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Evaluation of Antagonistic Activity of *Trichoderma harzianum* Against Various Pathogenic Fungi Infecting Dragon Fruit Stem

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ABSTRACT

Dragon fruit stem is prone to the infection of various pathogenic fungi and *Trichoderma harzianum* had been reported as an effective biological control agent against plant pathogenic fungi. This study was aimed to evaluate the ability of *T. harzianum* in controlling various pathogenic fungi infecting dragon fruit stem. This study was conducted using Completely Randomized Design (CRD) with three replicates. Pathogenic fungi was isolated from infected dragon fruit stem, then characterized morphologically to identify the species of those pathogenic fungi. The isolated fungi were subjected to in-vitro and in-planta antagonistic assay containing *T. harzianum* culture. The resulted growth inhibition in each fungal isolate was observed for a week by comparing the diameter of pathogenic fungi. Based on morphological characteristics, the dragon fruit stem was predicted to be infected by several pathogenic fungi, such as *Phytium* sp. (isolate A), *Fusarium* sp. (isolate B), *Alternaria* sp. (isolate C) and *Rhizoctonia* sp. (isolate D). Result of in-vitro antagonistic assay showed that *T. harzianum* conferred different suppression efficacy against all isolated pathogenic fungi ranging from 59-66%. Moreover, application of *T. harzianum* in-planta had resulted promising fungal growth inhibition up to 50% against all pathogenic fungi.

Keywords: Dragon fruit, *Trichoderma harzianum*, *Phytium* sp., *Fusarium* sp., *Alternaria* sp., *Rhizoctonia* sp.

1. INTRODUCTION

Dragon fruit is a cactus from the genera *Hylocereus* and *Selenicereus*. Dragon fruit comes from Mexico, Central America, and South America, but now dragon fruit has been cultivated in Asian countries, including Indonesia [1]. Dragon fruit has a high economic value compared with other fruits, thus offers a promising business opportunity for its large scale cultivation domestically. It is also predicted to be one of highly profitable export commodities [2]. Dragon fruit is highly preferred by local people due to its health benefits and it can be processed into various products.

Dragon fruit are sensitive to disease infection, including antrachnose, stem white disease, yellow rot disease, stem rot disease, stem canker and fruit spot disease [3-4]. These diseases caused huge loss on dragon fruit production. One of major disease in dragon fruit is stem rot disease. This disease infected young plants, thus might cause significant yield loss once it was not properly controlled. The infected stem would be unable to promote fruit formation, thus it is recommended to trim this infected stem in order to allow the fruit formation from the uninfected stem. Despite of the failure of fruit formation, this disease might also cause plant death.

Recently, management of plant disease are directing to be performed using more eco-friendly way to minimize the negative effect toward the environment. Chemical disease control mostly applied by local farmers could harm non target organism as well as farmers and increase the resistance of target organism [5]. Therefore, the use of biological control agents (BCA), such as *Trichoderma harzianum* could be more environmentally friendly strategy in managing plant diseases. *Trichoderma* sp. is known to be antagonistic against diverse array of pathogenic fungal species. Its antagonism was resulted from various mechanisms, such as competition, lysis, antibiosis and hyperparasitism [6]. The utilization of this species had been reported to be easily performed as this species had wide adaptability, fast growing, easily found in soil or plant roots and could be grown in various substrates.

Antagonistic effect of *T. harzianum* had been applied to control various species of pathogenic fungi, such as *Candida albicans, Rhizoctonia solani, Armillaria mellea, Lentinus edodes, Fusarium oxysporum, Athelia rolfsii, Pythium aphanidermatum* and *Sclerotinia*. Its application had been reported successfully reduced the severity of sudden death disease up to 78% in lettuce, sunflower, cauliflower and soybean [7]. Alfizar et al. [8] found that *Trichoderma* sp. could inhibit several phytopathogenic fungi (*C. capsici, Fusarium* sp., and *S. rolfsii*) in-vitro on seventh day after application. Other studies utilized *Trichoderma* sp. to manage the strawberry wilt disease caused by *Fusarium* sp. and resulted in 43.4 - 49.7% growth inhibition of *Fusarium* sp.

[9]. Similarly, Saravanakumar *et al.* [10] successfully control the severity of stem rot in maize up to 86.66% by applying *T. harzianum*. Application of *T. harzianum* had been reported to control *S. rolfsii* (the causal agent of stem and root rot disease as well as wilt) in green beans and cabbage [11]. Several studies also reported the efficacy of *Trichoderma* sp. in suppressing *Alternaria porri* [6, 12].

Considering its broad spectrum, *T. harzianum* might be potential to be used as biological control of various diseases infecting dragon fruit stem. Therefore, this study was aimed to evaluate the ability of *T. harzianum* in controlling various pathogenic fungi infecting dragon fruit stem.

