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

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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. harzanium*, (T7) – *F. oxysporum f. sp. lycopersici + T. viridae*, (T8) - *F. oxysporum f. sp. lycopersici + A. niger*, (T9) - *T. harzanium + T. viridae*, (T10) - *T. harzanium + A. niger*, (T11) – *T. viridae + A. niger*, (T12) - *F. oxysporum f. sp. lycopersici + T. harzanium + 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. harzanium + 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 cultivors. 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) [15].
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) [1]. 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 (Shekhawat *et al*., 1993 [40], 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 in each Petri-dish at equal distance and inoculated plates were incubated at 28ºC. The culture, *F. oxysporum f.sp lycopersici* identification was made based on the characters described by Booth (1971) [10]. The respective isolates of *F. oxysporum f.sp lycopersici* were used subsequently for further studies. The PDA slants containing *F. oxysporum f.sp lycopersici* isolate were stored in a refrigerator at 5ºC for further investigations and were sub cultured at regular interval during the course of investigation to maintain the virulence of the pathogen.

Identification and Characterization of pathogen

The observations were made on colour, septation, size and other morphological characters of conidia under high power objective lens. The size of the conidia was measured by using calibrated ocular micrometer and also cultural characters like growth, type colour of colony were observed (Madhukeshwara and Seshadri 2001) [27]. The spore dimensions were taken by micrometric technique (Tuite, 1969) [45].

Collection and maintainance of antagonistic micro organisms

The Bio-control agent used in this study i.e. *Trichoderma viride, Trichoderma harzianum and Aspergillus niger* were obtained from microbial culture collection bank (MCCB), Department of Industrial Microbiology, Jacob Institute of Biotechnology and Bioengineering. All Bio-control agent were maintained on PDA slants and were stored at 4°C till use.

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 28ºC 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 ± 2ºC. 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).

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

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

Sl. no	Treatment combination	Replications
1	*Trichoderma harzianum + Fusarium oxysporum*	5
2	*Trichoderma viridae + Fusarium oxysporum*	5
3	*Aspergillus niger + Fusarium oxysporum*	5

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 niger* were cultured on PD broth by aseptically punching out 5 mm of the agar plate culture with a cutter and incubate with 25^0C 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. The fungal formulation were mixed with 20g / liter of water before transplanting and seedlings were dip in this fungal formulation.

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*,

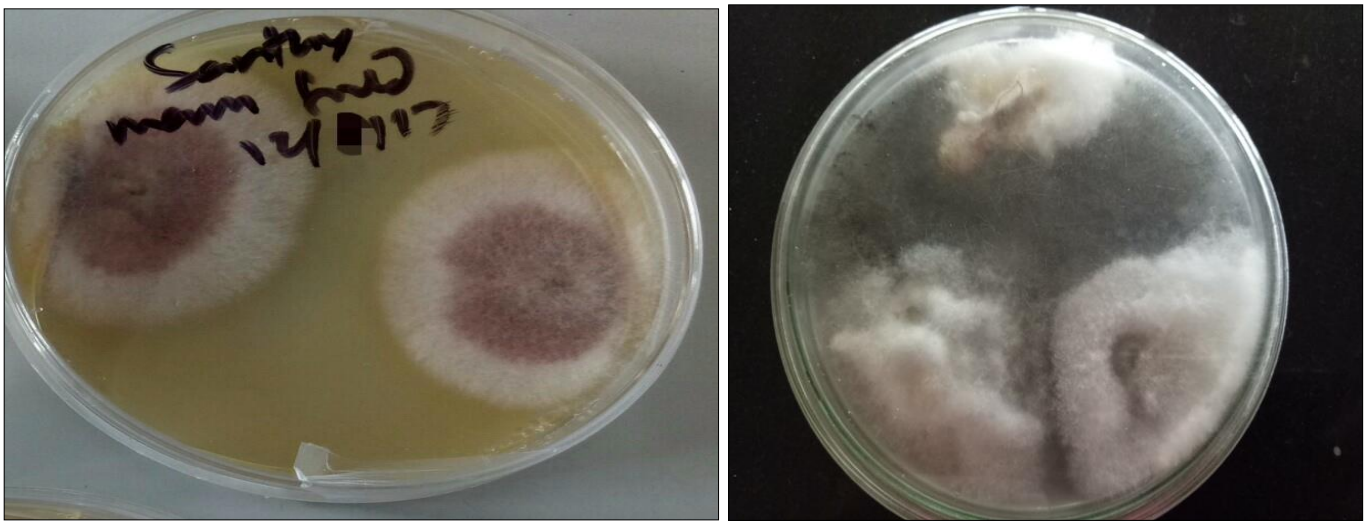
T10 - *T. harzanium* + *A. niger*, T11 - *T. viridae* + *A. niger*, T12 - *F. oxysporum f. sp. lycopersici* + *T. harzanium* + *T. viridae* + *A. niger*.

