Biological control of fusarium wilt of tomato (Solanum lycopersicum L.) by antagonistic fungi

Nikhat S Siddique, Vinod Hiremath, Pakkala Abhiram, Yashab Kumar, Ankit Khedikar, Akshay Kunghatkar and Singadi Spandhan Reddy

Abstract
A Field experiment was conducted during the Rabi season of 2017 at the main research field of department of horticulture, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad U. P. (India). To study the “Biological control of fusarium wilt of tomato (Solanum lycopersicum L.) By antagonistic fungi.” Under In Vitro, maximum radial growth was observed in Fusarium oxysporum f. sp. Lycopersici (90mm) and least radial growth was observed in Trichoderma harzianum (41.99mm). Among the three treatments viz., T1- Trichoderma harzianum, T2-Trichoderma viridae and T3- Aspergillus Niger. Maximum percent growth inhibition was recorded in Trichoderma harzianum (52.60%), which is followed by Trichoderma viridae percent growth inhibition (47.94%) and Aspergillus Niger percent growth inhibition (38.90%). Under In Vivo, twelve treatment i.e (T1 control), (T2) - F. oxysporum f. sp. lycopersici, (T3) - Trichoderma harzianum, (T4) - Trichoderma viridae, (T5) - Aspergillus niger, (T6) - F. oxysporum f. sp. lycopersici + T. harzianum, (T7) – F. oxysporum f. sp. lycopersici + T. viridae, (T8) - F. oxysporum f. sp. lycopersici + A. niger, (T9) - T. harzianum + T. viridae, (T10) - T. harzianum + A. niger, (T11) – T. viridae + A. niger, (T12) - F. oxysporum f. sp. lycopersici + T. harzianum + T. viridae + A. niger replicated three times each were carried out in the plot in Randomized block design. More over T12 treatment (F. oxysporum f. sp. lycopersici + T. harzianum + T. viridae + A. niger) showed better result followed by T10 and T9.

Keywords: Fusarium oxysporum, Trichoderma harzianum, Trichoderma viridae, inhibition, dual culture technique, yield parameters

Introduction
Fusarium oxysporum f. sp. Lycopersici is a known pathogen of tomato plant which is an economically important crop. Tomato yield is significantly reduced by F. oxysporum f. sp. Lycopersici because it can destroy the roots of tomato at growth stage. Numerous strategies has been proposed to control this fungal pathogen (Biondi et al., 2011). Trichoderma is a filamentous fungus which has attracted the attention because of their multi prong action against various plant pathogens (Harmam et al., 2004). Management of fusarium is mainly through chemical soil fumigations and resistance cultivars. The broad-spectrum biocides used to fumigate soil before planting (particularly methyl bromide) are environmentally damaging. On the contrary breeding for resistance can be very difficult when no dominant gene is known. In addition new races of pathogens overcoming host resistance can develop. The difficulty in controlling fusarium wilt has stimulated the research in biological control independently from the recent concern for environmental protection. (Demir et al., 2005) [13]. Tomato (solanum lycopersicum L.) is one of the world’s most cultivated vegetable crop in India and cultivated on an area of about 865 thousand ha. It is cultivated in essentially all countries either in fields or in protected culture. Its many varieties are now widely grown, sometimes in greenhouses in cooler climates (Abd-El Kareem et al., 2006) [11]. It is one of the most important vegetable crops of India and cultivated on an area of about 865 thousand ha (Anonymous et al., 2011). Biological control of plant pathogens is considered as a potential control strategy in recent years, because chemical control results in accumulation of harmful chemical residues, which may lead to serious ecological problems. At present, effective management of plant diseases & microbial contamination in several agricultural commodities is generally achieved by the use of synthetic pesticides. However, the incessant and indiscriminate application of these chemical fungicides has caused health hazards in animals and humans due to residual toxicity (Gaigole et al., 2001). In recent years, large number of synthetic fungicides has been banned in the western world because of their undesirable attributes such as high and acute toxicity (Dennis et al., 1971).
It is important to develop methods for evaluating antagonistic micro-organisms and incorporating them into successful disease management. Several antagonists have been evaluated with variable success (Shekhwat et al., 1993 [48], Lwin and Rana mukhaarachchi 2006) [26] reported a satisfactory suppression of the fungal wilt pathogen by the application of a commercially available mixture of effective microorganisms (EM).

Materials and Methods
The present study “Biological control of Fusarium wilt of tomato (Solanum lycopersicum L.) By antagonistic fungi.” was conducted at PG Laboratory Department of industrial Microbiology, Jacob institute of Biotechnology and Bioengineering, SHUATS, Allahabad, UP during kharif 2017-18. The details of the materials used and methodology followed during the course of investigations are described below.

