Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 616-619
Received: 25-05-2017
Accepted: 26-06-2017

Midhun Babychan
Dept. of Plant Pathology, SHUATS-Allahabad, Uttar Pradesh, India

Sobita Simon
Dept. of Plant Pathology, SHUATS-Allahabad, Uttar Pradesh, India

Correspondence
Midhun Babychan
Dept. of Plant Pathology, SHUATS-Allahabad, Uttar Pradesh, India

Efficacy of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici.* (FOL) infecting pre-and post-seedling of tomato

Midhun Babychan and Sobita Simon

Abstract
The antagonistic potential of eight *Trichoderma* isolates were evaluate the efficacy of the native isolates of *Trichoderma* species to inhibit the wilt and promote the growth parameters of tomato seedling and to manage the fusarium wilt disease as affected by *Fusarium oxysporum* f.sp. *lycopersici*. Under *in vitro* condition the results revealed that Trichoderma isolate MiT-4 was found to be effectively inhibiting the radial mycelial growth of the pathogen by 58.4%. Under greenhouse condition application of *Trichoderma* isolates (MiT-1 and MiT-4) exhibit no wilt incidence in tomato seedlings. The germination of tomato seeds is greatly influenced by *Trichoderma* isolate (MiT-3) over *Fusarium oxysporium* f. sp *lycopersici* by 83.66%. Application of *Trichoderma* isolates (MiT-3) on tomato seeds, seedlings showed a significant stimulatory effect on shoot and root height by 7.53 cm and 7.1 cm respectively which is higher than the control.

Keywords: *Trichoderma* isolates, antagonism, biological control, Fusarium wilt, *Lycopersicon esculentum*

1. Introduction
Trichoderma spp. are the most widely using biocontrol antagonists for controlling soil borne pathogens including *Fusarium oxysporium* f.sp. *lycopersici.* The application of *Trichoderma* is proven to improve root and plant growth and to induce resistance in plants. Trichoderma spp. are associated with root ecosystem and hence they promotegrowth and defense mechanism by production of several compounds (Rai *et al*. 2016) [14, 27]. The antagonistic mechanism exhibited by *Trichoderma* spp. are nutrient competition, antibiotic production, and mycoparasitism. Tomato (*Lycopersicon esculentum*) is one of the most common and commercially important vegetable crops grown all around the globe. This disease is highly destructive in both field and green house conditions and characterized by wilted plants, yellowed leaves and minimal or absent crop yield (Krishnakumar *et al*., 2008) [6, 15 19, 28].
Though fusarium is a soil borne pathogen chemical control is not economical preposition. Besides, these chemical leave residues which is harmful to human beings and can develop resistance by the pathogen on continuous use. Beside this, enhancement of plant growth of tomato has been observed after application of *Trichoderma* in field and greenhouse (Lubaina and Murugan, 2015) [20, 7]. With the view of biocontrol efficacy of Trichoderma isolates, the study was done to evaluate *Trichoderma* isolates against *Fusarium oxysporium* f.sp. *lycopersici* in both *invitro* and *invivo* conditions.

2. Methods
2.1. Isolation of pathogen and antagonist
Eight isolates *Trichoderma* was isolated from rhizosphere soil of various plant from erumad village of Tamilnadu by serial dilution plate technique on PDA medium (Rifai, 1969) [10, 23]. All the isolates were purified by using hyphal tip technique (Bissett, 1991) [3] and codded as MiT-1, MiT-2, MiT-3, MiT-4, MiT-5, MiT-6, MiT-7 and MiT-8. Five days old culture was used for experimentation.
Samples were collected from the wilted tomato plants and washed under tap water to remove the soil particles. The cut bits were sterilized with 10% sodium hypochlorite for 5-10 min and subsequently three passages of sterile distilled water. Then, they were placed on potato dextrose agar (PDA) medium separately and incubated at the laboratory conditions at 25 ± 3 °C, for five days. The fungi were purified by transferring the hyphal tip into PDA plates and stock were maintained (Nash and Snyder, 1965) [9, 22].