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

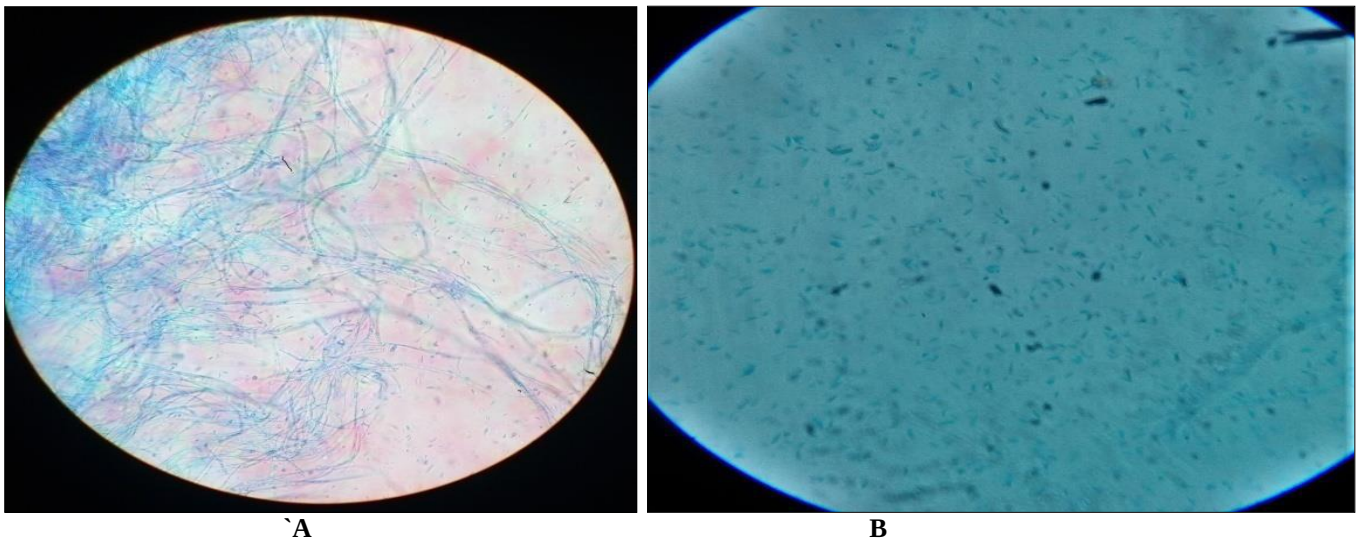
Table 1: *In vitro* evaluation of cultural and morphological characteristics of fusarium oxysporum f. Sp. Lycopersici

Media	Isolated from	Colony characteristics			Morphological characteristics					Organism
					Spores					
		Colour	Textue	Hyphae	Type	Size	Shape	Septation	Arrangemet	
PDA	stem and root of infected wilt diseased plant of Tomato	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*



A **B**

Fig 1: (AB) Colonies of *Fusarium oxysporum f. sp. Lycopersici* on PDA media



`A **B**

Fig 2: (AB) Conidia of *Fusarium oxysporum f. sp. Lycopersici* (10X and 40X)

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.

