Int. Journal of Universal Science and Technology
ISSN: 2454-7263, Volume: 05 No: 02 Published: Jan., 2019
Paper ID: IJUP3116, Web: www.universalprint.org
Title Key: In vitro Evaluation of Trichoderma…

In vitro evaluation of Trichoderma spp. against collar rot of tomato caused by Sclerotium rolfsii

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ABSTRACT

Trichoderma, a genus of asexually reproducing saprophytic fungi, frequently present in nearly all temperate and tropical soils. The strains of *Trichoderma* spp. are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers. *Trichoderma* is one of the common fungal biocontrol agent, is being used worldwide for suitable management of various foliar and soilborne plant pathogens. The distribution of several phytopathogenic fungi, such as *Fusarium*, *Pythium*, *Phytophthora*, *Botrytis* and *Rhizoctonia* has spread during the last few years due to changes introduced in farming with detrimental effects on crops of economic importance. Various isolates of *Trichoderma* spp were evaluated against *Sclerotium rolfsii* and significantly inhibited its growth over untreated control. Among the eight isolates tested, isolate *T. hamatum* (Thm-L) was found most effective shows 62.21% mycelial growth inhibition followed by *T. harzianum* (Thr-O) shows 56.33% mycelial growth inhibition.

Key words: Biocontrol, *Trichoderma* spp., soil borne pathogen, *Sclerotium rolfsii*

Introduction:

Trichoderma species are used as biocontrol agents in agriculture. *Trichoderma*, a genus of asexually reproducing saprophytic fungi, frequently present in nearly all temperate and tropical soils, decaying plant tissues and root ecosystems. The strains of *Trichoderma* spp. are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers. *Trichoderma* able to control a wide range of phytopathogenic fungi as antagonist. The antagonism of *Trichoderma* involves several mechanisms, such as competition for nutrient antibiosis and production of fungal cell wall degrading enzymes. The mycoparasitic ability of *Trichoderma* species against plant pathogenic filamentous fungi allows for development of biocontrol strategies (Benitez *et al*., 2004). The genus *Trichoderma* (Syn. *Hypocerea crassa*) belongs to the phyllum Ascomycetes, Class Sordriomycetes, Order Hypocreales and Family Hypocreaceae. The genus *Trichoderma* was first described more than two hundred years ago by Persoon (1794) in Germany. The potential for use of *Trichoderma* spp. as biocontrol agents was suggested more than 75 years ago by Weindling (1932) and Sanjeev, (2013) *Trichoderma* is one of the common fungal biocontrol agent, is being used worldwide for suitable management of various foliar and soil borne plant pathogens. Biocontrol agents like *Trichoderma* spp. are acclaimed as effective, ecofriendly and cheap, nullifying the ill effects of chemicals. *Trichoderma* is one of the common fungal biocontrol agents, is being used worldwide for suitable management of various foliar and soil borne plant pathogens. Therefore, of late, these biocontrol agents are identified to act against an array of important soil borne plant pathogens causing serious

diseases of crops (Khandelwal *et al.*, 2012). The distribution of several phytopathogenic fungi, such as *Fusarium*, *Pythium*, *Phytophthora*, *Botrytis* and *Rhizoctonia* has spread during the last few years due to changes introduced in farming with detrimental effects on crops of economic importance. Considering the seriousness of the pathogen/disease and economic importance and importance of local strains for management of plant diseases present research was carried out on isolation of local strains of *Trichoderma* spp. and its evaluation against collar rot of tomato caused by *Sclerotium rolfsii* isolated from tomato.

Materials and Methods

Serial dilution and plating technique was used to isolate the *Trichoderma* from the samples collected. The collected samples were air dried in shade and finely grind before serial dilutions. PDA media was used for Isolation. After isolation and identification of these *Trichoderma* spp. were used for further evaluation. For *In vitro* evaluation of biocontrol agents the test *Trichoderma* spp. were evaluated *in vitro* against *Sclerotium rolfsii* by dual culture technique (Dennis and Webster, 1971). Mycelial discs (5mm) taken from the margins of 6 day old colonies of *Trichoderma* isolates and *Sclerotium rolfsii* were transferred to the separate Petri dishes containing 20 ml PDA. One isolate of *Trichoderma* and *Sclerotium rolfsii* were placed simultaneously on opposite sides of each dish, 5cm apart. The control was prepared with only *Sclerotium rolfsii.* Three replications were used for each treatment. Mycelial growth of *Sclerotium rolfsii* was assessed by measuring the colony diameter in two perpendicular directions after 48hrs of incubation at 28^{o}C. Per cent inhibition of mycelial growth of *Sclerotium rolfsii* in the presence of *Trichoderma* spp. was determined as per the method given by Arora and Upadhyay, 1978.

