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Effect of *Trichoderma harzianum* on tomato plant growth and its antagonistic activity against *Phythium ultimum* and *Phytopthora capsici*

Muhammad Nazir Uddin*, Ubaid ur Rahman, Wajid khan, Nisar Uddin and Muhammad Muhammad

Abstract

Effect of *Trichoderma harzianum* was investigated on plant growth and inhibitory activity against *Phythium ultimum* and *Phytopthora capsici* under laboratory and greenhouse conditions. Data under lab conditions revealed that mycelial growth of both pathogens were inhibited by *T. harzianum* in vitro. The effect of *T. harzianum* on different plant growth parameters was assessed in the presence of *P. ultimum* and *P. capsici*. Fresh and dry shoot weight was reduced by both fungal strains. The fresh shoot weight was decreased by 38.8 and 44.4% in case of *P. capsici* and *P. ultimum*, respectively. *T. harzianum* improved the overall plant growth in the presence of *P. ultimum* and *P. capsici*. Histopathological observation of *P. ultimum* and *P. capsici* infected tissue of the root clearly indicated that both severely affected the epidermis and vascular bundle of the host plant. *T. harzianum* reduced the size of lesions caused by the two pathogens. Observation of hyphae interaction of the *T. harzianum* with pathogens demonstrated that it inhibited the entry of both pathogens to the vascular bundle of the host tissue. Furthermore, no effect was observed on the vascular bundle, pith and cortex of treated host plant inoculated with *T. harzianum* and pathogens.

Keywords: Histopathology, *Trichoderma harzianum*, *Phythium ultimum*, *Phytopthora capsici*, Bio control, Tomato

Background

Tomato (*Lycopersicum esculantum* Mill) is an important solanaceous vegetable crop all over the world including Pakistan. In Pakistan, its productivity and yield is low as compared to other developed countries due to many reasons (Heuvelink et al. 2003). Among one of them is soil-borne fungal diseases. *Phythium ultimum* and *Phytopthora capsici* are the most common destructive soil-borne pathogens having broad host range and causes severe crop loss to the farmers (Hausbeck and Lamour 2004).

P. capsici infects the underground part of plant, causing seed rot and seed blight as well as stem blight and overall stunted plant growth. Due to its severe infection, the fruits of the plant prematurely fall with low market value. *P. ultimum* usually destroys the conductive tissue of the root system which impairs translocation of water and mineral to the upper part of the plant producing stunted plant growth (Hendrix and Campbell 1973). Diseases caused by these soil-borne pathogens are complex in nature; therefore, their control and management is challenging task. Use of chemical pesticides is common and rapid mean of controlling soil-borne pathogens. However, the use of these chemicals are associated with negative impact like hazards to human, damage the beneficial soil micro-organisms, development of resistance by pathogen and also cause environmental pollution (Ragunathan and Divakar 1996).

Trichoderma species have long been identified and characterized as potential opportunistic, avirulent plant symbionts and biological agent against different soil-borne pathogens (Naseby et al. 2000; Harman et al. 2004). The important features of *Trichoderma*

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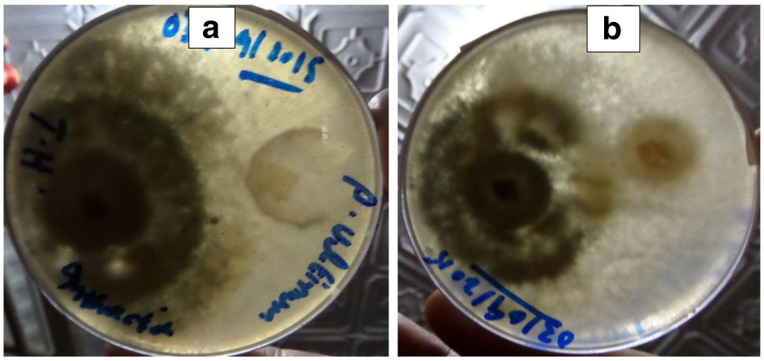

Fig. 1 **a** Interaction between *Trichoderma harzianum* and *Phythium ultimum*. **b** Interaction between *Trichoderma harzianum* and *Phytopthora capsici*

species are as follows: they rapidly colonize in the soil, favor wide range of environment, easy to isolate and culture, grow rapidly on many substrates, effective against a wide range of plant pathogens and rarely pathogenic to plants (Brotman et al. 2012; Khatabi et al. 2012). Histopathological study of host pathogen and biocontrol agent is very important to understand and visualize the efficacy of biological control agents (BCA) at a cellular level. It provides the base for searching the control measures of different diseases.