2.2. Antagonistic test

Isolates of *Trichoderma* was evaluated against FOL in the laboratory by dual culture technique (Morton andStroube, 1955) [8, 21] to screen out the most efficacious one. 5mm size discs of culture of eight *Trichoderma* isolates were placed opposite to the FOL discs at the periphery of 90 mm Petri plates containing PDA. For control *Fusarium oxysporium* f.sp. *lycopersici* was placed in same manner on PDA plates. All pairing was replicated thrice and incubated at 25°C. The effect of *Trichoderma* isolates on plant pathogens was determined by the percentage of mycelia growth inhibition in cm calculated with the follow formula (Abadi, 1990) [16, 29]:

Inhibition (%) = [(T_1-T_2) / T_1] ×100

Where,

T_1 = growth of the phytopathogen in the absence of antagonist and

T_2 = growth of the phytopathogen in the presence of antagonist.

2.3. Preparation of spore suspension:

Six pieces of agar discs (6 mm) were kept in a flask containing sterilized Richard's solution for each strains of *Trichoderma* with four replications. The flasks were incubated on a rotatory incubator at 80 rpm at 28 °C (Dennis and Webster, 1971) [4, 17]. The culture filtrates were collected after 20 days of incubation. These were then concentrated to about 50 % using a vacuum evaporator at 38-40 °C and finally filtered by sterilized membrane filter.

2.4. Germination test:

The seeds with no cracks were selected and surface sterilized with 1% sodium hypochlorite solution and rinsed with double distilled water and allowed for drying. The spore suspension of Fusarium was poured into steam sterilized soil filled trays of 1.5kg capacity. One week later, *Trichoderma* treated seeds were sow on the trays. The seeds were treated with the solution of 1×10^6 conidia per ml at the rate of 1 ml per 10g of seeds. A seed coating was prepared from *Trichoderma* culture supplemented with 2% of starch (w/v) as additive. Dry tomato seeds were dipped in culture filtrate supplemented with2% of starch (w/v) in *Trichoderma* for 1-2 minutes. 30 seeds were sown in each tray with three replications. Vigour index was calculated according to the formula suggested by (Adbul-Baki and Anderson, 1973).

Vigour index = [seedling height] × [percentage of seed germination]

Seedling height=Mean of root length (cm) + Mean of shoot length (cm)

3. Results

The antagonistic activity of *Trichoderma* isolates was screened against the soil borne plant pathogenic fungi *Fusarium oxysporum* f.sp. *lycopersici* were studied to record the mycelial inhibition and the results were summarized in Table 1. All the treatments were superior in managing the pathogen over control and all are at par with each other. Among the treatments, T_4 with MiT-4 (58.40%)followed by T_5 with MiT-5(57.33%), T_6 with MiT-6(57.33%), T_7 with MiT-7(57.33%), T_8 with MiT-1(55.32%), T_2 with MiT-2(55.2%), T_3 with MiT-3(55.2%) and T_1 with MiT-1(53.60%).

Result shown in Table2 gives the significant increase in germination percentage against *Fusarium oxysporum* f.sp. *lycopersici* in treatment T_3(86.663%) as highest and least in T9 (36.66%). The significant increase in the root length when *Trichoderma* is applied to soil as a bioagent with T3 soil application of MiT-3 (7.10cm) when compared with all other treatments. And also, the shoot length increases when *Trichoderma* is applied to soil as a bioagent with T3 soil application of MiT-3(7.53cm) when compared with all other treatments. Treatments are effective in suppressing the wilt incidence (by 0.00%). Conspicuously, an application of MiT-1, MiT-4 shows no wilt incidence in the seedling stages. Control shows maximum wilt incidence (64.4%).

4. Discussion

Significant increase in inhibiting percentage against *Fusarium oxysporum* f.sp. *lycopersici* was obtained in MiT-4(T4) when compared to all other treatments. The similar findings were given by (Sundaramoorthy and Balabaskar, 2013) [13, 26]. Increase in germination percentage against *Fusarium oxysporum* f.sp. *lycopersici* was reported in MiT-3(T3) when compared to all other treatments. The similar findings were given by (Patel, 2017) [11, 24]. The Vigour index was reported to be greater in MiT-3(1258.43) followed by MiT-4(1015.33), MiT-2(904.20) and least among the treatments was MiT-6(758.29) and the vigour index for control (271.18). The similar findings were given by (Sundaramoorthy and Balabaskar, 2013) [13, 26] and (Ramezani, 2010) [12, 25].

