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# Management of Tomato Wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersci* with different bio-agents

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## Abstract

Tomato is an important fruit providing all essential nutrients. *Fusarium oxysporum* f.sp. *lycopersici* (FOL), causing wilting in tomato plants. The mode of survival of this fungus is vascular; so not easy to control and identify at the beginning stage. Many chemicals are present in markets to control this disease but are expensive and are also causing hazardous effects on the lives of the people and the environment. Hence, there is a need to apply biological strategies to control this disease. In this experiment, six biological agents *Fusicola incarnatum,Trichoderma harzianum, Trichoderma viride, Fusarium equisetti, Alternaria alternate* and *Nigrospora oryzae* have been tested *in vitro*; among them, *T. viride* and *F. incarnatum* were found best to inhibited FOL, while after the application of bioagents *T. viride* and *F. incarnatum in vivo*. The present results showed that *T. viride* and *F. incarnatum* can control FOL.

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## Introduction

Tomato (*Lycopersicon esculentum* L.) is a member of *Solanaceae* family. It is mostly available all over the world (Pritesh *et al.*, 2011). It was found 1st time in Mexico and Perue (Verma *et al.*, 2018). Tomato production in the world is 130 million tons while its area is about 160 thousand hectares. The crop is cultivated in Pakistan on 63 thousand hectors and production is 95279 kg/ha (FAO, 2018).

Tomato is essential in our food as salads, cooked with vegetables like tomato puree, sauces, and is used in making ketchup. It is providing important vitamins like A and C (Abdullah *et al.,* 2013). Tomatoes are a good source of lycopene, which prevents cancer, heart disorders and age-related disorders (AVRDC, 2003). Tomato is very necessary to our lives because it has important amino acids, glucose, fructose, and minerals which include Mg, Ca, P, Fe, Na, K, Cu and S. It is an important source of proteins, minerals, fibers and carbohydrates, which have following ratios 1.9 g, 0.6 g, 0.7 g and 3.7 g per 100 g of edible portion, respectively (Nikhate, 2012).

FOL is a very devastating fungus and its widespread is all over the world. This fungus causes tomato wilt in tomato (Abdallah *et al.,* 2016) and losses due to this disease are 10 to 50% in tomato (Ghazalibiglar *et al.,* 2016). This fungus is not easy to handle due to its mode of survival in the vascular system. It is the reason why the effectiveness of fungicides is less against this fungus (Verma *et al.*, 2018). Among all soil-borne fungi, FOL plays a significant role in causing diseases in plants due to its saprophytic nature which enables it to survive for a longer time on the organic matter (Fravel *et al.*, 2003).

Different chemicals are being used for the control of pests and pathogens, but these chemicals are very costly and dangerous for the environment (Song *et al.*, 2001). The extreme use of chemicals causes effects on the non-target population, makes the pathogens resistant which enables them to live many years and thus remains a continuous threat for the crops (Bawa 2016).

For the last two decades, biological methods for the control of plant diseases have been very common (Omar *et al.*, 2016) and considered as safe strategy; because, chemicals affect humans as well as animals leading towards ecological troubles (Banerjee *et al.*, 2016).

Biological control is safe as well as effective for the control of diseases in plants. *Trichoderma* spp. are found in soil all over the world, their mode of living is free and highly compatible with roots, soil and foliar atmospheres. This fungus is famous due to having antibiotic properties against different pathogenic fungi (Omar *et al.,* 2016). *Trichoderma* spp. compete with the fungal pathogens for nutrition and parasitism, degrade their cell wall, and produce resistance in the plants (Taghdi*et al.,* 2015). The objective of the current research was to investigate the potential of different fungal antagonists against FOL *in vitro* and *in vivo*.

## Materials and methods

The experiments were conducted *in vitro* in Tree Pathology Lab., and *in vivo* at the research area of the Department of Plant Pathology, University of Agriculture, Faisalabad. Pakistan.

*Collection of pathogenic fungi (Fusarium spp.) and identification*

We isolated the fungus from the diseased samples of tomato wilt affected plants. Root and shoot samples were cut into approximately 1cm length and surface-sterilized for 30-40 seconds. These pieces were placed onto potato dextrose medium (PDA) in petri-plates and incubated for 7 days at 28°C. Fungal isolates appearing on the plant pieces were identified and transferred to a fresh PDA medium. The fungus was identified based on the cultural and morphological characters. Morphological characters of the fungus were studied by slide culture technique under the microscope. Purification was done of each colony obtained from a single spore or hyphal tip method. Identification of *Fusarium* sp. was done using illustrated genera of imperfect fungi (Verma *et al.*, 2018).