The present study was conducted to assess the effect of *Trichoderma harzianum* and its inhibitory activity against *P. ultimum* and *P. capsici* on tomato plants under lab conditions. Moreover, the histopathology of host roots was also performed to explore the interactions of *T. harzianum*, *P. ultimum*, and *P. capsici*.

Materials and methods

Isolates of *P. capsici*, *P. ultimum*, and *T. harzianum* were obtained from the Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan.

In vitro assay

In vitro antagonistic activity of *T. harzianum* was tested against *P. ultimum* and *P. capsici* according to the standard procedure of Dennis and Webster (1971). Mycelial disc of 5 mm (diameter) of *T. harzianum* and pathogens were taken from 7-day-old culture and placed in a petri dish containing potato dextrose agar (PDA) at an equal distance in opposite direction. The control (pathogen alone) was also run in this assay. All the petri dishes were incubated in the dark at 28 ± 2 °C for 5 days. In vitro antagonistic activity was measured in terms of percentage inhibition using the following formula.

$$I = (C - T)/C \times 100$$

where I refers to percent inhibition, C is control, and T radial growth of pathogen (mm) in the presence of *T. harzianum.*

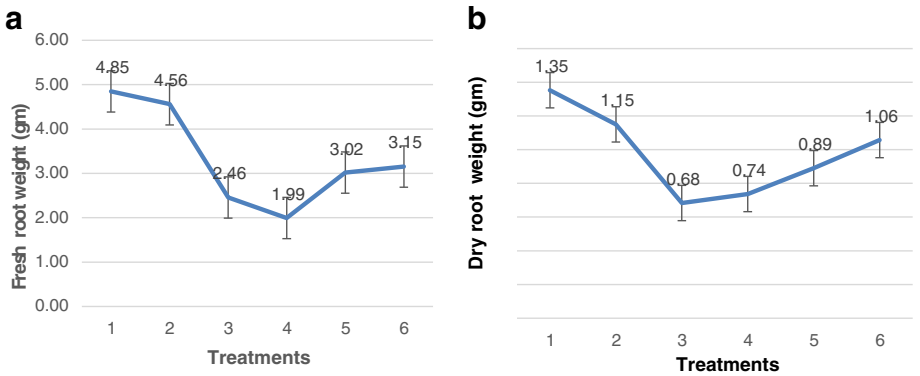

Fig. 2 Effect of soil infection with *Trichoderma harzianum*, *Phythium ultimum*, and *Phytopthora capsici* on tomato fresh weight (g) in a pot experiment (**a**). Effect of soil infestation with *T. harzianum* on *P. ultimum* and *P. capsici* on tomato dry root (g) in a pot experiment (**b**). 1, uninoculated control (healthy); 2, *T. harzianum* (alone); 3, inoculated with *P. capsici* (alone); 4, inoculated with *P. ultimum* (alone); 5, inoculated of *P. capsici* with *T. harzianum*; 6, *P. ultimum* with *T. harzianum*

