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Study of Antagonistic Effects of *Trichoderma* Species on Growth of *Verticillium dahliae*, the Causal Agent of VerticilliumWilt of Pistachio under Laboratory Condition

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Abstract

Verticillium wilt is a serious disease of pistachio caused by *Verticilliumdahliae*. Control of the disease is difficult due to soil borne nature of the causal agent. Verticillium wilt has been biologically controlled by *Trichoderma* spp. In the present study, *Trichoderma* spp. was isolated from soils of pistachio orchards and their effect was investigated on radial growth of *Verticillium dahliae* by using dual culture and production of volatile and non-volatile metabolites. Five isolates of *T. harzianum* and one isolate of *T. asperellum*,*T.koningii* and *T. crassum* were used in the present study. All the isolates of *Trichoderma* produced volatile and non-volatile metabolites that reduced radial growth of the pathogen. *T. harzianum* 30 and *T. koningii* isolates had the highest effect on radial growth of the pathogen but *T. harzianum* 42 and *T. crassum* showed the lowest effect in non-volatile test. Six days after culture of pathogen on the medium was the best time for study of effect of the non-volatile metabolites. In volatile metabolite test, *T. harzianum* 85 and *T. koningii* reduced radial growth of *V. dahliae* further than others. Overally, *T. harzianum* 30, 85 and *T. koningii* isolates showed the highest inhibitory effect of radial growth of *V. dahliae*.

Keywords: Antagonistic Effects, Pistachio, *Trichoderma*, *Verticillium dahliae*.

Introduction

Verticillium dahliae Kleb. is one of the most important pathogens that limit planting of pistachio trees in some countries such as the United States. The use of biological control methods is one of the best ways for control of soil-borne diseases caused by fungi. One of the most important biocontrol agents against pathogenic fungi is *Trichoderma* (Cohen-Kupiec and Chet, 1998). Various species of *Trichoderma* has been reported as antagonist agents against various pathogens due to soil-borne properties and rapid establishment in soil (Mohamadi, 2003).

In an investigation, systemic resistance in cotton caused by seed treatment with strains of *T. virens* and decreased the severity of Verticillium wilt (Linda and Hanson, 2000). In another study, it was observed that three species of T. *koningii*, *F. solani* and *A. alternate* could colonize microsclerotia of V. dahliae (Grunden *et al.*, 2001). Furthermore three species of *Trichoderma* including *T. viride*, *T. virens* and *T. harzianum* prevented the activity of microsclerotia and reduced the mycelial growth of Verticillium wilt in tomato by production of metabolites and volatile compounds (Jabnoun-Khiareddine *et al.*, 2009).

Materials and Methods

Dual culture of Trichoderma isolates and Verticilliumdahliae

In this experiment, the ability of *Trichoderma*species was examined on the growth inhibitory of *V. dahliae* in culture medium based on Jabnoun-Khiareddine et al (2009). For this purpose, mycelia disks (5 mm in diameter) of *V. dahliae* was cultured in the center of the 10-cm Petri dish containing PDA (Potato Dextrose Agar) medium. *Trichoderma* species were cultured in both sides of colonies of *V. dahliae* after colony diameter reached to 1.5 cm. Petri dishes were incubated at 25 °C. Then, the isolates were selectedwith inhibitory effect on radial growth of *V. dahliae*.

For study of effects of secondary metabolites (Non-volatile) produced by different species of *Trichoderma* on mycelial growth of *Verticillium dahlia*, erlenmeyer flasks (250 ml) containing 100 ml PDB (Potato Dextrose Broth) were sterilized and four mycelial disks (5 mm in diameter) of the 3-day colonies of Trichoderma species were placed in each flask. Four mycelial disks were added to PDA medium in Erlenmeyer flask of control. Erlenmeyer flasks were shaken in a rotary shaker at 90 rpm for 10 days. The contents of each Erlenmeyer flask were passed through two layers of sterile filter paper by Buchner funnel and vacuum pump for separation of solid particles in a liquid medium. Then prepared liquid was passed through the milipore filter (0.2 µ in diameter) for sterilization. This extraction was mixed into ratios of 10, 20 and 30% with PDA medium and was added in Petri dish (Haghdel, 2009). Then, a mycelium disk (5 mm in diameter) of the V. dahliae was cultured in center of each Petri dish. After 7 days, colony diameter of Verticillium was recorded. Percentage of inhibition of Trichoderma extraction on vegetative growth of Verticillium was calculated based on the following formula:

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$$\frac{A-B}{C}\times 100$$

A: The average diameter of the control colony
B: The average diameter of the treatment colony
C: The average diameter of the control colony

For study of volatile compounds effects on the growth of *V. dahliae*, a mycelial disk (5 mm in diameter) of the pathogen was cultured in center of 8-cm Petri dishes containing PDA medium. After the colonies diameter reached to 1 cm, a mycelial disk (5 mm in diameter) of *Trichoderma* was cultured in center of another Petri dish. After removing the lids of the Petri dishes under sterile conditions, two Petri dishes were placed on each other which *Trichoderma* plates were placed below and the around of Petri dishes was completely blocked by Para film. The Petri dishes were incubated at 25 ° C. Then, the radial growth of *V. dahliae* was measured after 48, 72 and 96 hours. Eventually, percentage of inhibition of volatile compounds produced by *Trichoderma*species on growth of *V.dahliae* was calculated as described above (Haghdel, 2009).

Results

The effect of 10, 20 and 30% of secondary metabolites of *Trichoderma* isolates has been showed in (Fig.1). All ratios of the metabolites reduced radial growth of*V. dahliae. T. harzianum* 30 and *T. harzianum* 42 isolates showed the highest and lowest inhibition of radial growth, respectively. *T. crassum* and *T. harzianum* 42 had the lowest effect on growth of the pathogen. These results showed that the ability of secondary metabolites production is different in *Trichoderma*species and isolates.

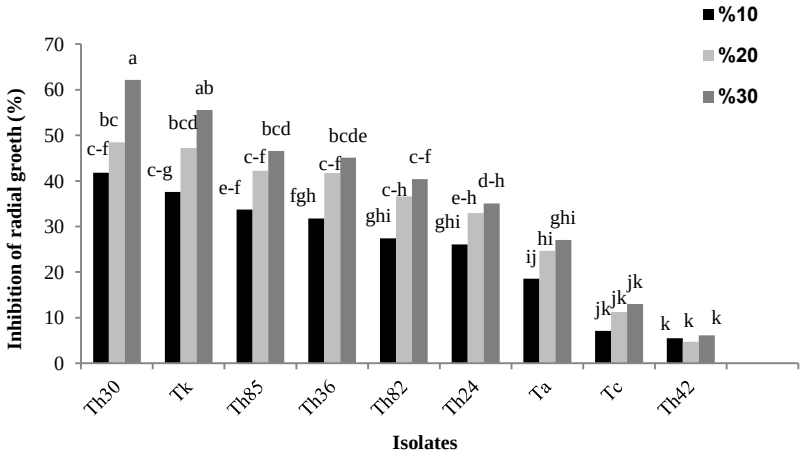

Fig.1. The effect of 10, 20 and 30 percentage mixture of secondary metabolites of *Trichoderma* isolates with medium on inhibition of radial growth of *Verticilliumdahliae* (*T. harzianum* 30, *T. koningii*, *T. harzianum* 85, *T. harzianum* 36, *T. harzianum* 82, *T. harzianum* 24, *T. asperellum*, *T. harzianum* 42).

Maximum inhibition of radial growth of the pathogen was observed at sixth day after inoculation of *V. dahliae* (Fig.2). The difference between the sixth days with other days for radial growth inhibition was significant only in *T. harzianum* 30, 85, 36, 82 and *T. koningii* isolates. The percentage of radial growth inhibition had no significant difference between *Trichoderna*isolates after 2-days. So, this time is not sufficient for study the effects of *Trichoderma* metabolites on radial growth of *V. dahliae*. This result was agreed well with Henni (1987) and El Rafai *et al.* (2003). One reason for this could be increase of secondary metabolites produced by *Trichoderma* isolates over the time.

