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Research Article
In vitro Evaluation of *Trichoderma* Species Against *Fusarium oxysporum* f. sp. *lycopersici* Causing Tomato Wilt

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Abstract

Background and Objective: Wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W., is one of the most economically important disease of tomato occurring world-wide. In the present study, the mycoparasitism inhibitory affects of three *Trichoderma* species (*T. viride*, *T. harzianum* and *T. koningi*) on the growth of the causal agent of tomato *Fusarium* wilt (*Fusarium oxysporum* f.sp. *lycopersici*) were investigated by dual culture under *in vitro* conditions. **Materials and Methods:** Already identified *Trichoderma* species viz. *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma koningii* were taken for the present experiment used to check their relative viability against *Fusarium oxysporum* f. sp. *lycoperisci* by dual culture techniques. All the isolates of *Trichoderma* were evaluated for the growth pattern on five different media along with different temperature and pH range. **Results:** *Trichoderma harzianum* inhibited maximum radial growth (75.7%) of *Fusarium oxysporum* f. sp. *lycopersici* pathogen during the experiment followed *T. viride*. During the same study all these *Trichoderma* isolates were evaluated on five different solid media for assessing growth and sporulation and the maximum radial growth of *Trichoderma harzianum* were found in Potato Dextrose agar whereas the lowest growth was observed in *Trichoderma* selective medium. Similarly, excellent growth of *Trichoderma* spp. was found at temperature range of 25±2°C, while 5.0-7.0 pH was found the most favourable for the growth and sporulation of *Trichoderma* spp. **Conclusion:** Out of all the three species of *Trichoderma*, the maximum inhibition of the test pathogen was furnished by *Trichoderma harzianum*. On the basis of present study the fungal bioagents, might be exploited for future plant disease management programs (DMP) to save environmental risk.

Key words: Tomato, *Fusarium oxysporum*, fungal bioagent, environmental risks, *Trichoderma*, bio-efficacy, mycoparasitism

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

India ranks second in the area as well as in production of Tomato. The major tomato growing countries are China (30.7%), India (11.5%), USA (8.1%), Turkey (7.0%) and Egypt (5.3%). In India Andhra Pradesh is the leading state in both area and production of tomato which contributes 17.90% to the total production of tomato in India. It is the most important tropical vegetable crop widely used throughout the world[1].

Tomato plants are affected by a wide array of pathogens. The most common diseases of tomato include early blight, anthracnose, bacterial wilt, bacterial canker, tomato spotted wilt, *Verticillium* wilt and *Fusarium* wilt, the wilt diseases of tomato are caused by bacteria (*Pseudomonas* spp.) and fungi (*Fusarium* and *Verticillium* spp.)[2]. *Fusariam* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici*. This disease was first described by G.E. Massee 1895 in England[3]. The disease was first reported in India from Pusa Bihar as *Fusarium oxysporum* f. sp. *lycopersici*[4].

Fusarium wilt is soil-borne in nature application of fungicides to control this disease is not very effective. However, the regular use of chemical fungicides is harmful for the environment[5]. Hence, there is a need to develop eco-friendly practices for the control of soil borne phytopathogens. Recent trends favor the use of natural plant extracts, biological control agents and several others components. The various types of biological control agents such as bacteria and fungi are involved in biocontrol activity. Members of the genus *Trichoderma* are well known bio-control agents (BCAs)[6-7]. It has been very effectively used for the control of large number of soil borne plant pathogen like *Phytophthora*, *Rhizoctonia*, *Sclerotium*, *Phythium*, *Fusarium*, *Sclerotinia* and *Galumannomyces*. Presently, for commercialization purpose there are mainly three species used viz. *Trichoderma harzianum, Trichoderma viride* and *Trichoderma koningii*. Considering the wide occurrence of the wilt disease, the present investigation was undertaken in the Department of Plant Pathology, Lovely Professional University, Phagwara, India with the objective to evaluate the antagonistic activity of three *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

The present investigations on the wilt disease complex in tomato (*Lycopersicon esculentum* Mill., *Solanum lycopersicum* L.) and it's management were carried out in the Department of Plant Pathology, Lovely Professional University, Phagwara during the year 2017-2018.

Collection of wilt infected tomato plants: Tomato plants showing characteristic symptoms of *Fusarium* wilt were collected from Lovely Professional University, Infected parts of tomato plants were cut from roots and stems and kept in rough dry envelopes especially meant for the purpose. Each envelope was marked clearly mentioning location, variety, date of collection etc. and were brought to the laboratory. The samples were dried for 24 h in shade in order to remove excess surface moisture. After drying, the samples were kept in BOD incubator in paper envelop and maintained at 6-8°C for isolation and further studies.