Isolation, identification and maintenance of Fusarium oxysporum f. sp. Lycopersici.
Fusarium oxysporum f.sp. Lycopersici was isolated from naturally infected tomato plants collected from different field viz., central and Horticulture field. The plant parts showing brown discoloration of vascular tissues were cut into small pieces (1 cm). Such pieces were aseptically transferred to sterile potato dextrose agar medium. Both pathogen and antagonistic micro organisms were placed equidistant from the periphery so that they would get equal opportunity for their growth. After the incubation period, the radial growth of Fusarium oxysporum strains. In control, as well as in treatment plate was measured and the per cent inhibition was calculated using the formula (Webster et al., 2013).

\[ I = \frac{(C - T) \times 100}{C} \]

Where,
I = per cent inhibition
C = Growth of the pathogen in control plate (mm)
T = Growth of the pathogen in dual culture plate (mm)

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Treatment combination</th>
<th>Replications</th>
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<tbody>
<tr>
<td>1</td>
<td>Trichoderma harzianum + Fusarium oxysporum</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Trichoderma viridae + Fusarium oxysporum</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>Aspergillus niger + Fusarium oxysporum</td>
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Formulation preparation of biocontrol agents
The fungal biocontrol agents, Trichoderma viridae, T. harzianum and Aspergillus Niger. Were cultured on potato dextrose agar (PDA). Two day old culture of Trichoderma viridae, T. harzianum and Aspergillus nigra were cultured on PD broth by aseptically punching out 5 mm of the agar plate culture with a cutter and incubate with 25°C upto 15 days fungal growth were centrifuged at 2000 rpm for 5 min. the supernatant was discarded and the pellets were washed in sterilized distilled water repeatedly thrice and filtration through a What man No. 1 filter paper to get spore masses and concentration of conidia was adjusted to 2.5X10^7 spores/ml (Sivan et al., 1984). The mycelial pellet was mixed with talc powder in 1:2 ratio. It was air dried and stored in polyethylene bags at 4°C.

Field Experiment
Three replicate were specified for each treatment in Randomized Block design. The experiment included the following treatments:-T1 - Non infested soil (control), T2 - F. oxysporum f. sp. Lycopersic, T3 - Trichoderma harzianum, T4 - Trichoderma viridae, T5 - Aspergillus Niger, T6 - F. oxysporum f. sp. lycopersici + T. harzanium, T7 - F. oxysporum f. sp. lycopersici + T. viridae, T8 - F. oxysporum f. sp. lycopersici + A. niger, T9 - T. harzanium + T. viridae, T10 - F. oxysporum f. sp. lycopersici + A. niger + T. harzanium + T. viridae.

In vitro evaluation of bio agents against Fusarium oxysporum f. sp. lycopersici
The above mentioned fungal bio agents were evaluated in vitro for their antagonistic effect against F. oxysporum f. sp. lycopersici by dual culture technique (Dennis and Webster 1971) on potato dextrose agar medium. 15ml of potato dextrose agar medium was poured into sterile petriplate and allowed for solidification. Seven days old 5 mm disc of F. oxysporum f. sp. lycopersici was cut with a sterile Cork borer and placed near the periphery on one side of PDA plate. A plate without antagonist was maintained as control. The inoculated plates were incubated at 28°C for seven days. Each treatment was done on five replicate.

The antagonistic activity of Trichoderma viride, T. harzianum and Aspergillus Niger was screened in vitro against Fusarium oxysporum sp. by dual culture plate technique. The antagonistic efficacy against tested pathogens was evaluated on PDA medium. Both pathogen and antagonists were grown on sterilized PDA plates separately for 7 days. For testing antagonism in dual culture method, a mycelial disk of 5 mm in diameter of antagonist were excised from the edge of an actively growing 7day old culture plate and inoculated opposite to the pathogenic fungi in the same plate 1cm away from the edge inoculated. For each treatment five replicates were maintained and incubated at 26 ± 2°C. The test pathogen was inoculated in the middle of the plate in duplicates these paired cultures of antagonist and test pathogen were placed equidistant from the periphery so that they would get equal opportunity for their growth. After the incubation period, the radial growth of Fusarium oxysporum strains. In control, as well as in treatment plate was measured and the per cent inhibition was calculated using the formula (Webster et al., 2013).

\[ I = \frac{(C - T) \times 100}{C} \]

Where,
I = per cent inhibition
C = Growth of the pathogen in control plate (mm)
T = Growth of the pathogen in dual culture plate (mm)

Table 1: Treatments details of in vitro evaluation
T10 - *T. harzanium* + *A. niger*, T11 - *T. viridae* + *A. niger*, T12 - *F. oxysporum* f. sp. *lycopersici* + *T. harzanium* + *T. viridae* + *A. niger*.