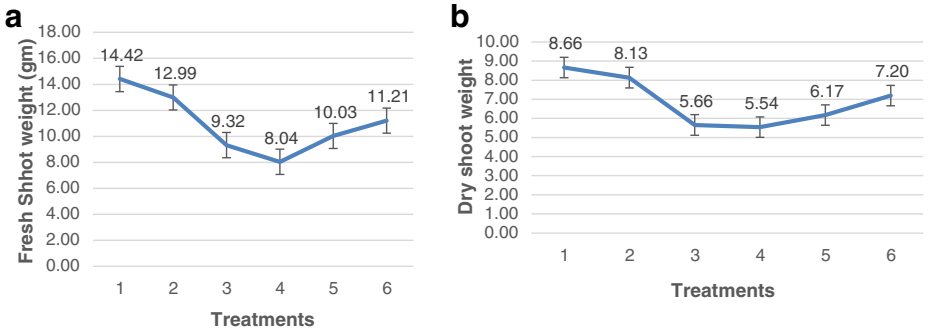

Fig. 3 Effect of soil infection with *Trichoderma harzianum*, *Phythium ultimum*, and *Phytopthora capsici* on tomato fresh shoot weight (g) in a pot experiment (**a**). Effect of soil infestation with *T. harzianum* on *P. ultimum* and *P. capsici* on tomato dry shoot weight (g) in a pot experiment (**b**). 1, uninoculated control (healthy); 2, *T. harzianum* (alone); 3, inoculated with *P. capsici* (alone); 4, inoculated with *P. ultimum* (alone); 5, inoculated of *P. capsici* with *T. harzianum*; 6, *P. ultimum* with *T. harzianum*

Green house experiment

Tomato germplasm, i.e. money maker, was obtained from Tarnab Agriculture Station, Peshawar, Pakistan. Nursery was raised in earthen pots from 3-week-old seedlings, transplanted to the 8.8inches diameter pots containing 2.5 kg of sterilized soil having sand, clay, and silt at a ratio of 2:1:1. The pure cultures of *P. capsici*, *P. ultimum*, and *T. harzianum* were refreshed on PDA media for 4 days at 25 °C. The inoculum was prepared in potato dextrose broth and placed in a shaking incubator for 1 week at 25 °C ± 2 °C. The flask containing the culture media was then seeded with disks (7 mm diameter) of 4-day-old culture (Margaret et al. 2011). Holes were made in rhizosphere, and 5 ml of conidial suspension of 107 ml^{-1} was poured in each hole.

Both pathogens and biological agent were applied to the rhizosphere after 10 days of transplantation in a greenhouse of the Centre for Biotechnology and Microbiology (CB&M), University of Swat, Pakistan.

The experiment was designed in randomized complete block design (RCBD) with six treatments and three replications. The treatments were categorized in to the following:

Un-inoculated control (healthy)

1. *T. harzianum* (alone).
2. Inoculated with *P. capsici* (alone)
3. Inoculated with *P. ultimum* (alone)
4. Inoculated with *P. capsici* and *T. harzianum*
5. *P. ultimum* with *T. harzianum*

The experiments were terminated after 40 days of inoculation. Plants of each pot were carefully uprooted, separately labeled, and brought to the laboratory. Data on different agronomic traits were recorded and analyzed by Statistic 8.1 Program. Means of the results were compared by using least significant difference (LSD) test (Steel et al. 1997).

Histopathological study was conducted. A comparative study of the root tissues in each treatment was carried out at 30 and 40 days after inoculation. The uprooted samples were thoroughly washed with tap water and excised into small pieces for fixation in F.A.A. (formaldehyde/acetic acid/alcohol, 3:1:6) and processed for histopathological studies (Sass 1958). Following fixation, the samples were dehydrated in 10, 20, 30, 40, and 50% ethanol. Roots were then transferred to butanol/ethanol/water solution prepared in the following ratio.

Butanol	Ethanol	Water
25	30	45
55	25	20
85	15	0
100	0	0

Roots were kept in each of the above solutions for 2 h at room temperature. Dehydrated root tissues were infiltrated and imbedded in paraffin wax at 52 ° C for 10 days. Air bubbles were removed from the roots during the wax infiltration process. Sections of 12-μm thicknesses were cut with a rotary microtome which were then affixed on slides with the help of Mayer's Albumin adhesive and stained with safranin and fast green (Sass 1958). The stained sections were mounted in Canada balsam and examined. Photographs were taken using Olympus digital camera at 4 ×, 10 ×, and 100 × magnifications.

