Ability of *Trichoderma harzianum* in carbon fiber and silica nano particles formulation to control *Fusarium oxysporum* in vitro

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Abstract. Basal rot disease caused by *Fusarium oxysporum* f. sp. *cepae* is one of the major diseases that cause yield loss on shallot. Utilization of biocontrol agents can be applied as an environmentally safe control method. The antagonistic microorganism that has the potency to control fusarial diseases is *Trichoderma harzianum*. A carrier is required in preparing a formulation of this antagonistic fungus as a biocontrol delivery system (BDS). Carbon fiber was proven suitable as a carrier of some antagonistic bacteria. A formulation can also be supplemented with plant micronutrient. Addition of silica nano particles (Si NPs.) in the formulation did not reduce the viability of the antagonistic bacteria. An experiment was carried out to determine the ability of *T. harzianum* in the formulation with carbon fiber and Si NPs. to suppress the in-vitro growth of *F. oxysporum*. The experiment was arranged in the completely randomized design with 5 treatments and 5 replications. The treatments were challenging *F. oxysporum* by *T. harzianum* in different composition of formulation on potato dextrose agar. The compositions consisted of *T. harzianum* + 0.5% Si NPs., *T. harzianum* + 1% Si NPs., *T. harzianum* + 0.5% Si NPs. + 5% carbon fiber, *T. harzianum* + 1% Si NPs. + 5% carbon fiber, and *T. harzianum* only (control). The results showed that each treatment with *T. harzianum* in the formulation of carbon fiber + various concentrations of Si NPs. was able to suppress the in vitro growth of *F. oxysporum* by 58.39-60.92%. The BDS with carbon fiber and Si NPs. did not significantly reduced the ability of *T. harzianum* to antagonize *F. oxysporum*. The control treatment of single *T. harzianum* caused the highest suppression on the growth of *F. oxysporum*, up to 60.93%.

1. Introduction

Shallot (*Allium ascalonicum* L.) is a horticultural crop with many beneficial uses. It is not surprising if the demand of shallots is increasing with time. One of the constraints in shallot cultivation is plant disease. Fusarium basal rot or fusarium wilt caused by *Fusarium oxysporum* f.sp. *cepae* is a major shallot disease [1]. The symptoms of fusarium wilt on shallot are leaf wilting and curling, start from the tip of the leaf, and bulb and root rot [2].

Synthetic fungicide is still widely used to control fusarium wilt on shallot, although this method is not environmentally friendly. Utilization of biocontrol agents is a good alternative in controlling plant diseases. *T. harzianum* is a biocontrol agent of several soilborne pathogens such as *Sclerotinia* sp.,
Fusarium sp., Pythium sp., Rhizoctonia sp., Ganoderma sp. and Rigidoporus microporus [3]. T. harzianum T35 controlled Fusarium oxysporum by colonizing the rhizosphere and competing in nutrition uptake.

Biocontrol agents should be formulated in a biocontrol delivery system (BDS) to manage a simpler handling. The formulation can be in form of solid powder or liquid (suspension). Beside the biocontrol agent as the main ingredient, other component in a BDS is the carrier. Carbon fiber as carrier was proven to maintain the viability of some antagonistic bacteria in a formulation [4]. Carbon fiber is a strong and durable composite material. In a BDS, the formulation can be supplemented with plant micronutrient such as silica. The roles of silica are to neutralize the soil acidity and strengthen the plant tissues, which then develop a more resistant plant to pathogens [5]. The use of silica nanoparticles (NPs.) can be beneficial to enhance the effectiveness of the BDS. In the previous study, Djaya et al. [6] reported that bacterial isolates of Lysinibacillus, B. subtilis, and P. fluorescens, which were antagonistic to R. solanacearum and able to reduce the wilt disease incidence on potato plant, were viable in the BDS formulated with graphitic and silica NPs. Those bacterial isolates in the formulation were also able to inhibit the in vitro growth of R. solanacearum, indicated that the delivery system significantly affects the mechanism of biocontrol. In this study, T. harzianum, an antagonistic fungus to F. oxysporum f.sp. cepae was tested. The objective was to determine the ability of T. harzianum fungus in the BDS with carbon fiber and silica NPs. to suppress the in-vitro growth of F. oxysporum

2. Materials and Methods
The experiment was carried out at the Laboratory of Phytopathology, Faculty of Agriculture, Universitas Padjadjaran, Indonesia. It was arranged in the completely randomized design with 5 treatments and 5 replications. The treatments were challenging F. oxysporum by T. harzianum on potato dextrose agar in different composition of formulation, as follow: A) T. harzianum + 0.5% silica NPs., B) T. harzianum + 1% silica NPs., C) T. harzianum + 0.5% silica NPs. + 5% carbon fiber, D) T. harzianum + 1% silica NPs. + 5% carbon fiber, and E) control (T. harzianum only). The size of the silica NPs. was 100 nm. The carbon fiber was 80 mesh (0.177 mm).

Both fungi isolates were the collection of Laboratory of Phytopathology, Faculty of Agriculture, Universitas Padjadjaran. F. oxysporum was isolated from infected shallot plant in Kabupaten Brebes.

2.1. Preparation of fungal isolates
F. oxysporum was cultured on PDA. One piece of F. oxysporum culture (0.5 cm in diameter) was cut by using a cork borer and put in the middle of PDA in a petri dish, and incubated for 7 days. T. harzianum was also cultured on PDA in the same way as F. oxysporum.

The suspension of T. harzianum was prepared by collecting the culture on PDA. Sterile distilled water was added to the culture in petri dish, 10 ml/dish, and then the culture was scrapped by using an L shape glass rod. The spore density of the suspension was counted by using a haemacytometer. It was 8.30 x 10^8 conidia/ml. This suspension was mixed with silica NPs. and Carbon fiber in different composition as the treatments.