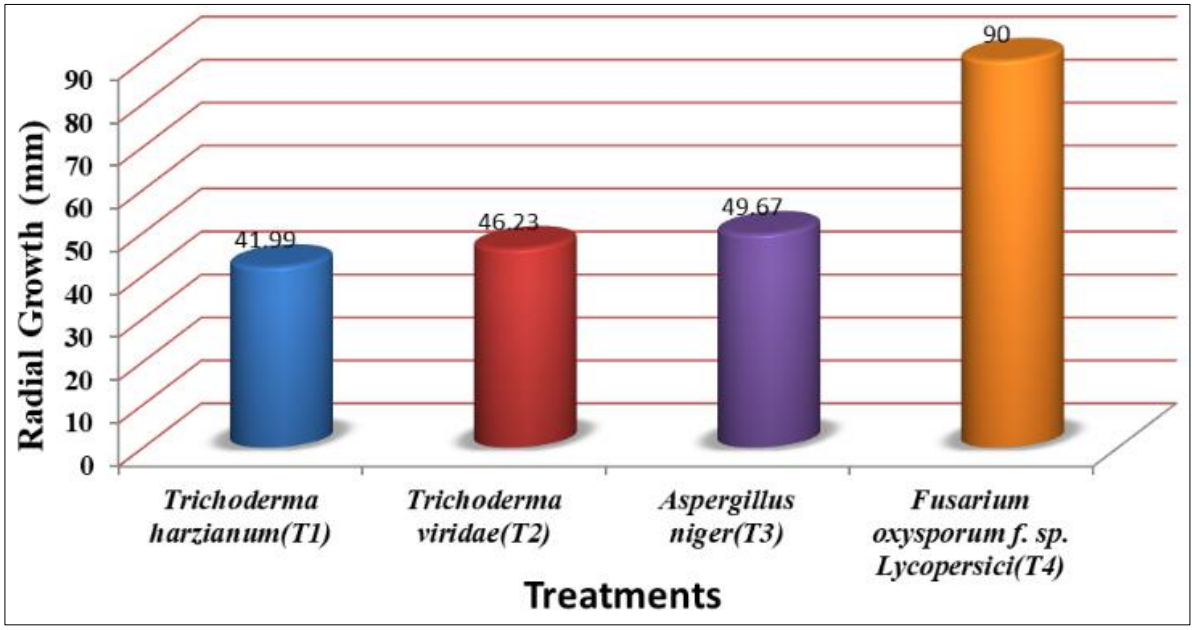
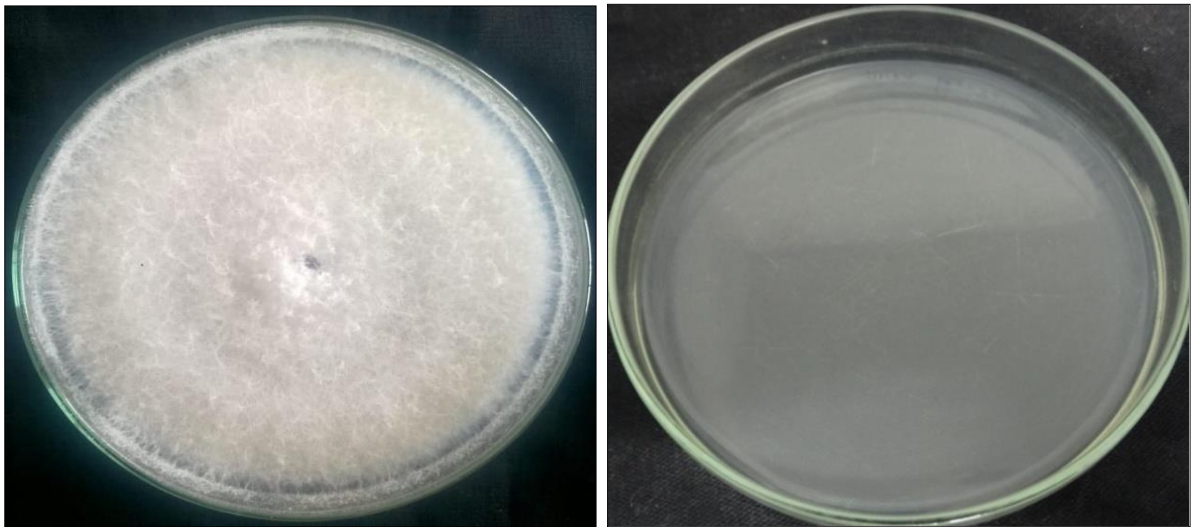
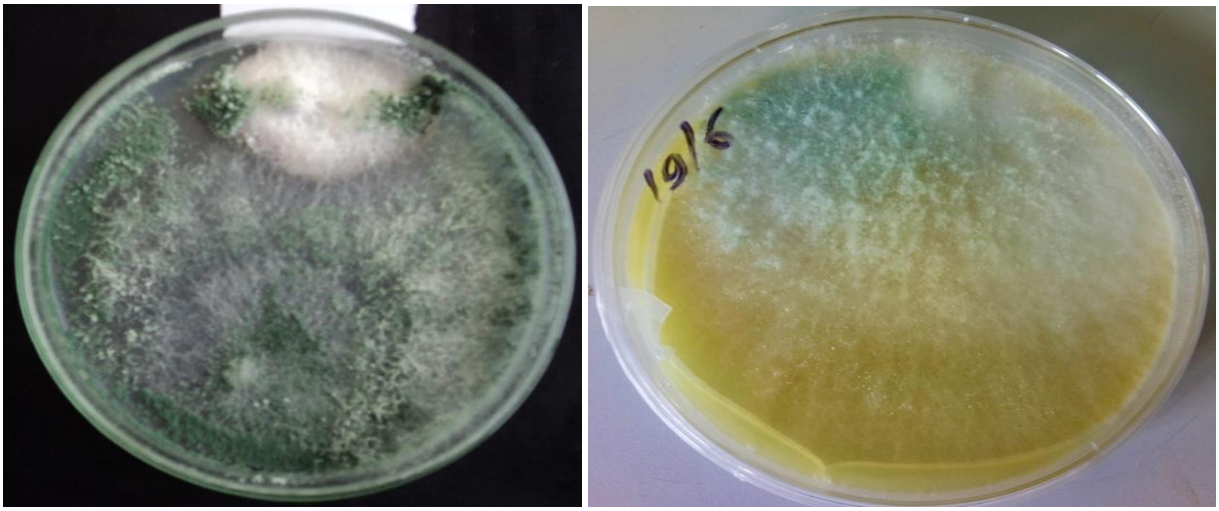


