyphae growths of *Trichoderma sp*. and pathogens and isolates. Macroscopic (B) and microscopic morphologies (B) of *Trichoderma* isolate which matched with that of *Trichoderma harzianum*.

Antagonism of *Trichoderma* isolate against shallot pathogens

Dual cultures consisting of *Trichoderma* isolate and a shallot pathogen isolate are presented in **Figure 4A**. Overwhelming growth of *Trichoderma* isolate hyphae was clearly observed in culture containing isolate PT3 (*Phytium* sp.) and PT4 (*Penicillium* sp.). Hyphae growths of *Trichoderma* isolate were observed in cultures containing isolate PT1 (*Aspergillus* sp.) but having a limited area coverage. As in culture containing isolate PT2 (*Colletotrichum* sp.), the hyphae growth of *Trichoderma* isolate was less unclear and obscured by that of PT2. Quantitatively, inhibition of shallot pathogens by *Trichoderma* isolate is presented in **Figure 4B**. The highest inhibition was experienced by PT3 and followed by PT4 (75.4% and 40.38%, respectively). The inhibitions of PT1 and PT2 only reached 23.45% and 26.19% respectively. Based on these findings, *Trichoderma* isolate was categorized as having strong activity against PT3 (*Phytium* sp.), moderate activity against PT4 (*Penicillium* sp.), and weak activity against PT1 (*Aspergillus* sp.) and PT2 (*Colletotrichum* sp.).

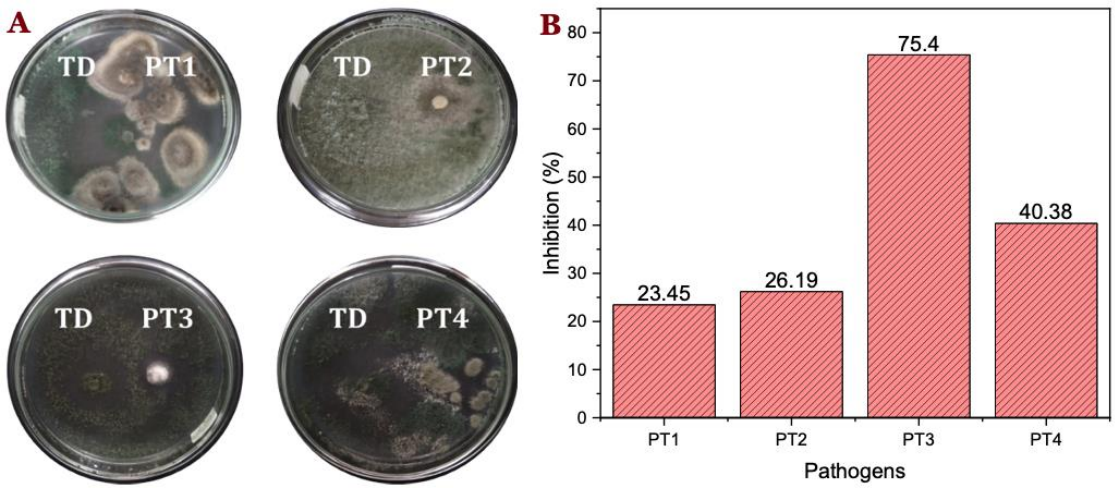

Figure 4. Antagonistic activity of *Trichoderma* isolates. (A) Antagonistic activity of *Trichoderma* isolate (TD) against shallot pathogens (PT1–4) based on dual culture method. (B) Mean inhibition of shallot pathogens by the *Trichoderma* isolates as determined using dual culture method.

Discussion

Identified pathogens in shallot

Four isolates were obtained from the leave and root system of shallot, namely PT1, PT2, PT3, and PT4, which were then identified as *Aspergillus* sp., *Colletotrichum* sp., *Phytium* sp., and *Penicillium* sp., respectively. According to a previous report, *Aspergillus* spp., *Colletotrichum* sp. and *Fusarium* spp. are common pathogens in shallot [17]. However, another study suggested that the *Fusarium* sp., *Aspergillus* sp. and *Colletotrichum* sp. were predominantly found in shallot [18]. A study conducted in Kalimantan Selatan (a province in Indonesia) successfully identified *Fusarium oxysporum*, *Penicillium* sp. and *Colletotrichum* sp. in shallot [19]. Taken altogether, pathogens identified in the present study could represent common fungi infecting shallots.