Results and discussion

Genus *Trichoderma* is mostly used as biocontrol agent against different soil-borne pathogens (Ranasingh et al. 2006; Moubarak and Abdel-Monaim 2011). *Trichoderma spp.* as a biocontrol agent are known to compact the

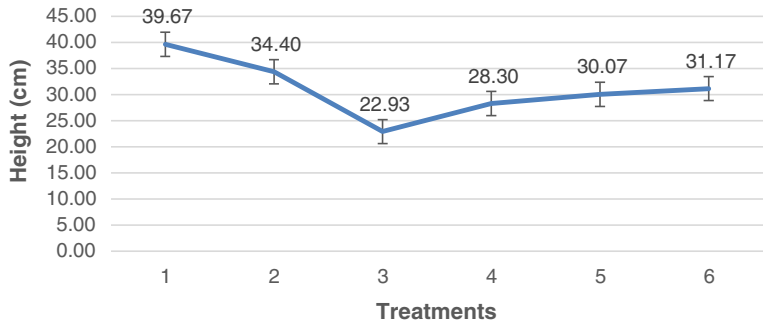

Fig. 4 Effect of soil infection with *Trichoderma harzianum*, *Phythium ultimum*, and *Phytopthora capsici* on tomato plant height (cm) in a pot experiment. 1, uninoculated control (healthy); 2, *T. harzianum* (alone); 3, inoculated with *P. capsici* (alone); 4, inoculated with *P. ultimum* (alone); 5, inoculated of *P. capsici* with *T. harzianum*; 6, *P. ultimum* with *T. harzianum*

plant pathogenic attack and improve the plant growth and yield by enhancing the growth hormones and increment of plant beneficial microbiome (Dubey et al. 2007; Khatabi et al. 2012).

Under lab condition, the *T. harzianum* inhibited the radial growth of both pathogens and showed more antagonistic activity against *P. ultimum* as compared to *P. capsici* (Fig. 1a, b). *T. harzianum* caused lysis of pathogen mycelium and thus inhibited its radial growth. Similar finding has also been reported in various studies (Ahmed et al. 1999; Rey et al. 2001). This antagonistic mechanism of *T. harzianum* involved the production of antibiotics, competition for nutrients, and production of hydrolytic enzymes (Howell 2003; Zeilinger and Omann 2007).

Under greenhouse conditions, both pathogens adversely affected the growth of host plant resulting in decreased fresh and dry weights of root and shoot (Spies 2011; Lamour et al. 2012). Figure 2a, b shows fresh and dry root weights in response to applied treatments. *P. ultimum* reduced the fresh root weight by 58.9% as compared to healthy control. Similarly, decreased in fresh root weight by 49.2% was noted in the presence of *P. capsici*. On the other hand, inoculation of *T. harzianum* increased the fresh root weight as compared to tomato plants inoculated with the two pathogens alone (Fig. 2a). *P. ultimum* inoculation reduced the dry root weight by 45.2%. Similarly, the dry root weight was reduced by 49.6% in the presence of *P. capsici* as compared to healthy plants. Application of *T. harzianum* increased dry root weight in the presence of *P. ultimum* and *P. capsici* (Fig. 2b). Fresh and dry shoot weights were reduced by both pathogens. The fresh shoot weight was decreased by 38.8 and 44.4% in the case of *P. capsici* and *P. ultimum*, respectively. Similarly, *P. ultimum* inoculation reduced the dry shoot weight by 36% while 34.6% decrease in dry shoot was noted in the presence of *P.capsici* as compared to healthy plants. The artificial infestation of soil with *T. harzianum* significantly increased the fresh shoot weight in the soil infected with *P. ultimum* and *P.capsici* (Fig. 3a). However, dry shoot weight was less affected by *T. harzianum* inoculation (Fig. 3b). Both pathogens affect the height of plants as *P. capsici* and *P. ultimum* reduced the height by 42 and 28.6% respectively than in the healthy control (Fig. 4). This was reduced with artificial infestation of soil with *T.*