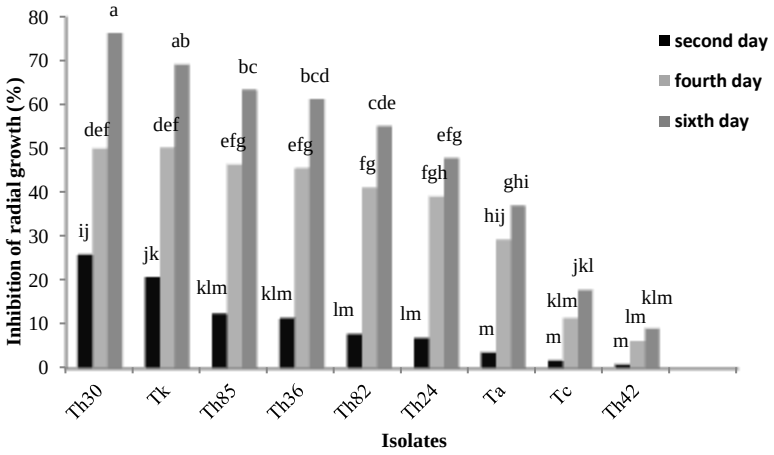

Fig.2. Inhibition percentage of radial growth of *Verticilliumdahliae* at different times (2, 4 and 6 days) of pathogen culture on medium containing non-volatile metabolites of *Trichoderma* isolates.

The ability of volatile compounds production was different in *Trichoderma* species and isolates (Fig.3). Volatile compounds were reduced radial growth of pathogen significantly at sixth day compare with other times. The difference was not significant in *T. crassum* and *T. harzianum* 42 isolates. It seems that these two strains have been little ability in production of volatile compounds and inhibition of pathogen growth. Etebarian *et al.* (2003) reported that volatile compound produced by *T. virens* can inhibit growth of *Phytophthoradrechsleri*.

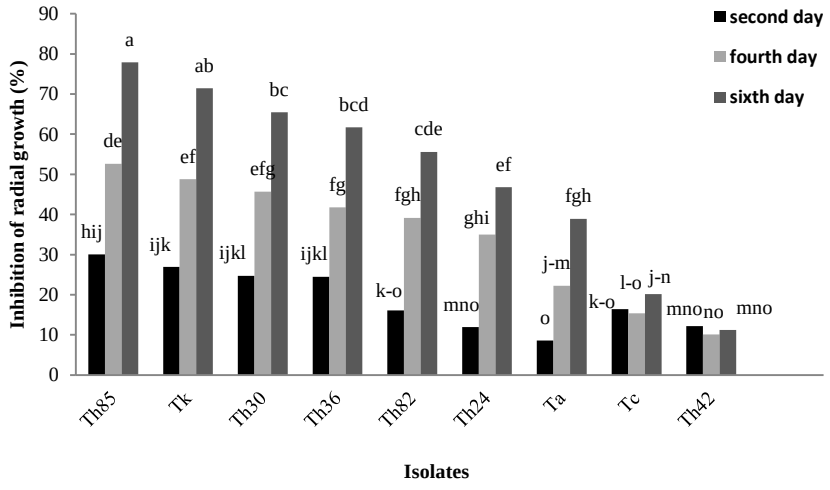

Fig.3. Effect of volatile secretions of *Trichoderma* isolates on inhibition of mycelial growth of *Verticilliumdahliae* under laboratory condition

Discussion

The present research showed *Trichoderma* species from soils of pistachio orchards can produce volatile and non-volatile compounds. Exactly similar to non-volatile, these compounds inhibit radial growth of pathogenic fungi (Dennis and Webster, 1971; Jabnoun-Khiareddine *et al.*, 2009). The present study showed that antagonistic activity of *T. harzianum* 85 and *T. koningii* can reduce radial growth of *V. dahliae* more than other isolates and species. It seems that these species can be used for the control of Verticillium wilt disease in greenhouse and field conditions.

Several volatile compounds included Lactones, Alcohols, Terpene derivatives and α-Pieron derivatives were obtained from *Trichoderma* species under different culture conditions (Zeppa *et al.*, 1991). As well as volatile compounds, lipids and amino acids of *T. harzianum* have been isolated and reduced radial growth of pathogenic fungi (Zeid *et al.*, 1998).

It seems that the ability of radial growth inhibition of *V. dahliae* is different among isolates and species of *Trichoderma*. So for biological control of verticillium wilt, volatile and non-volatile compounds production were examined in collected isolates of *Trichoderma*.

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