5. Conclusion

It may be concluded that the *Trichoderma* isolates control the wilt causing agent *Fusarium oxysporium* f. sp *lycopersici* on various levels of inhibition. Culture filtrates of *Trichoderma* strains have potential to enhance the germination in tomato seeds. Thus, *Trichoderma* has a large potential effect as biocontrol antagonist in control *Fusarium oxysporium* f. sp *lycopersici* causing wilt disease of tomato and also it enhances all the growth parameters of crop and increase the yield.

Table 1: Percent mycelial inhibition of *Fusarium oxysporium* f. sp *lycopersici* against *Trichoderma* isolates.

Treatments	Replication			Growthof Pathogen	Mycelial Inhibition
T-1 (MiT-1 with FOL)	3.0	2.8	2.9	2.90	53.60^{c}
T-2 (MiT-2 with FOL)	2.8	2.7	2.9	2.80	55.2^{bc}
T-3 (MiT-3 with FOL)	2.7	2.8	2.9	2.80	55.2^{bc}
T-4 (MiT-4 with FOL)	2.3	2.8	2.7	2.60	58.40^{a}
T-5 (MiT-5 with FOL)	2.7	2.6	2.7	2.67	57.33^{ab}
T-6 (MiT-6 with FOL)	2.7	2.7	2.8	2.73	56.26^{abc}
T-7 (MiT-7 with FOL)	2.6	2.7	2.7	2.67	56.26^{abc}
T-8 (MiT-8 with FOL)	2.7	2.75	2.8	2.77	55.32^{abc}
T-9 (Only FOL)	5.6	6.7	6.3	6.20	0

Means in the column followed by different letters indicate highly significances according to DMRT (P<0.05).

Table 2: Growth parameters of tomato seedlings as affected by *Fusarium oxysporium* f. sp *lycopersici* against *Trichoderma* isolates.

Treatments	Germination %	Root length	Shoot length	Seedling length	Vigour index	Wilt incidence
MiT-1	66	4.66	5.70	10.36	684.15	No incidence
MiT-2	66	6.93	6.76	13.70	904.20	10.75
MiT-3	86	7.10	7.53	14.63	1258.43	5.7
MiT-4	83	6.00	6.23	12.23	1015.33	No incidence
MiT-5	53	5.23	6.90	12.13	643.04	15.91
MiT-6	65	5.33	6.33	11.66	758.29	35.89
MiT-7	66	6.66	6.13	12.80	844.80	25.25
MiT-8	77	5.46	5.63	11.10	854.70	37.26
Control	36	2.93	4.60	7.53	271.18	64.4

6. Acknowledgement
I acknowledge department of plant pathology, Sam Higginbottom University of Agriculture Science and Technology for providing the necessary facilities for this work.