Isolation and identification of test pathogen: Modified Czapek Dox Agar media was used for isolation of wilt pathogen of tomato[8]. Potato-Dextrose-Agar medium was used for maintaining of pure culture of the wilt pathogen. Pure culture of pathogen was done by single spore isolation[9]. Identification of the pathogen was made by looking at the social and morphological characters of the organism with that of depicted by Booth in his monograph for *Fusarium oxysporum* f. sp. *lycoperisci*, the accompanying development propensity, social and morphological characters were considered on PDA for its confirmation[10].

Screening of *Trichoderma* spp. for their antagonistic potential: Three *Trichoderma* species viz. *Trichoderma harzianum, Trichoderma viride* and *Trichoderma koningii* were taken from Biocontrol lab. of C.S. Azad University and Technology, Kanpur. Cultures were already identified from Indian Type Culture Collection (ITCC), New Delhi. So, the culture have revived for further studies.

Dual culture assay of *Trichoderma* against the test pathogen: *Trichoderma* species was evaluated for relative viability against *Fusarium oxysporum* f. sp. *lycoperisci* by dual culture techniques presented in Fig. 1. About 9 mm breadth circle of test growth and the hostile organisms, cut as bit from the edge of five days old culture were put inverse to each other at a separation of 5 mm from the fringe of Petri plate. Same circle of test organism was set another Petri plate one side on PDA plate, which filled in as control. Every treatment was recreated 3 times and hatched at 25±2°C. The plates were analyzed following 7 days for the development of

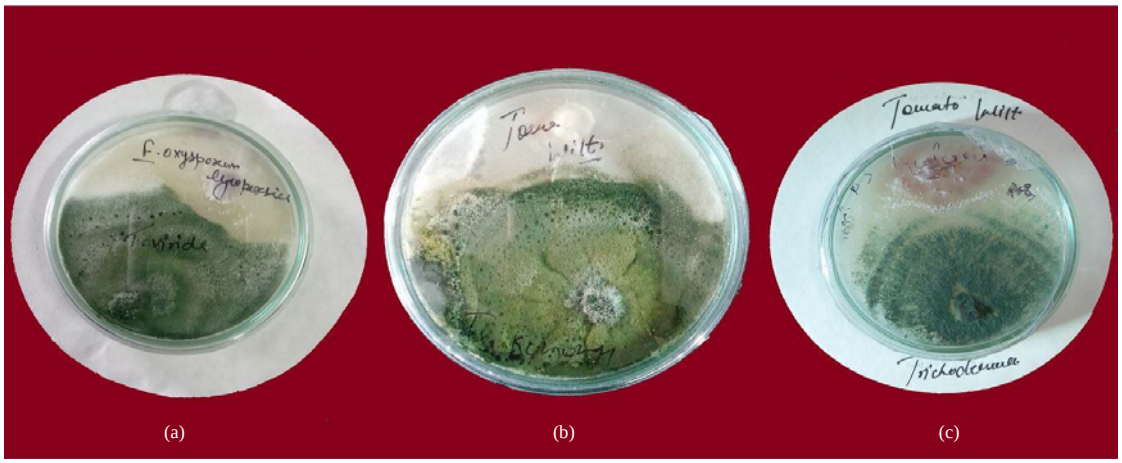

Fig. 1(a-c): Dual culture assay of *Trichoderma* sp. against *Fusarium oxysporum* f. sp. *lycoperisci*, (a) Tomato wilt against *Trichoderma viride*, (b) Tomato wilt against *Trichoderma konigii* and (c) Tomato wilt against *Trichoderma harzianum*

restraint zones amongst *Trichoderma* and pathogen. Toward the finish of brooding period spiral development was estimated. The experiment was replicated thrice and percent growth inhibition was calculated by using the Eq.[11]:

$$L = \frac{C - T}{C} \times 100$$

Where:

L = Inhibition of radial mycelial growth
C = Radial growth measurement of the pathogen in the control
T = Radial growth measurement of the pathogen in the presence of antagonists

Evaluation of the growth pattern of *Trichoderma* isolates on different culture media: *Trichoderma* isolates were evaluated for the growth pattern on five different media namely Potato dextrose agar, Rose Bengal Agar, Malt Extract Agar and Czapek's (Dox) Agar medium and Trichoderma Selective Medium (TSEM). After 3 days of incubation linear growth (cm) of *Trichoderma* was recorded. Linear growth was measured by averaging 3 diameters taken from each colony.

***In vitro* effect of temperature on growth and sporulation of *Trichoderma* spp.:** Influence of temperatures on the linear hyphal growth of *Trichoderma viride* was studied *in vitro* on PDA (15 mL) in 90 mm Petri plates adjusted for each temperature. The fungal growth was observed by placing a bit (9 mm) of pure culture at the centre of the Petri plates with the help of sterile cork borer and three replications were taken for each treatment. The cultures were incubated at 15, 20, 25 and room temperature in BOD incubator and daily observation on mycelial growth of *T. viride* was recorded at every 24 h up to 7 days.