*In vitro evaluation of the antagonistic effect of biological agents by dual culture technique.*

Efficacy of biological agents, *F. incarnatum, T. harzianum, T. viridie, F. equisetti, A. alternate* and *N. oryzae* were tested in vitro to check their antagonistic effect on the fungal isolates of TWD by dual culture method (Morton & Stroube 1955). Discs of fungal culture were obtained with the help of a cork borer and were placed on fresh petri-plates having PDA and incubated at 25±1˚C in an incubator for 48 hours. Pure cultures of biological agents were obtained, and mycelial discs of these antagonistic agents were cut and transferred to the opposite edges of the plates which were already inoculated with FOL at equal distance from their centers. Control was maintained without inoculating with any antagonist. Radial growth of the FOL fungus was measured and percent inhibition of mycelia growth over control was calculated using the following formula (Vincet 1947):

$$\text{Inhibition Percentage} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment} \times 100}{\text{Radial growth in control}}$$

*In vivo evaluation of the antagonistic effect of biological agents*

For the *in vivo* experiment, FOL, *T.viride*and *F. incarnatum* were grown on PDA broth. For the preparation of PDA broth, one-liter water was first boiled and then cooled down at room temperature, then PDA 40g was added in the boiled-cooled water and autoclaved for 20 minutes. FOL plug was added in a flask and mixed well, the flask was then placed on a shaker at 200rpm for one week and filtered with cheesecloth and removed the plug. The suspension was measured with the help of a hemocytometer; 3 microliter suspension contains approximately 150 spores (Mj *et al.*, 2017).

The potting mixture was autoclaved consecutively for 1 hour for 3 days, pots were filled with that material, and tomato seeds were sown in the pots. After 15 days of sowing, inoculum suspension $1x10^9$ was poured in the pots, and after 3 days of inoculation, agonistic agents were applied. The data was recorded on 3rd, 6th and 9th day and measured by the following formula (Lindow, 1983):

$$\text{DSI Percentage} = \frac{\text{Sum(class frequency} \times \text{score of rating class)} \times 100}{\text{Total number of plnts} \times \text{maximum disease index}}$$

*Statistical analysis*

The data of the experiment were analyzed statistically using analysis of variance (ANOVA) with SAS (SAS Institute, NC, USA 1990). Whereas, the testing of the significance of each bio-agent against FOL andFWD was performed with Duncan's Multiple Range Test at a 5% level of significance (Steel *et al.*,1996).

**Results**

*Identification of FOL*

The mycelium characteristics were observed under the microscope and mycelium was found branched and septate. Characteristics of microspores, macrospores and chlamydospores were also studied. Spores were found hyaline, microconidia were non-septate and their size varied between 1-3µm, whereas macrospores were septate and their length was 24.31 to 40.95 µm while their width was 3.32 to 5.04 µm. Chlamydospores were hyaline to pale brown and were found single-celled or in the chain with a diameter of 8-10 µm (Fig. 1). For the *in vitro* evolution of bio-agents, dual culture technique was used and bio-agents were evaluated consecutively for two years. *T. viride* significantly reduced the mycelial diameter of FOL followed by *F. incarnatum, A. alternate, N. oryzae, T. harzianum* and *F.* equiseti, respectively (Fig. 2). *T. viride* reduced the mycelial growth of FOL to 36, 69 and 98 % on 3rd, 6th and 9th day, respectively in the first year, and 33, 67 and 97 % on 3rd, 6th and 9th day in second year (Fig. 2).

The second best bio-agent *in vitro* was *F. incarnatum* against FOL (Fig. 2). Bio-agents *T. viride* and *F. incarnatum*, found best in vitro, were also tested in vivo.*T. viride* showed significant result against TWD in vivo and reduced TWD severity to 2.01, 5.1, 8.82. 12.43, 15.3, 18.27 and 23.75%, from the first week to seven-week, respectively, in the first year, and 3.01, 5.17, 9, 12.4,15.3,17.26 and 24.75%, from the first week to seven weeks, respectively, in the second year (Fig. 3). Similarly, *F. incarnatum* also effectively controlled TWD in vivo during both years (Fig. 3).