Among the fungal pathogens found in shallots in the present study, *Aspergillus* sp. is known to cause diseases in various fruits and vegetables, leading to wilting of plants and the development of dark brown infected tissues due to the accumulation of black-colored fungal spores [20]. Another identified pathogen, *Colletotrichum* sp., induces anthracnose disease in horticultural commodities, resulting in plant wilting, leaf yellowing and twisting, organ rot, and brown discoloration of roots, especially at the lower stem neck in contact with the soil [21]. *Pythium* sp. is a soilborne pathogen that causes stem and seed to rot [22]. Infections of *Pythium* sp. typically occur before plants emerge from the growing media, resulting in premature rotting of seeds and seedlings [23]. Meanwhile, *Penicillium* sp. is mostly known to be responsible for post-harvest disease in shallot bulbs, characterized by spot formation with varying sizes, initially yellowish but later becoming somewhat rounded with a light brown center surrounded by a dark brown edge [24].

Trichoderma sp. as potential bioagent

Findings from the present study revealed that the isolated *T. harzianum* exhibited varying degrees of antagonism towards different pathogens in shallots. Notably, when confronted with (*Phytium* sp.), *T. harzianum* demonstrated the highest level of antagonism, with an average inhibition rate of 75.40%. Conversely, its antagonism against PT1 (*Aspergillus* sp.) showed the lowest efficacy, with an average inhibition rate of 23.45%. Previous studies have suggested that the antagonistic activity of *T. harzianum* could range from strong to weak depending on the percentage of inhibition, with values between 70% and 100% classified as strong inhibition. This corroborates with earlier research indicating that the mycelial growth of *T. harzianum* has relatively weak antagonistic activity against *Aspergillus* sp., possibly due to the faster colonization rate of the pathogenic fungus [25].

In the present study, the antagonism test also revealed that the antagonistic activity of *T. harzianum* against *Colletotrichum* sp. was categorized as weak. As for the *Penicillium* sp., the antagonistic activity of *T. harzianum* was categorized as moderate. The inhibition of the two pathogens by *T. harzianum* was ineffective because of the rapid and extensive growth of the pathogens. As evidenced by a visible discoloration, *T. harzianum* hyphae was overgrown by that of pathogens resulting in insufficient growing space and nutrients. Moderate inhibition activity of *T. harzianum* against *Penicillium* sp. has also been witnessed previously, where the average inhibition area only reached 26.38% [26]. As for *Colletotrichum* sp., however, a previous study found that *T. harzianum* had a moderate activity against the pathogen with 64.2% inhibition as observed on day 168 [27].

Limitations

Herein, we have successfully investigated the antagonistic activity of *T. harzianum* against various shallot pathogens. However, there are several limitations which should not be overlooked when interpreting the findings. First, species or genus identifications were based on visible macroscopic and microscopic characteristics, which are prone to false identification. Molecular techniques are required to accurately determine the species and strains of pathogens and *Trichoderma* isolate. Secondly, the sampling was only carried out in a single location, where multiple locations and more isolates are required to yield representative results. Thirdly, our study only utilized a single observation time, where assessing antagonistic activity over varying time intervals would provide more comprehensive and informative data. Taken altogether, future

research is still required to validate the potential of the isolated *T. harzianum* in controlling shallot pathogens.

Conclusion

T. harzianum isolated in the present study could strongly inhibit the growth of *Phytium* sp. but showed a moderate to weak activity against *Aspergillus* sp., *Colletotrichum* sp., or *Penicillium* sp. Findings in the present study suggested the complex interactions between *T. harzianum* and various shallot pathogens, which warrants future research on the antagonistic mechanisms and potential applications in controlling plant pathogens.

Ethics approval

Not required.

Acknowledgments

None.

Competing interests

The authors declare that there is no conflict of interest.

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Underlying data

All underlying data have been presented.

How to cite

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