Fig 3: Radial growth of *Fusarium oxysporum f. sp. Lycopersici* (mm) in dual culture technique



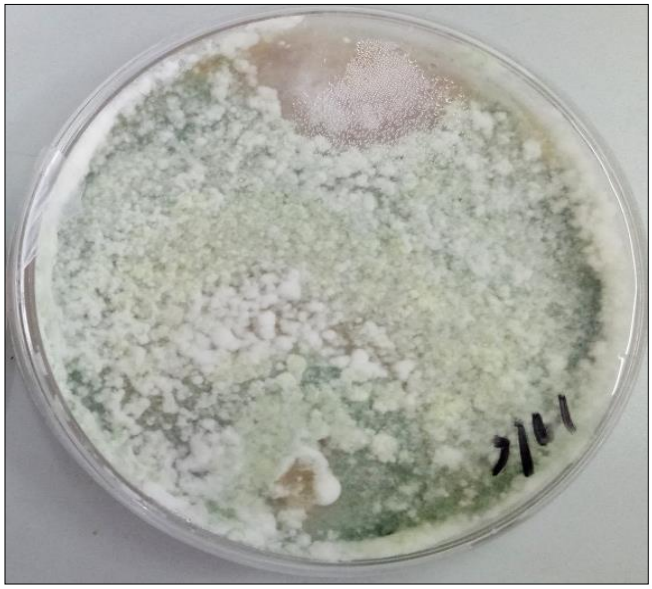
A **B**

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



A **B**

Fig 5: (a) Antagonistic effect of *Trichoderma harzianum* against *F. oxysporum* on PDA media.
(b) Radial growth of *Trichoderma harzianum* on PDA media.