***In vitro* effect of pH on growth and sporulation of *Trichoderma* spp.:** Potato dextrose broths were set at different pH levels 3.0, 5.0, 7.0 and 9.0. The pH was adjusted according to the requirement. All the treatments were carried out in triplicates. About 1 mL spore suspension containing 1×105 spore mL^{-1} recorded by haemocytometer was then added in PDB set at different pH ranges and incubated in BOD at 25 ± 2°C for 7 days. The mycelial mat after 7 days was then harvested from the flask by collecting the culture filtrate through sterilized filter paper (Whatman Filter Paper No. 4). The harvested mycelium was kept in hot air oven at 35°C for 48 h. The biomass was calculated by obtaining the dry weight of mycelium using oven dry method.

Statistical analysis: The analysis of variance technique was applied for drawing conclusions from the data. The calculated value was compared with tabulated value at 5% level of probability for the appropriate degree of freedom[12].

RESULTS

***In vitro* evaluation of different biocontrol agents against *F. oxysporum* f. sp. *lycopersici*:** Biological management of plant pathogens by employing potential bioagents has been an important component of non-chemical plant

disease management. Extensive study was undertaken through *in vitro* screening of three bioagents to ascertain their potential as suitable bio-pesticides against *F. oxysporum* f. sp. *lycopersici*. Analysis of data presented in Table 1 indicated that *Trichoderma harzianum* was very effective in controlling *F. oxysporum* f. sp. *lycopersici* where inhibition zone formation was highest (75.9%) followed by *Trichoderma viride and Trichoderma koningii* with inhibition zone of 67.7 and 55.6%, respectively.

Growth pattern of *Trichoderma* isolates on different culture media: For growth and physiological studies of antagonistic five different culture media were tested and present in Table 2. All the culture media used showed significant effect at on mycelium growth, sporulation of the *Trichoderma* spp. the medium was potato Dextrose Agar supporting maximum growth was observed of *Trichoderma harzianum* and *Trichoderma viride* is 8.1 cm followed by *Trichoderma koningii* is 7.9 cm and minimum growth rate was observed for all species on rose bengal agar was observed of *Trichoderma koningii* is 7 cm followed by *Trichoderma harzianum* is 6.3 cm.

Effect of different temperatures on growth of *Trichoderma* spp: Study was undertaken to find out the optimum as well as the best temperature for the growth of *Trichoderma* spp. by growing at different temperatures (15, 20, 25°C and also at room temperature) on Potato Dextrose agar medium. After 5 days of incubation the average radial growth (cm) was recorded and presented in Table 3. From the perusal of the data presented in Table 3, it is evident that maximum growth was evaluated of all *Trichoderma* spp. at room temperature which ranges between 25±2°C.

Effect of different pH on growth and sporulation of *Trichoderma viride*: Like other physiological parameters, pH also plays an important role in the growth and sporulation. For assessing the optimum pH for the growth and sporulation of *Trichoderma* spp. a set of different pH values (3.0, 5.0, 7.0 and 9.0) were maintained on potato dextrose agar media in three replicates. The pH were adjusted by adding appropriate amount of NaOH and HCl. After 5 days the growth of *Trichoderma* spp. were shown in Table 4. Data presented in Table 4 indicated that all three species of *Trichoderma* could grow well at pH range of 5.0-7.0.

DISCUSSION

From the above findings it was proved that *Trichoderma harzianum* was very effective in controlling *F. oxysporum* f. sp. *lycopersici*. Potential bioagents have been identified for the control of *Fusarium* wilt diseases. Among fungal antagonists, *Trichoderma* spp. have been the most commercialized and efficacious inoculants used world over for the control of soil borne fungal plant pathogens[13-14]. Biocontrol agents are also known to provide habitat specific suppressive effects. During the present experiment it was found that *Trichoderma harzianum* was very effective in controlling *F. oxysporum* f. sp. *lycopersici*[15-16]. In India, *Trichoderma viride* and other several bacterial species such as *Streptomyces gougeroti* were found to be antagonistic to *Fusarium oxysporum* f. sp. *lycopersici*[17]. Bioagents like *Aspergillus niger*, *Trichoderma* sp. and *Penicillium citrinum* and some bio-dynamic antagonists have shown their effectiveness towards the control of wilt pathogens of guava[18]. Several reports indicated that *Trichoderma* species can effectively suppress *Fusarium* wilt pathogens[19-20]. *Trichoderma* species has multiple mechanisms of action, including coparasitism via production of chitinases, β-1-3glucanases and β-1-4glucanases, antibiotics, competition, solubilization of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process[21].