2. METHODS

2.1. Plant Collection

Dragon fruit used in this study was red type with purplish red flesh. Plants were collected from Bona Mitra Farm, Kaliurang, Yogyakarta, Indonesia. Sampling was performed in three different blocks. Infected stems were collected where each plant was represented by three infected stems.

2.2. Characterization of Plant Pathogenic Fungi Infecting Dragon Fruit Stem

Infected stems were surface sterilized then grown on PDA medium for 7 days at room temperature and dark condition. Single colonies were picked and subcultured onto new medium. Pure culture of each isolated fungus was further characterized microscopically based on its cell shape and the morphology of its conidia, hyphae and spore. Prediction of each isolated pathogenic fungi was identified based on the morphological similarity with those reported in previous studies.

2.3. Preparation of T. harzianum

T. harzianum used in this study was collected from internal collection of Agrobiotechnology Laboratory, Faculty of Agriculture, Universitas Muhammadiyah Yogyakarta. *T. harzianum* was refreshed onto new PDA medium and grown for 7 days at room temperature and dark condition.

2.4. In-vitro Antagonistic Assay

In-vitro antagonistic assay was performed using dual culture method by culturing both *T. harzianum* and each isolated pathogenic fungi in the same plate containing PDA medium. The antagonistic effect was observed after a week by measuring the radial growth of both pathogenic fungi and *T. harzianum*. Inhibition efficacy was then calculated using the formula described by Dwiastuti *et al.* [9] as followed:

$$\text{Growth inhibition (\%)} = \frac{R1-R2}{R1} \text{ x } 100 \quad (1)$$

where **R1** represented diameter of non dual culture *T. harzianum* and **R2** represented diameter of dual culture *T. harzianum*.

2.5. In-planta Antagonistic Assay

Dragon fruit was grown in soil mixed with compost fertilizer and the inoculum of *T. harzianum* (2.6 x 10^7). After a week, tendrils of dragon fruit were inoculated with each isolated pathogenic fungus. This inoculation was repeated three times for each pathogen isolates. Disease development were observed regularly for a month. Disease incidence and severity were determined using the formula proposed by Resti *et al.* [13] as followed:

$$\text{DI (\%)} = \left[\frac{T1}{T2}\right] \times 100 \quad (2)$$

where **DI** was disease incidence, **T1** represented number of infected plants and **T2** represented total number of plants observed.

$$\text{DSI (\%)} = \frac{\sum(n \times V)}{Z \times N} \times 100 \quad (3)$$

where **DSI** was disease severity index, **n** represented symptom scale, **V** represented number of infected plants representing each scale, **Z** represented the highest symptom scale and **N** represented total number of plants observed.

Symptom scale used in this study was based on specific criteria mentioned in Table 1.

Table 1. Criteria used to determine the scale of disease symptoms.

Scale	Symptom Description
0	No visible symptom
1	Mild infection, showing <5 cm lesion length in the infected stem
2	Moderate infection, showing 5-10 cm lesion length in the infected stem
3	Severe infection, showing >10 cm lesion length

2.5. Data Analysis

Data collected from both in-vitro and in-planta antagonistic assay were statistically analyzed using one-way analysis of variance (ANOVA). Significance among treatments was further analyzed using DMRT with a $p<0.05$.

3. FINDING AND DISCUSSION

3.1. Characteristics of Dragon Fruit Stem-infecting Pathogenic Fungi

The infected stems collected showed different symptom indicating different possible causal agent. This study found 4 isolates of pathogenic fungi conferring different morphological characteristics (Figure 1). Based on its morphology, those four fungi were predicted to be *Phytium* sp. (A), *Fusarium* sp. (B), *Alternaria* sp. (C) and *Rhizoctonia* sp. (D).

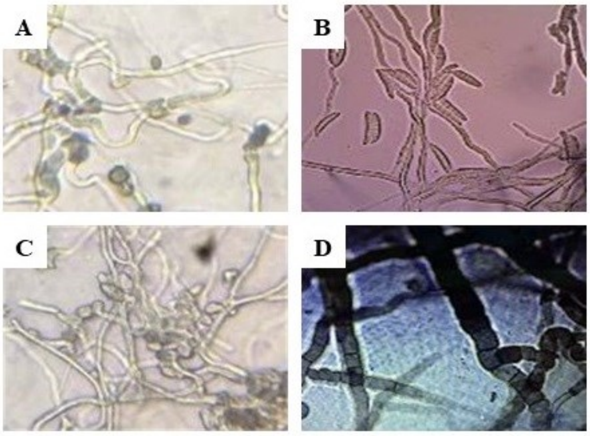

Figure 1. Microscopic visual of pathogenic fungi isolated from the infected stem. (A) *Phytium* sp., (B) *Fusarium* sp., (C) *Alternaria* sp. and (D) *Rhizoctonia* sp.

Isolate A was predicted to be *Phytium* sp. [14] due to its white mycelia with stringy surface and black colored spores. Hyphae was found to be not insulated and conidia was also found in this isolate. This isolate also formed an irregular shape of sporangium with branch-like structure separated from the tip of the hyphae.