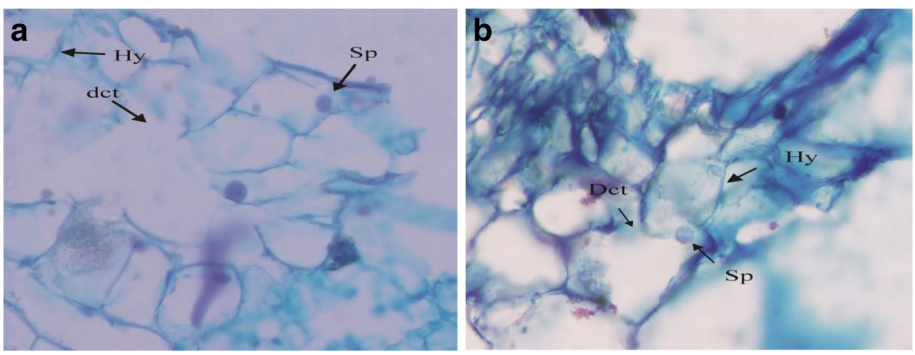

Fig. 5 **a** Histopathological interaction of *Phythium ultimum* with roots tissue of tomato plants (longitudinal section, 40 ×). **b** Histopathological interaction of *Phytopthora capsici* with roots tissue of tomato plants (longitudinal section, 40 ×)

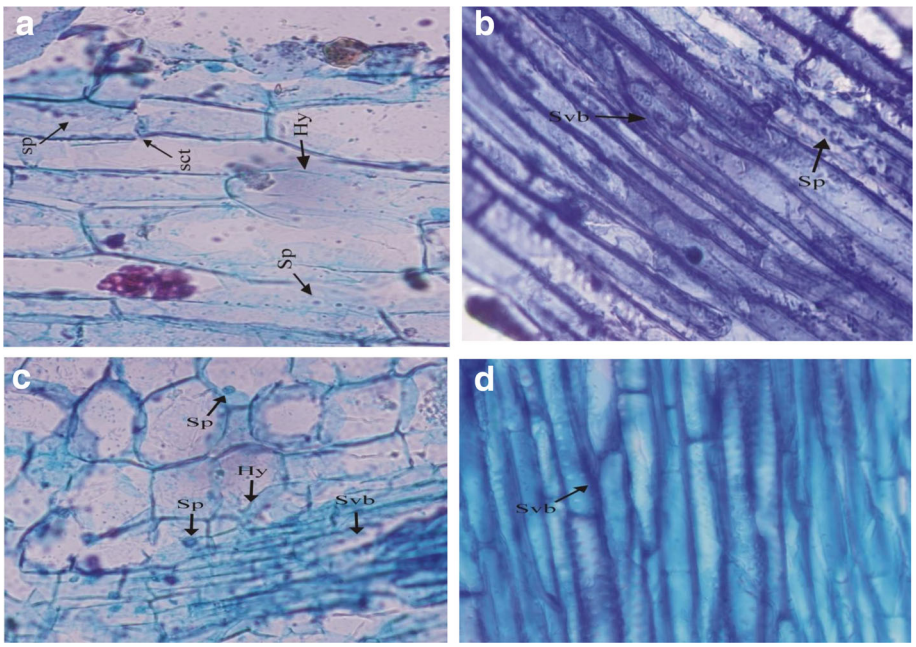

Fig. 6 **a** Histopathological interaction of *T. harzianum* and *Phythium ultimum* in cortex region of roots tissue of tomato plants (longitudinal section, 40 ×). **b** Histopathological interaction of *T. harzianum* and *Phythium ultimum* in vascular bundle of roots tissue of tomato plants (longitudinal section, 40 ×). **c** Histopathological interaction of *Trichoderma harzianum* and *Phytopthora capsici* in cortex region of roots tissue of tomato plants (longitudinal section, 40 ×). **d** Histopathological interaction of *Trichoderma* and *Phytopthora capsici* in vascular bundle of roots tissue of tomato plants (longitudinal section, 40 ×)

harzianum in the presence of both pathogens. Improvement in agronomic traits of host plant in the presence of pathogen is attributed to *T. harzianum* that increased water uptake and translocation of nutrients (Hoyos-Carvajal et al. 2009).