Observation on Yield and yield attributing parameters viz. No. of plants observed, No. of plants wilted, % wilt incidence, No. of fruits per plant, Weight of single fruit (g), Fruit yield per plant (kg per plants). Yield per plot (kg), Fruit yield per hectare (tonnes per ha). The data were statistically analysed using ANOVA.

**Results and Discussion**

| Table 1: In vitro evaluation of cultural and morphological characteristics of fusarium oxysporum f. Sp. Lycopersici |
|---|---|---|---|---|
| Media | Isolated from stem and root of infected wilt diseased plant of Tomato | Colony characteristics | Morphological characteristics | Organism |
| Colour | Textue | Hyphae | Spores | Type | Size | Shape | Septation | Arrangemet |
| PDA | Whitish rosy | Felty woolly | Aerial | Chlamydospores | Conidia 5.50 - 30.0 × 2.0 - 12.0 μm | Spindle, Sickle shaped or curved | Septate | Monophialids | *Fusarium oxysporum* |

*Fusarium sp.* was isolated repeatedly from wilted plants. Fungus isolated from wilted plants was identified as *F. oxysporum* f. sp. *lycopersici* based on the morphological and cultural characters as described by Butler (1910), Pad wick (1940) [32] and Booth (1971) [10]. Similarly Nirmala devi and Srinivas (2012) [30] identified the *Fusarium oxysporum* isolated from wilt affected Tomato plant. Hussain et al., (2012) [21] also characterized the *Fusarium oxysporum* isolated from Guava wilt in Bangladesh.
**Fig 3:** Radial growth of *Fusarium oxysporum f. sp. Lycopersici* (mm) in dual culture technique.

**Fig 4:** (a) Radial growth of *F. oxysporum* culture on PDA. (b) PDA media.

**Fig 5:** (a) Antagonistic effect of *Trichoderma harzianum* against *F. oxysporum* on PDA media. (b) Radial growth of *Trichoderma harzianum* on PDA media.
Among the three treatments viz., T1- *Trichoderma harzianum*, T2- *Trichoderma viridae* and T3- *Aspergillus Niger* evaluated for fungal growth inhibition of *Fusarium oxysporum f. sp. Lycopersici*. Maximum percent growth inhibition was recorded in *Trichoderma harzianum* 52.60%, followed by *Trichoderma viridae* percent growth inhibition 47.94% and *Aspergillus Niger* percent growth inhibition 38.90%. Compared to untreated control where full colony growth of *Fusarium oxysporum f. sp. Lycopersici* was recorded and there was no per cent inhibition observed [Fig no. 1, Plate no. 4(a), 5(a), 6(a)].

Among the various antagonists used for the management of plant diseases, *Trichoderma* sp. plays a vital role. Recently, it was suggested that, *Trichoderma* affects induced systemic resistance mechanism in plants against pathogens (Haggag and Amin, 2001, Prasad et al., 2002, Hibar et al., 2007, Jayalakshmi et al., 2009) [24]. Among the various isolates of *Trichoderma*, *T. Asperellum, T. harzianum, T. virens, T. viride*, and *T. hamatum* are used against the management of various diseases of crop plants especially with soil borne pathogens. These filamentous fungi are very common in nature, with high population densities in soil and plant litters (Samuels, 1996) [39]. Many studies have proved the potential of *Trichoderma* sp. as biological agents antagonistic to several plant pathogens (Sivan and Chet, 1986, Naseby et al., 2000, Tondje et al., 2007, Houssien, et al., 2010) [22] and use
of *Trichoderma* spp. on banana (Thangavelu 2004) [43] arbuscular mycorrhiza (AM) on banana (Jaizme-Vega et al., 1998) [23], an soil amendment of Lettuce on cucumber has also been reported. In the presented study, a promising strategy for biocontrol of Fusarium wilt of tomato observed. The dual treatment with *T. harzianum* and *T. viride* strains may be due to their abilities to produce phytohormones, vitamins and solubilizing minerals besides, their role in direct inhibition of pathogen growth (Morsy, 2009 [28] and Zaghloul et al., 2007) [47], Niknejad et al. (2000) [31] and Zaghloul et al. (2007) [47] reported that application of selected antagonists (*T. harzianum*, *T. viride*) either individually or in combination has significantly increased the number of fruits/plant, weight of fruits and the total yield of tomato fruits. It could be concluded that the dual treatment with *T. harzianum* combined with *T. viride* has a significant and more feasible to control root rot disease and increased the yield components of tomato comparing with the individual treatments, because of their potential to produce plant growth promoting substances (Bochow et al., 2001 and Morsy, 2005), which might create favourable conditions for improving minerals uptake by plants.

**Conclusion**

On the basis of present investigation it may be concluded that treatment T12 = *F. oxysporum* + *T. harzianum* + *T. Viridae* + *A. Niger* showed superior performance in terms of yield & yield attributes.

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**References**


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