Isolate B was predicted to be *Fusarium* sp. [14] due to its white mycelia with velvety surface and white-colored spores. Hyphae was found to be not insulated with slender, simple, irregular branching structure. The conidia had a crescent moon-like curve structure with pointed tip.

Isolate C was predicted to be *Alternaria* sp. [14] due to its greyish white mycelia with black spores and filamentous structure. Hyphae of this isolate was found to be insulated. The conidia had an inverted club shape with peak at the end and mulberry-like branching structure.

Isolate D was predicted to be *Rhizoctonia* sp. [14] due to its brownish white mycelia with black spores and filamentous structure. Hyphae of this isolate was found to be insulated with the elbow-shaped hyphal branching and thick wall where the branches were notched at the base. This isolate did not show any conidia formed.

3.2. Comparison of T. harzianum In-Vitro Biocontrol Efficacy Against Several Pathogenic Fungi

T. harzianum showed different growth inhibition efficacy against each pathogenic fungus (Table 2). *T. harzianum* showed the highest inhibition on *Alternaria* sp. (isolate C), while the lowest one was recorded from *Rhizoctonia* sp. (isolate D). This finding was in accordance with several previous studies related to each pathogenic fungus.

Table 2. Difference of in-vitro growth inhibition effect of *T. harzianum* against several pathogenic fungi.

Pathogenic fungi	Growth inhibition (%)
Phytium sp.	62
Fusarium sp.	62
Alternaria sp.	66
Rhizoctonia sp.	59

Kamo *et al.* [15] reported the in-vitro biocontrol efficacy of *T. polysporum* against the causal agents of snow rot (*P. iwayamai*). It was predicted that the biocontrol effect was resulted from antibiosis mechanism. Other study also reported the high antagonistic activity of *T. harzianum* in-vitro up to 96.30 against maize-infecting *F. oxysporum* [10]. Similar finding reported the high biocontrol effect of *T. harzianum* and T. *longibrachiatum* against *A. porri* in shallot up to 73.12% [6]. Kobori et al. [16] also highlighted the excellent antagonism of *T. harzianum* in reducing the infection of damping off disease caused by *R. solani*.

3.3. Effect of In-planta T. harzianum Application on Disease Severity

Dragon fruits exhibited visible symptom after re-inoculation of each pathogenic fungus (Figure 2). Significant reduction on lesion length as well as disease incidence and severity were recorded in *T. harzianum*-treated plants compared to the untreated one, except in *R. solani*-infected plants (Table 3). These findings emphasized the efficacy of *T. harzianum* both for in-vitro and in-planta application indicating its powerful characteristics as a biocontrol agent for disease management.

Pathogenic Fungi	Disease incidence (%)		Disease severity (%)	
	Untreated*	Treated**	Untreated	Treated
Phytium sp.	66	33	66	33
Fusarium sp.	33	33	33	33
Alternaria sp.	66	33	66	33
Rhizoctonia sp.	66	33	66	66

Table 3 revealed that incidence of *Pythium* sp., *Alternaria* sp. and *Rhizoctonia* sp. was recorded up to 66% in the absence of *T. harzianum*. Remarkable reduction of those three diseases was spotted in *T. harzianum*-treated plants, thus indicating the efficacy of this beneficial fungi in suppressing the disease development. In contrast, *Fusarium* sp. infected plants showed no difference on disease incidence between the *T. harzianum* treated and untreated ones (Table 3). Regarding the disease severity, in-planta application of *T. harzianum* successfully reduced the severity of *Phytium* sp. and *Alternaria* sp. infection up to 50% compared to untreated one (Table 4). However, the severity of *Fusarium sp.* and

Rhizoctonia sp. infection showed no effect after the application of *T. harzianum*. It was assumed that *T. harzianum* might prevent the disease occurrence, but seemed unable to significantly controlled the disease development.

Disease suppression induced by *T. harzianum* might be associated with several mechanisms, such as niche competition, lysis, antibiosis and hyperparasitism [6]. Other studies also reported the capability of *T. harzianum* in producing siderophore that inhibit the growth of the other competing fungus. In addition, previous studies also mentioned that the niche competition occurred between Trichoderma species and pathogenic fungi was commonly resulted in nutritional deficiency thus triggering the growth suppression of pathogenic fungi [17]. Several antibiotic compounds produced by T. harzianum were able to degrade the fungal cell wall and might interfere some vital cellular processes [18].

4. CONCLUSION

This study highlighted the efficacy of *T. harzianum* in controlling the disease infection caused by various pathogenic fungal species in dragon fruit stem. In-vitro application of T. harzianum showed high suppression effect to all pathogenic fungi ranging from 59-66%. However, the in-planta application resulted in remarkable severity reduction of *Alternaria* sp. and *Phytium* sp. infection.

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