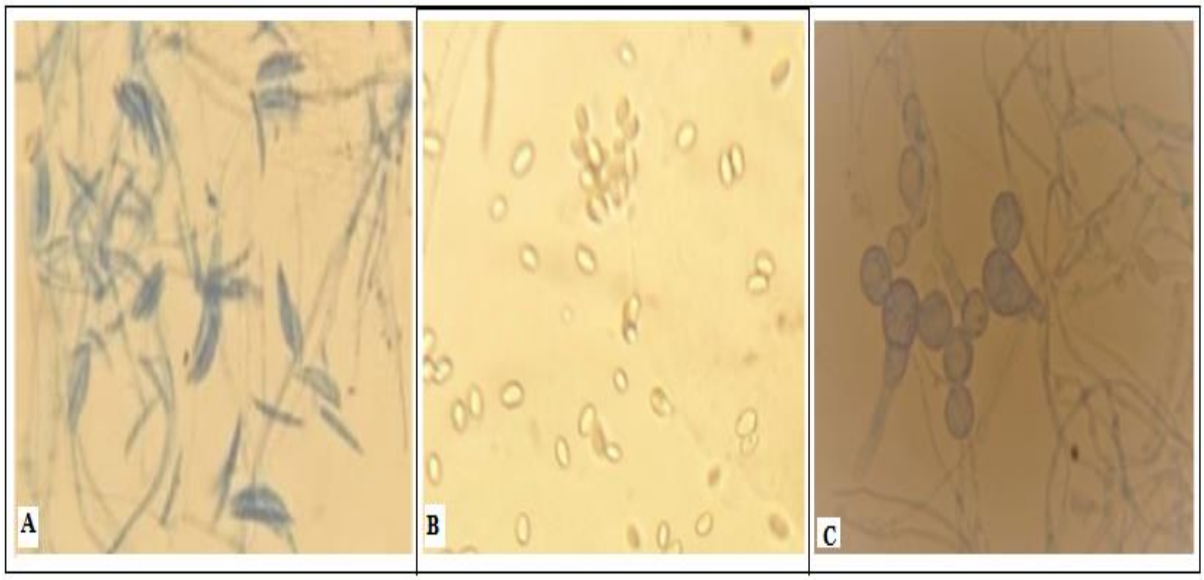


**Fig. 1.** a) Macrospores, b) microspores and c) chlamydospore of FOL under microscope.

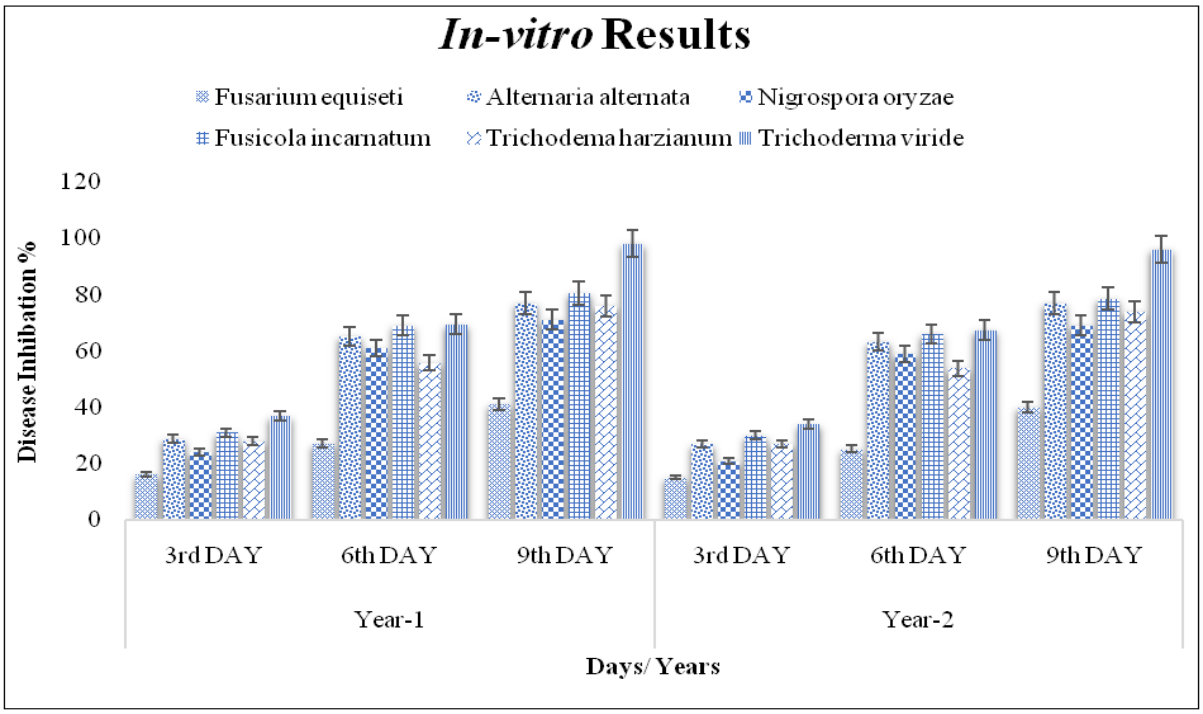


**Fig. 2.** *In vitro* mean antagonistic effect of different bio-agents on the mycelial growth of FOL for two years.

**Discussion**

*Trichoderma* spp. is present worldwide and easily found in all types of soils. There are several reports that *Trichoderma* spp. has an antagonistic effect against different plant diseases (Sundaramoorthy *et al.,* 2013). *Trichoderma* produces many types of enzymes, antibiotics and toxins which damage the cell wall and create competition between bioagent and pathogen for the nutrients (Taghdi *et al.*, 2015). In present study, *T. viride* significantly reduced mycelial growth in vitro and significantly controlled TWD in vivo. This control may be attributed to the mechanism of antagonism of *Trichoderma* spp. including secretion of hydrolytic enzymes, antibiotics and antifungal metabolites against pathogenic fungi. *Trichoderma* spp. have been reported to inhibit the growth of *F. oxysporum* by antimicrobial activity which halts mycelial and radial growth, germ tube length and spore germination of *F. xysporum* (Mj *et al.*, 2017).

