# The Effects of Mycorrhizal Fungi and Trichoderma harzianum on Verticillium dahliae in Cucumber

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**Abstract:** *Verticillum dahliae* is the important soil-borne pathogen and infects wide variety of hosts. In this study, the effects of *Trichoderma harziarum* and mycorrhizal fungi on Verticillium wilt caused by *Verticillium dahliae* in cucumber was investigated. *T. harzianum* restricted the mycelial development in dual culture in vitro. In the pot experiment, *Glomus mosseae*, mycorrhizal preparation and *T. harzianum* reduced the diseases severity by 61.4%, 56.1% and 66.7%, respectively. As a result of the study, bioagents could be used against Verticillium wilt and detailed research are required to elucidate resistance mechanism

**Key Words:** Mycorrhizal fungi, *Trichoderma harzianum, Verticillium dahliae*, cucumber

## Introduction

*Verticillum dahliae* is an important soil borne pathogen which has large host range and maintain viability for 10-15 years in the soil as microsclerot. The disease prevent the exchange of plant nutrients and water in the plant and cause wilting (Roustaee and Baghdadi, 2007). There is no effective control methods against diseases including fungicides. Biological agents are used against diseases in recent years especially where good agricultural treatments are practiced. Mycorhizal fungi are symbiotic organisms living with root of many plants and by means of spores which exist in the soil enter into root and continue to colonize. Mycorrhizal fungi enhanced the development of plants by water absorption and nutrients from the soil (Smith and Read, 1997). It covers the root of plants so it makes protective physical barrier against diseases also (McAllister et al. 1997; Karagiannidis et al, 2002)

*Trichoderma harzianum* is an important biological control agent effective to plant pathogents via hyperparasitism. *Trichoderma* spp. also produce some bioactive substances has an antagonistic effect. It was determined that some isolates of Trichoderma control effectively some fungal pathogens including *Rhizoctonia solani, Sclerotium rolfsii, Pythium* spp. *Trichoderma* spp. enhanced the development of plats and induces the resistance mechanisms by several ways (Harman, 2006; Akrami et al., 2009).

The aim of this study was to determined efficiency of mycorrhizal fungi and *Trichoderma harzianum* against *Verticillum dahliae* in cucumber.

## The Study

### Materials

Cucumber (*Cucumis sativus* L.) cv. Beith Alpha F1 was used in the pot experiments. *Verticillium dahliae* was isolated from diseased tissues of naturally infected plants on Potato Dextrose Agar (PDA). *Trichoderma harzianum* was obtained from pepper grown area by soil isolation. *Glomus moseae* was bulked up on maize and used as mycoorhizal fungal inoculum. The other biological fungal preparation contained 23.5% Mycorrhizae (including 18 mycorrhizal fungi belongs to *Pisolithus* spp. *Rhizopogon* spp *Scleroderma* spp., *Laccaria* spp., *Glomus* spp., *Gigaspora* spp*)* and 27.0% Cross linked polyacrylamide polymer, 14.0% Cold Water Kelp Extracts, 10.0% Humic Acids, 9.0% Ascorbic Acid (Vitamin C), 6.4% Dry Humus, 4.0% Amino Acids, 2.4% Myo-Inositol, 1.2% Thiamine (Vitamin B1), 1.2% Thickener, 1.0% Surfactant, 0.5% Alpha-tocopherol (Vitamin E) was used.

### Methods

#### Dual Culture Tests Of Trichoderma Harzianum And Verticillium Dahliae In Vitro

First step of dual culture test were conducted using colonized plate method. *V. dahliae* and *T. harzianum* were cultured on PDA at 24°C. 10ml PDA was prepared in test tubes and poured in 9cm-diameter Petri dishes and signs were put 3cm from the edge at both side by measuring point at the bottom of Petri dishes. 6mm discs of both fungi placed opposed in Petri dishes. For the control plates, V. dahliae was cultured without *T. harzianum*. Cultures were incubated at 24°C. After 1 week inhibition zones were measured and developping area was evaluated according to 1-5 scales (Bell et al., 1982)

Class 1: The hyperparasyte completely overgrown the pathogen (100% overgrowth)
Class 2: The hyperparasyte overgrown at least 2/3th of the pathogen
Class 3: The hyperparasyte and pathogen colonised on half of the Petri dishes
Class 4: The pathogen overgrown at least 2/3th of the hyperparasyte
Class 5: The pathogen completely overgrown the hyperparasyte (100% overgrowth)

At the same time, the effects of volatile compounds of *T. harzianum* was determined. 6 mm mycelial discs of *T. harzianum* and *V. dahliae* were cultured on PDA singly. Lids of petri dishes were removed and culture plates were immadiately placed over T. harzianum plates and sealed with parafilm. Cultures were incubated at 24°C for 1 week and colony diameters measured. Control petri dishes were included in two experiments. Experiments was repeated three times and 5 Petri dishes in each.