The disease control by *Trichoderma* species involves multifarious interaction between the pathogen, biocontrol agent, and host plant (Harman 2006; Hoitink et al. 2006; Alfano et al. 2007). Histopathological interaction of *T. harzianum* with host and *P. ultimum* and *P. capsici* showed that the treatment involving only pathogens result in visible cellular alterations in the host root system. Data regarding histopathological study indicated that *T. harzianum* significantly reduced the size of the lesion caused by *P. ultimum* and *P. capsici*. The stained root sections of tomato plant revealed visible cellular alterations in different treatments (tomato plants inoculated with the two pathogens alone). The plants inoculated only with *P. ultimum* and *P. capsici* possessed damaged cells of the epidermis, cortex, and vascular bundles. Hyphae were seen in the epidermis, and spores were present in the damage area of both cortical and vascular regions of the root. The boundaries of several cells were broken, and thus, deformed xylem was not arranged longitudinally but was dispersed in a diffused and disconnected manner (Fig. 5a, b). But when both pathogens were applied separately in the presence of *Trichoderma harzianum,* hyphae were entered into the epidermis of tomato root without damaging the epidermal cells. The epidermal cells were intact and the tissues were healthy. Mycelium and spores of BCA were observed in the cortex region (Fig. 6a–d). Histopathological observation of their roots showed little damages to the cortex and vascular bundles which might be the excretion of *T. harzianum* that inhibit the population of both pathogens. Secondly, the biocontrol agent restricted the pathogen to cortex

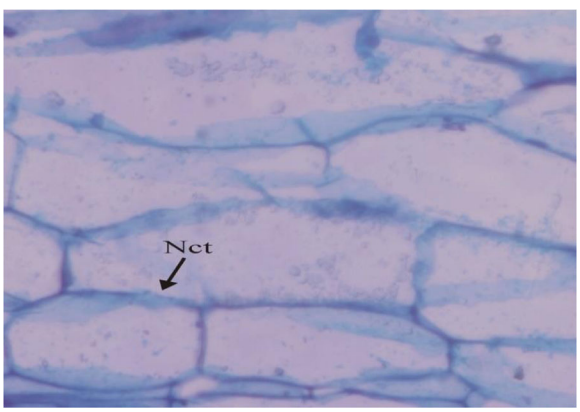

Fig. 7 Histopathological section of roots tissue of healthy tomato plants (longitudinal section, 40 ×)

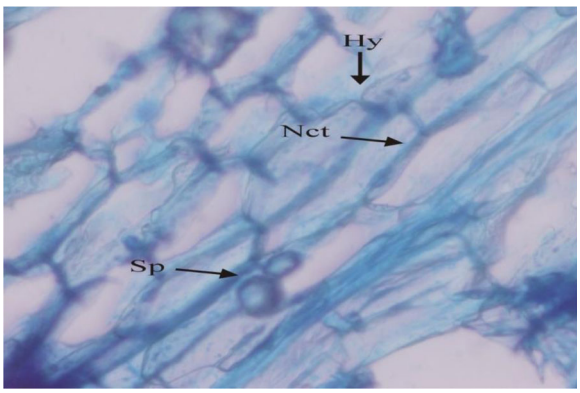

Fig. 8 Histopathological interaction of *Trichoderma harzianum* with roots tissue of tomato plants (longitudinal section, 40 ×)

region and hence the cells of vascular bundle remain intact and healthy and showed no malformation.

No histological changes were observed in longitudinal and transverse sections of stained roots of healthy plants (Fig. 7). However, for plants treated with *T. harzianum* alone, the biological control agent entered into the epidermis and cortex region of root tissue. Mycelium and dark round spores of *T. harzianum* were seen in those regions (Fig. 8).

Conclusions

The present evaluation clearly indicated that *T. harzianum* had strong antagonistic activity against *P. ultimum* than against *P. capsici.* Therefore, it could be recommended to be used for management of the diseases caused by *P. ultimum* and *P. capsici.*

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Authors' contributions

MNU conceived idea and designing of experiment. WK management of article, analysis of data and critical revision. UuR conduct experiments. NU participated in experiments design and coordination. MM drafting of manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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