2.2. Antagonism test of T. harzianum in the BDS
The BDS consisted of T. harzianum, carbon fiber and silica NPs. The in-vitro antagonism between T. harzianum against F. oxysporum was tested on PDA. One piece of F. oxysporum culture (0.5 cm in diameter) was placed on 3cm from the side the petri dish (9 cm diameter). One drop of the suspension of treatments was placed 3 cm across the F. oxysporum towards the other side. The radius of the colony of F. oxysporum on each petri dish was measured. The inhibition rate was calculated by the equation:

\[ I = \frac{R_1 - R_2}{R_1} \]
Where $I$ is inhibition rate; $R1$ is radius of $F. oxysporum$ colony at the opposite direction of $T. harzianum$ colony; and $R2$ is radius of $F. oxysporum$ at the direction to the center of $T. harzianum$ colony.

3. Results and Discussion

3.1. In-vitro antagonism test between $T. harzianum$ in the formulation with carbon fiber and silica NPs. against $F. oxysporum$

Results of the experiment showed that $T. harzianum$ in the formulation with carbon fiber and silica NPs. had the potency to control $F. oxysporum$. This was proven by the inhibition of the pathogen growth on the dual culture, which ranged from 58.39 to 60.93% (Table 1). $T. harzianum$ in the formulation inhibited the in vitro growth of $F. oxysporum$ (Figure 1), as $T. harzianum$ grew faster than the pathogen. Trichoderma spp. is a saprophytic soil inhabitant that has the ability to parasitize and antagonistic to many fungi. The mechanism of Trichoderma spp. To antagonize plant pathogenic fungi is mycoparasitism and competition [7]. In this study, all treatments of the formulation with carbon fiber and silica NPs. suppressed the in vitro growth of $F. oxysporum$ (Figure 1).

![Figure 1](image)

Figure 1. Inhibition of $T. harzianum$ in the BDS against $F. oxysporum$ on day 4.
1) $F. oxysporum$, 2) $T. harzianum$, A) $T. harzianum$ + 3lica NPs. 0.5%, B). $T. harzianum$ + 3lica nano 1%, C). $T. harzianum$ + 3lica NPs. 0.5% + serat karbon 5%, D). $T. harzianum$ + 3lica NPs. 1% + carbon fiber 5%, and E). $T. harzianum$.

Results of the in-vitro antagonism test showed that each treatment was able to inhibit the growth of $F. oxysporum$ on day 1, 2, 3, 4, and 5. On day 1 and 2, treatment D ($T. harzianum$ + Si 1% + C 5%) caused the highest inhibition i.e. 25.50% and 29.97% respectively. On day 3 and 4 treatment, the highest inhibition was caused by treatment B ($T. harzianum$ + Si 1%) i.e. 54.94% and 56.72% respectively. On day 5, treatment E ($T. harzianum$) caused the highest inhibition (60.93%), although it was not significantly different with other treatments. The antagonism was indicated by inhibition of the colony
growth of the pathogen. Dendang [8] reported that *T. harzianum* caused the highest inhibition (74%) on the growth of *Ganoderma* sp. colonies compared with *T. viridae* and *T. pseudokoningii*.

*T. harzianum* is widely used as biocontrol agent against soil borne plant pathogens, and able to increase the plant growth and yield [9]. The observation on the in vitro growth of the *F. oxysporum* showed that the antagonistic mechanism was competition on growth space and nutrition. Chet *et al.* [10] reported that *T. harzianum* outgrew the space for the growth of *Rhizoctonia solani* mycelia. *T. harzianum* degraded the cell wall of the fungal host, by the production of chitinase, glucanase and protease.

### Table 1. Inhibition rate of *F. oxysporum* growth by *T. harzianum* in the BDS.

| Treatments | Inhibition rate* (%) on day: |
|------------|-------------------------------|
|            | 1  | 2  | 3  | 4  | 5  |
| A (T. harzianum + Si 0.5 %) | 9.7 a | 26.52 ab | 47.65 bc | 55.66 a | 58.39 a |
| B (T. harzianum + Si 1 %) | 25.22 a | 29.00 a | 54.94 a | 56.72 a | 60.60 a |
| C (T. harzianum + Si 0.5% + C 5%) | 24.23 a | 20.23 ab | 50.18 ab | 55.04 a | 58.90 a |
| D (T. Harzianum + Si 1% + C 5%) | 25.50 a | 29.97 a | 51.52 ab | 55.15 a | 60.92 a |
| E (T. harzianum) | 16.98 a | 14.89 b | 41.07 c | 43.66 b | 60.93 a |

*Numbers followed by the same letter within a column are not significantly different according to Duncan’s multiple range test (5%).

![Figure 2. SEM image of the BDS. A) T. harzianum + Silica NPs. B) T. harzianum + Carbon fiber + Silica NPs. C) T. harzianum.](image)

Under Scanning Electron Microscope (SEM), the spores of *T. Harzianum* is cup shape (Figure 2). The spore shape is the same as described by previous studies [11, 12].

### 4. Conclusion

*Trichoderma harzianum* in the BDS consisted of carbon fiber and silica NPs. was able to suppress the *in vitro* growth of *Fusarium oxysporum* by 58.39% to 60.92%. The BDS with carbon fiber and Si NPs. did not significantly reduced the ability of *T. harzianum* to antagonize *F. oxysporum*. BDS with 1% of
silica NPs. and 5% of carbon fiber was the best for *T. harzianum* to antagonize *F. oxysporum* in vitro; the inhibition was 60.92%.

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