Among all the media used during study, the maximum radial growth of *Trichoderma harzianum* was found in Potato Dextrose agar whereas the lowest growth was observed in *Trichoderma* selective medium. The growth of the three

Table 1: *In vitro* antagonistic activity of *Trichoderma* against *F. oxysporum* f. sp. *lycopersici*

Trichoderma species	Radial average growth of pathogen	Inhibitions of mycelial growth (%)
Trichoderma viride	28.5	67.7
Trichoderma harzianum	21.2	75.9
Trichoderma koningii	39.2	55.6
Control	88.3	00.0

CD at 5% 0.366

Table 2: Radial growth (cm) of *Trichoderma* spp. on different media

Trichoderma species	Potato dextrose agar	Czapek dox agar	Rose bengal agar	Malt extract agar	*Trichoderma* specific media
Trichoderma viride	8.1	7.9	4.5	7.8	3.6
Trichoderma harzianum	8.1	6.6	6.3	7.9	3.1
Trichoderma koningii	7.9	7.1	7.0	7.6	2.4

CD at 5%, 0.097, 0.126 and 0.218

Table 3: Radial growth (cm) of *Trichoderma* spp. at different temperature range

	Temperature range (°C)			
Trichoderma species	15	20	25	Room temperature
Trichoderma viride	6.6	8.0	8.2	8.1
Trichoderma harzianum	6.3	8.1	8.0	8.1
Trichoderma koningii	5.7	7.8	7.8	7.9

CD at 5%, 0.116, 0.134 and 0.231

Table 4: Effect of different pH range on growth (mg) and sporulation of three species of *Trichoderma*

	pH range			
Trichoderma species	3.0	5.0	7.0	9.0
Trichoderma viride	4.2	8.1	8.4	5.3
Trichoderma harzianum	3.4	8.0	7.9	4.3
Trichoderma koningii	3.8	8.1	7.8	5.3

CD at 5%, 0.112, 0.129 and 0.224

species of *Trichoderma* sp. viz. *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma koningii* were tested on five different solid media in which Potato dextrose agar in solid supported excellent radial growth and sporulation[22]. The findings of present studies are consistent with Jahan *et al.*[23] as they found the highest linear growth of *Trichoderma* sp. in potato dextrose agar[23]. The best mycelium growth of *Trichoderma* sp. was observed in PDA. Very dense, mycelium has grown in concentric rings, dark green pigmentation on the 6th day of incubation[24]. An earlier experiment conducted on physiological aspect of *Trichoderma* sp. against different liquid media, temp, pH, whereas PDB reveals the excellent average mycelium weight (190.9 mg)[25].

Data of above findings revealed that maximum growth of all *Trichoderma* spp. was evaluated at room temperature which ranges between 25±2°C. During the studies, it was found that temperature affected the linear hyphal growth of *Trichoderma* isolates. After 3 days of inoculation, the growth rate decreased when temperature was kept below or above 25 and 30°C. Sharma *et al.*[26] also reported similar findings, which confirmed that *Trichoderma viride* was grown better between 25-30°C and slow growth was recorded[26] at 35°C. Similarly, Sobieralski[27] reported the fastest growth of the mycelium of *Trichoderma* isolates at the temperature ranges of 25-30°C. Similar results were also obtained by Szczech and Shoda[28] in the experiment conducted in Poland[28].

All three species of *Trichoderma* could grow well at pH range of 5.0-7.0. Variation in the biomass of *Trichoderma* isolates was observed at different (3.0, 5.0, 7.0 and 9.0) pH range. Maximum number of isolates showed high biomass production at pH 6.5 followed by 7.5 and 5.5 while minimum at pH 4.5. The excellent growth of *Trichoderma* was found at temperature range of 25-30°C, while 6.5 pH (Slightly acidic) was found the most favorable for the growth and sporulation of *Trichoderma* isolates[29]. Moreno-Mateos *et al.*[30] also found variation among the isolates within the *Trichoderma* species[30]. The studies showed that acidic media favored fungal growth than alkaline. Benitez *et al.*[31] reported that growth of *Trichoderma* is more efficient in acidic than alkaline soils and they modify the rhizosphere soil by acidifying the soil[31].

CONCLUSION

This study concluded the mycoparasitism inhibitory affect of three trichoderma species on the causal agent growth of tomato fusarium wilt. Out of three species the maximum inhibition was showed by *Trichoderma harzianum*. So on the basis of the current study fungal bioagent may be exploited for plant disease management programs in future to save the environmental risk.

SIGNIFICANCE STATEMENT

This study discover that the use biocontrol agents such as *Trichoderma viride*, *Trichoderma harzianum* etc along with the recommended range of temperature and pH will be very beneficial which also the salient findings of the present study. So, this study will help the researcher to uncover the critical areas of the growth of crop without affecting our natural ecosystem that many researchers were not able to explore. On the basis of present study the fungal bioagents, might be exploited for future plant disease management programs (DMP) to save environmental risk.

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