7. Reference

1. Abdul-Baki AA, Anderson JD. Vigor determination in soybean seed by multiple criteria. Crop science, 1973, 13(6):630-633.
2. Barbari H. Biocontrol of Tomato Fusarium Wilt by *Trichoderma* Species under *In vitro* and *in vivo* Conditions. Cercetări Agronomice În Moldova, 1. 2016; (165):91-98.
3. Bissett J. A revision of the genus 39. *Trichoderma.* II: Infragenic classification. Canadian Journal of Botany. 1991; 69:2357-2317.
4. Dennis C, Webster J. Antagonistic properties of species-groups of Trichoderma: I. Production of non-volatile antibiotics. Transactions of the British Mycological Society, 1971; 57(1), 25IN3-39.
5. Harman GE, Myths and dogmas of biocontrol - Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease, 2000; 84(4):377-393.
6. Krishnakumar K, Chou KC, Martinetz T, Möller S, Suganthan PN, Sridharan S *et al.* AFP-Pred: A random forest approach for predicting antifreeze proteins from sequence-derived properties. Journal of theoretical biology, 2008; 270(1):56-62.
7. Lubaina AS, Murugan K. Evaluation of Biological Efficacy of Trichoderma Species Isolates against Alternaria Leaf Spot Disease of Sesame, 2015.
8. Morton D1, Stroube WH. Antagonistic and stimulatory effects of soil microorganisms upon *Sclerotium rolfsii*. Phytopathology, 1955; 45:417-420.
9. Nash SM, Snyder WC. Quantitative and qualitative comparisons of Fusarium populations in cultivated fields and non-cultivated parent soils. Canadian Journal of Botany, 1965; 43(8):939-945.
10. Rifai MA. Revision of the genus Trichoderma, 1969.
11. Patel, Stuti. Interaction of root colonizing biocontrol agents demonstrates the antagonistic effect against *Fusarium oxysporium* f.sp. *lycopersici* on tomato. European Journal of plant pathology, 2017.
12. Ramezani, Hesamadin. Antagonistic effects of *Trichoderma spp* against *Fusarium oxysporum* f.sp. *lycopersici* causal agent of tomato wilt. Plant Protection Journal. 2010; 2 (1):167-173.
13. Sundaramoorthy S, Balabaskar P. Biocontrol efficacy of *Trichoderma spp* against wilt of tomato caused by *Fusarium oxysporum f. sp. lycopersici*. Journal of Applied Biology Biotechnology, 2013; 1:36-40.
14. Rai Shalini, Kashyap LP, Kumar, Sudheer, Srivastava KA, Pramod RV *et al.* Identification, characterization and phylogenetic analysis of antifungal *Trichoderma* from tomato rhizosphere. Springer Plus, 2016, 5:1939.
15. Krishnakumar K, Chou KC, Martinetz T, Möller S, Suganthan PN, Sridharan S *et al.* Pugalenthi G. AFP-Pred: A random forest approach for predicting antifreeze proteins from sequence-derived properties. Journal of theoretical biology. 2008; 270(1):56-62.
16. Abadi AL. Antagonistic effect of four fungal isolates to *Ganoderma boninense*, the causal agent of Basal stem rot of oil palm. *Biotropia,* 1990; 3:41-49.
17. Dennis C, Webster J. Antagonistic properties of species-groups of Trichoderma: I. Production of non-volatile antibiotics. Transactions of the British Mycological Society, 1971; 57(1):25, 3-39
18. Harman GE. Myths and dogmas of biocontrol - Changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease. 2000; 84(4):377-393.
19. Krishnakumar K, Chou KC, Martinetz T, Möller S, Suganthan PN, Sridharan S *et al.* AFP-Pred: A random forest approach for predicting antifreeze proteins from sequence-derived properties. Journal of theoretical biology. 2008; 270(1):56-62.
20. Lubaina AS, Murugan K. Evaluation of Biological Efficacy of Trichoderma Species Isolates against Alternaria Leaf Spot Disease of Sesame, 2015.
21. Morton D, Stroube WH. Antagonistic and stimulatory effects of soil microorganisms upon *Sclerotium rolfsii*. Phytopathology, 1955; 45:417-420.
22. Nash SM, Snyder WC. Quantitative and qualitative comparisons of Fusarium populations in cultivated fields and non-cultivated parent soils. Canadian Journal of Botany. 1965; 43(8):939-945.
23. Rifai MA. Revision of the genus Trichoderma, 1969.
24. Patel, Stuti. Interaction of root colonizing biocontrol agents demonstrates the antagonistic effect against *Fusarium oxysporium* f. sp. *lycopersici* on tomato. European Journal of plant pathology, 2017.
25. Ramezani, Hesamadin. Antagonistic effects of *Trichoderma spp* against *Fusarium oxysporum* f. sp. *lycopersici* causal agent of tomato wilt. Plant Protection Journal, 2010; 2(1):167-173.
26. Sundaramoorthy S, Balabaskar P. Biocontrol efficacy of *Trichoderma spp* against wilt of tomato caused by *Fusarium oxysporum f. sp. lycopersici*. Journal of Applied Biology Biotechnology, 2013, 1:36-40.
27. Shalini Rai, Kashyap LP, Sudheer Kumar, Srivastava K A, Pramod RV. Identification, characterization and phylogenetic analysis of antifungal *Trichoderma* from tomato rhizosphere. SpringerPlus, 2016, 5:1939.
28. Krishnakumar K, Chou KC, Martinetz T, Möller S, Suganthan PN, Sridharan S *et al.* AFP-Pred: A random forest approach for predicting antifreeze proteins from

sequence-derived properties. Journal of theoretical biology. 2008; 270(1):56-62.
29. Abadi AL. Antagonistic effect of four fungal isolates to *Ganoderma boninense*, the causal agent of Basal stem rot of oil palm. Biotropia, 1990; 3:41-49.