## Pot Experiments

Cucumber seeds were surface disinfested in 1% NaOCl solution for 3 min and washed twice with sterile distilled water. The mixture of soil, sand, and pumice (1/1/1, v/v/v) was autoclaved at121ºC twice for 1 h and used as growth medium. Mycorrhizal seedling produced by incorporating the mycorrhizal inoculum including soil infested with spores mixed with root fragments, 2-3 cm below the seeds (Menge and Timmer, 1982). Inoculum amount was determined as 1000 spores 10 $g^{-1}$ for each plant. Cucumber seeds were sown in containers without mycorrhizal fungal inoculations. Containers were placed in growth room at 25±2ºC tempertures until 3-4 leaf stage. Plants with 3-4 leaves with ot without mycorrhizal fungi were transplanted into 15cm diameter pots containing same mixture of soil. The treatments were as follows: *Glomus mosseae*, Mycorrhizal preparation, *Trichoderme harzianum*, *Verticillium dahliae*, Control. Pots were maintained in in a growth room 25±2ºC 12 h photoperiod. Experiments were designed as compeletely randomized block design with four replications and 5 plants in each.

Mycorrhizal colonisation was determined 4 week after transplanting and colonisation percentages (%) were calculated . the roots were cleared and stained as described by Koske and Gemma (1989) and the percentage of root colonisation (%) was determined by gridline intersection method (Giovannetti and Mosse, 1980).

*T. harzianum* was maintained on PDA at 24ºC for 1 week. Culture plates were scraped from the surface using spatula for collecting the spores. Released spores were collected by filtering through two

layers of cheese cloth and conidia concentration was adjusted to $10^6$ conidia $ml^{-1}$ using haemocytometer. 10 ml suspension were applied to rhizosphere of plants.

*Verticillum dahliae* oat medium: Oat seeds were boiled to be used for inoculation. Boiled seeds were placed into Erlen mayer and autoclaved at 121°C 1 kPa for 20 min. After cooling, *V dahliae* mycelial disc were added and incubated at 24ºC for 3 weeks.

Plants were inoculated after 4 week with *V. dahliae*. For inoculation, 2g oat medium incorporated to soil around roots. Disease severity was evaluated using the following 0-5 scale (Huang et al, 2006) which 0: Healty plants, 1: <25% of the plant wilted and browning of crown; 2: 25-50% of plant wilted and slight browning; 3: 50-75% of the plant wilted and progressive browning; 4: ≥75% of plant wilted and complete browning 5: Dead plant

Diseases index were calculated using scale value and disease severity (%) was determined (Karman, 1971)

**Findings**

In vitro studies*T. harzianum* restricted the *V. dahliae* mycelial growth in dual culture (Table 1).

| *T. harzianum* | Mycelial Diameter (mm) | | Score of antagonistic activity |
|---|---|---|---|
| Volatile compounds test | Control | 45 | Scale 2<br>The hyperparasyte overgrown at least 2/3th of the pathogen |
| | *T. harzianum* + *V. dahliae* | 15 | |

**Table 1:** The characteristics of *T. harzianum* in dual culture test

In dual culture test *T. harzianum* overgrown at least 2/3th of the pathogen and placed Class 2. The average mycelial diameter of *V. dahliae* was 15mm while *T. harzianum* covered the rest of the medium completely. In volatile compound test, the average mycelial diameter of *V. dahliae* was 45mm while 15mm together with *T. harzianum* application.

In a previous study, *Trichoderma* spp. results parasitation of the hypal growth of *Sclerotium rolfsii* by different mechanisms and showed antagonistic effect (Shaigan et al., 2008).

**Pot experiments**

Biological control agents were used to determined the effects on Verticillium wilt caused by *V. dahliae* in cucumber. Results shown in Table 2.

| Treatments | Colonisation (%) | Disease index | Diseases severity (%)* | % Effect |
|---|---|---|---|---|
| *Glomus mossea* | 60 | 1.10 | 22 ab | 61.4 |
| Mycorrhizal preparation | 57 | 1.25 | 25 b | 56.1 |
| *Trichoderma harzianum* | - | 0.95 | 19 a | 66.7 |
| *V. dahliae* | - | 2.85 | 57 c | - |

*Means within column followed by different letters are significantly different *P (0.05)* according to Fishers LSD test.

**Table 2:** The effects of mycorrhizal fungi and *Trichoderma harzianum* on *Verticillium dahliae* in cucumber.

Root colonisation of *G. mosseae* and mycorrhizal preparation were found as 60 and 57% respectively after 4 weeks. The diseases severity of *V. dahliae* inoculated plant was 57%, while the diseases severity of mycorrhizal fungi and *V. dahlia* inoculated plant were 22 and 25%, respectively. The diseases severity of *T. harzianum* applied plant was lower than other treatments and was 19%. In previous studies also revelaed that Glomus species are good colonizer of many plants (Dell'Amico et al, 2002; Karagiannidis et al., 2002).

*G. moseae*, mycorrhizal preparation and *T. harzianum* reduced the disease severity of *V. dahliae*. Especially, *T. harzianum* reduced external browning of crown effectively. Some investigations concluded that arbuscular mycorrhizal fungi could reduce the soilborme fungal plant pathogens (Azcon-Aguilar and Barea, 1996; Inbar et al, 1996; Akköprü et al., 2005; Arıcı, 2009)

## Conclusion

The biocontrol agents could be used against Verticillium wilt caused by *V. dahliae*. Soil factors is the basic elements for plant development in agriculture and disease are the main limiting factors in crop production. In addition the traditional control method, application of biological agents provide the renewal of the soil as well as supporting the control methods in integrated diseases management.

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