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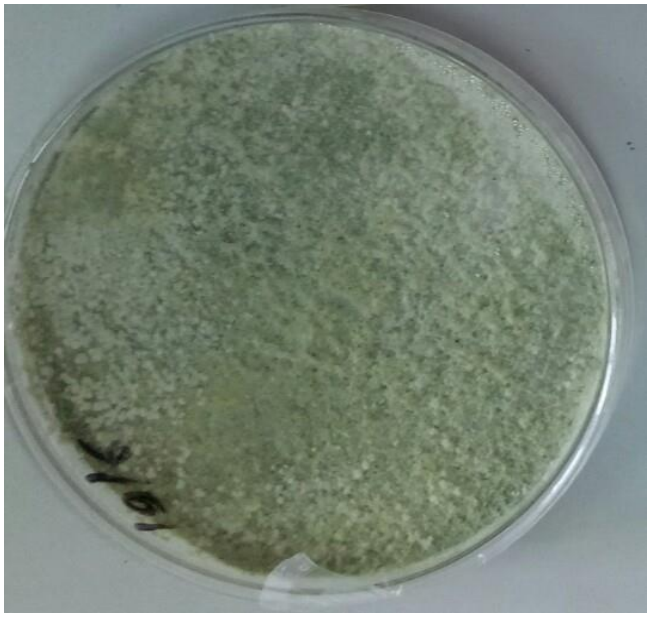
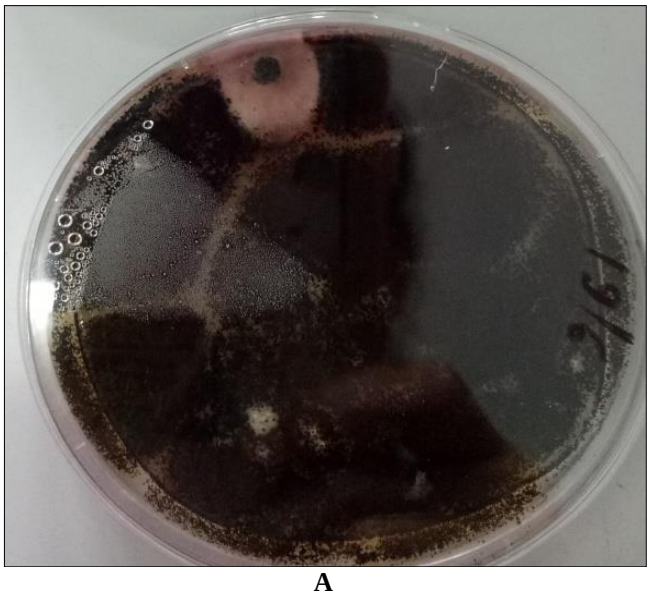


B

Fig 6: (a) Antagonistic effect of *Trichoderma viridae* against *F. oxysporum* on PDA media
(b) Radial growth of *Trichoderma viridae* on PDA media.



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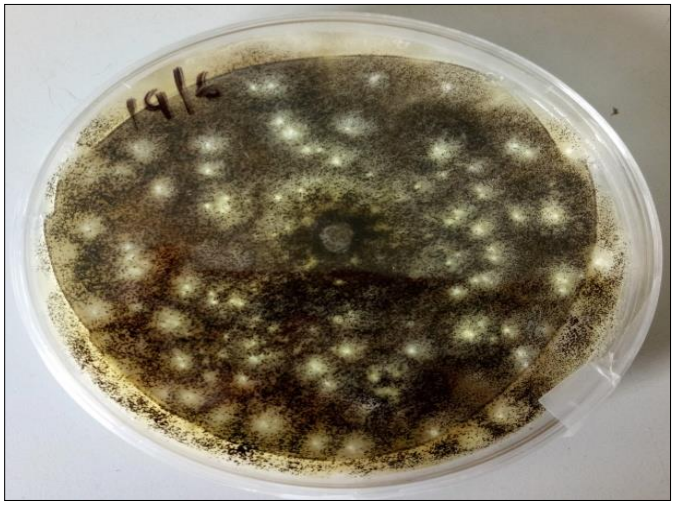

B

Fig 7: (a) Antagonistic effect of *Aspergillus Niger* against *F. oxysporum* on PDA media (b) Radial growth of *Aspergillus Niger* 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)}.

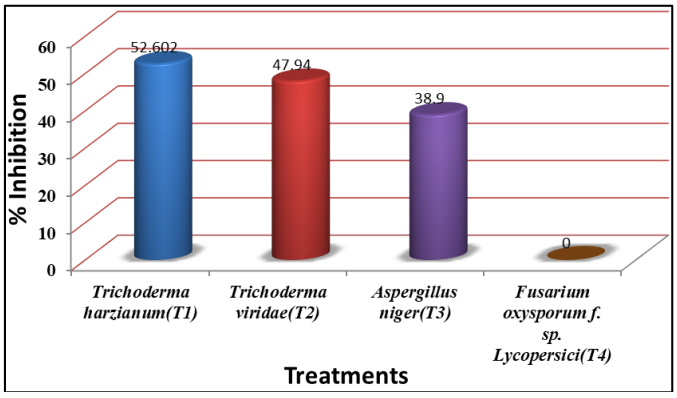

Fig 8: *In vitro* evaluation of bio agents against *Fusarium oxysporum f. sp. Lycopersici*

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 bio control disease agents such as *F. solani* and *F. oxysporum* that are exposed to tomato field has been implemented by three *Trichoderma* spp., *T. Asperellum, T. harzianum* and *T. virens*.