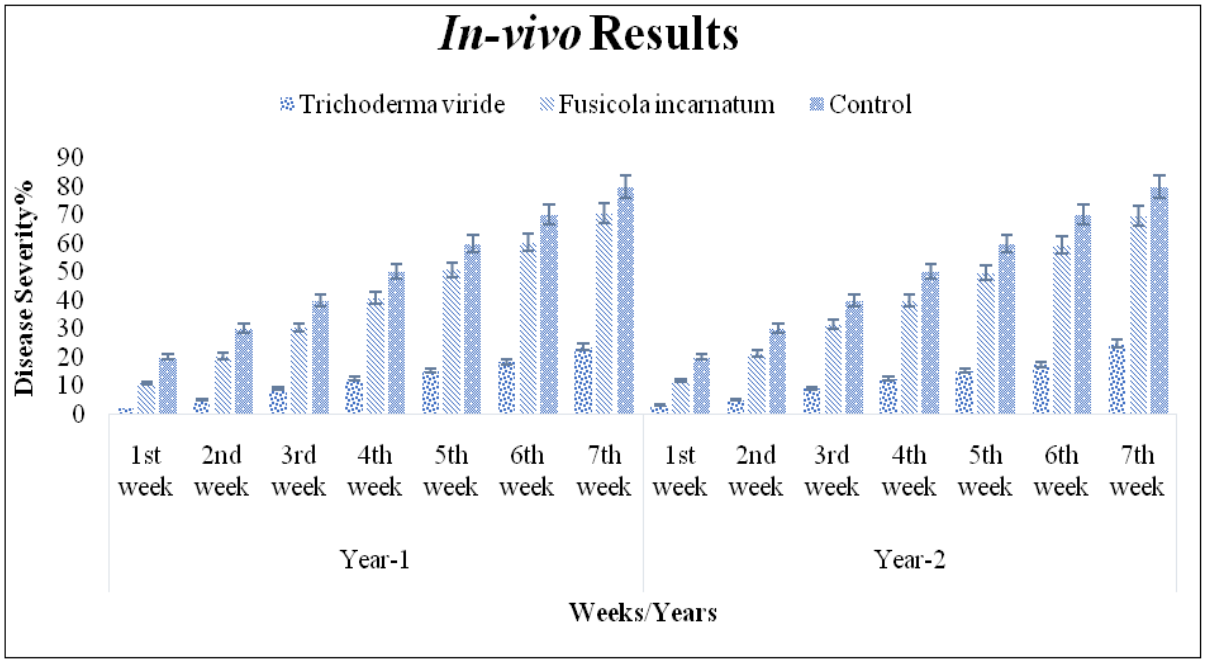


**Fig. 3.** In vivo mean antagonistic effect of T. viride and F. incarnatum bio-agents on TWD for two years.

There are reports that local strains of *Trichoderma* spp. in different parts of the world have effectively controlled tomato wilt caused by FOL (Larkin and Fravel, 2002; Akköprü and Demir, 2005; Alwathnani and Perveen, 2012; Mohammad *et al.*, 2019). Further, *Trichoderma* spp. do not have effects on the ecosystem, therefore it is recommended as biocontrol agents against different diseases (Verma *et al.*, 2018). Antagonistic potential of *F. incarnatum* against FOL found during this study is the first time being reported and needs to be investigated in future studies to reveal its potential as an antagonist against TWD. *F. incarnatum* has been reported to produce different types of toxins on different types of plants (Villani *et al.,* 2019), it is the possibility that these toxins might have killed FOL and reduced the severity of TWD *in vivo*. But, still, this needs to be investigated.

**Conflict of interest**

The authors declare that there is no conflict of interest to publish the article.

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