Demands for *in vitro* effectiveness of *Trichoderma* against species of *Fusarium* have been reported (Jee and Kim, 1987; Calvet *et al.*, 1990; Reddy, 1996; Morsy *et al.*, 2009 [28]; Padmadaya and Ramezani, 2009; Sabalpara *et al.*, 2009) [38]. The antagonist *Trichoderma harzianum*, *T. coningi* and *T. viride* were reported to be equally antagonistic to *F. udum in vitro* (Bahatnagar, 1986) [3]. Results of this evaluation were similar to the finding of Siven and Chet (1987) and Somasekhar *et al.* (1996) [42] Biswas and Das (1999) [8], Cal *et al.* (2004) [12], Morsy *et al.* (2009) [28], Ramezani (2009) [35]. The present investigation showed that *T. hamatum*, *T. harzianum* and *T. longiconis* were more or less equally effective, *T. koningi* was less effective, whereas the least was noticed in case of *T. viride*. It was reported that isolates of the *T. harzianum* collected from different soil samples was not equally effective to inhibit the growth of *F. udum* (Bell *et al*., 1982 and Biswas, 1992) [9]. It was found that one isolate (T1) among the 10 isolates of this antagonist was effective. So, there is need to search a very specific isolate(s) of *Trichoderma* sp. for successful control of *Fusarium oxysporum* f. sp. *lycopersici* (Biswas, 1992) [9].

In vivo evaluation of different treatments in field experiments

Among twelve treatments evaluated to record wilt incidence of *Fusarium oxysporum f. sp. Lycopersici* under field condition number of wilted plants 3.69 (avg) and Maximum % wilt incidence 58.12% were recorded in T2- seedling dip with *Fusarium oxysporum f. sp. Lycopersici* @ 20 g/litre. Which is followed by T8- seedling dip with *Fusarium oxysporum f. sp. Lycopersici + A. niger* @ 20 g/litre with number of wilted plants 0.67 (avg) and 12.29% of wilt incidence were recorded. However T1- untreated (control), T7- seedling dip with *Fusarium oxysporum f. sp. Lycopersici + T. viridae* @ 20 g/litre and T6- seedling dip with *Fusarium oxysporum f. sp. Lycopersici + T. harzianum* @ 20 g/litre recorded number of wilted plants 0.38 (avg), 0.46 (avg) and 0.38 (avg) % wilt incidence of 6.02%, 5.78% and 5.56% respectively. However remaining treatments no plants were wilted, which were treated as best treatments for the management of Fusarium wilt of tomato observed.

Among the twelve treatments imposed to know their effectiveness on yield parameters of tomato. Maximum mean number of fruits 53.35 fruits/plant, single fruit weight of 54.79g and harvested maximum fruit yield of 3.12 fruits kg/plant were recorded in T-12, Which is followed by number of fruits 50.5 (avg) fruits/plant, 52 g/single fruit weight and harvested 2.97 kg of fruit/plant were recorded in T-9. However, least number of fruits/plants was recorded in T2 with average 11.67 fruits/plant, average single fruit weight of 30.02 g and harvested average 0.62 kg fruits/plant was observed.

Maximum plot yield and per hectare yield was recorded in T12 with average 17.06 kg of fruits yield/plot and harvested 52.52 tons/hectare respectively. T9 and T10 these two treatments recorded yield of fruits 16.04 kg/plot and 15.36 kg/plot and harvested 50.36 tons/ha and 48.47 tons/ha respectively. However least yield per plot and per hectare was recorded in T2 with average yield of fruits 3.99 kg/plot and harvested average yield of fruits 11.6 tons/ha was observed**.**

The promotion of tomato growth parameters by *T. harzianum* and *T. viride* strains may be due to their abilities to produce phytohormones, vitamins and solublizing 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.

Whipps and Mc Quilken (1993) [46] reported that *A. Niger, A. terreus, G. virens, P. citrinum, T. harzianum* and species of *Bacillus* control soil-borne diseases. Bashar and Rai (1994) [5] observed that *A. flavus, A. Niger,* and *T. viride* amended in soil suppressed the growth of *F. oxysporum* f. sp. *ciceri* and exhibited strong fungistatic activity against germination of conidia of test pathogen.

Conclusion

On the basis of present investigation it may be concluded that treatment T_{12} = *F. oxysporum + T. harzianum + T. Viridae + A. Niger* showed superior performance in terms of yield & yield attributes.

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