Result and Discussion

Results (Table 1 and Fig. 1) revealed that, all the isolates of *Trichoderma* exhibited fungistatic / antifungal activity against *Sclerotium rolfsii* and significantly inhibited its growth over untreated control (Fig. 1)

At 48 hrs, maximum colony diameter 6.96 mm was observed in *T. harzianum* (Pbn-1) with 6.46 per cent mycelial inhibition and least linear mycelial growth 3.13 mm was observed in *T. hamatum* (Nand-1) with per cent inhibition mycelial growth was 8.43. At 72 hrs , highest linear mycelia growth 31.38 mm was observed in *T. harzianum* (Pbn-1) with per cent inhibition mycelial growth was 54.47 and lowest linear mycelial growth 25.51 mm was observed in *T. harzianum* (Thr-J) with 61.22 per cent inhibition mycelial growth . At 96 hrs, highest linear mycelial growth 47.02 mm was in *T. viride* (Tv-B) with 40.55 per cent inhibition mycelial growth and least linear mycelial growth 38.72 mm with highest mycelial growth inhibition 56.06 was observed in *T. viride* (Tv-H).

The second and third best antagonists found were Tv-H (T2) and Thr-O (T5), which were recorded mycelial growth of 3.76, 26.52 and 38.72 mm and 3.76, 25.89 and 38.78 mm of test pathogen, respectively and average mycelial inhibition of 8.11, 60.11, 56.06 and 8.11, 60.82 and 56.33 percent, respectively. This was followed by Thr-J (T6) Colony diameter: 6.83, 25.51 and 40.35 and in Thr-Ab (T8). 4.73, 28.18 and 40.36 mm. Inhibition of mycelial growth in Thr-J (T6) was 6.58, 61.22, 53.36 and in Thr-Ab (T8) 7.63, 58.15 and 53.36 percent, respectively. It was observed that, treatment Thm-L (T3) was found most effective isolate which showed highest average zone of inhibition i.e. 62.21 percent followed by Tv-H (T2) isolate i.e. 56.06 percent and Thr-O (T5) isolate i.e. 56.33 percent for *Sclerotium rolfsii*, respectively.

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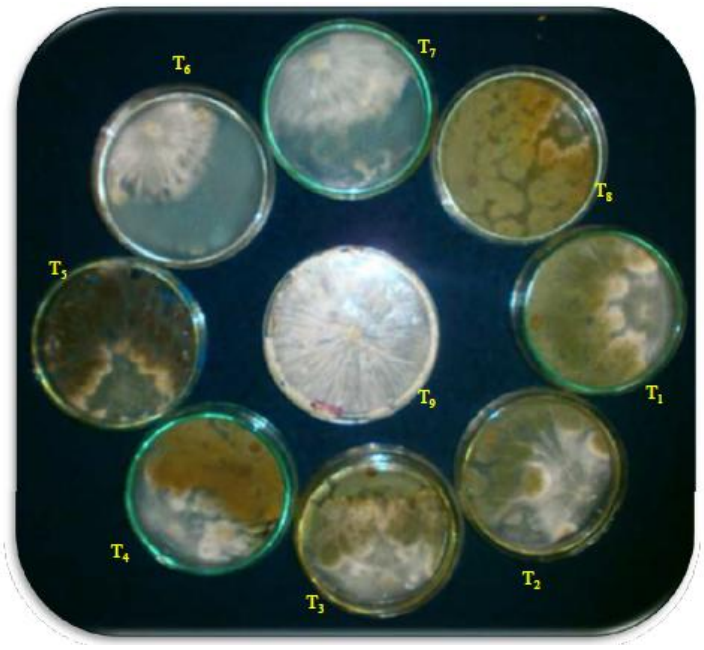
Among the isolates tested, isolate Thm-L (T3) was found most effective and test pathogen was recorded least linear mycelial growth at 48, 72 and 96 hrs interval i.e. 3.33, 25.67 and 40.79 mm, respectively with highest mycelial inhibition i.e 8.33, 61.78 and 62.21percent, respectively. Hence, from this study concluded that isolate Thm-L (T3) from Latur region was found most effective for the management of collar rot of tomato. Among the local strains of *Trichoderma* isolated from different districts of Marathwada region the strains of latur district was found efficient to manage the coilor rot of tomato caused by *Sclerotium rolfsii.*



T1= Beed-1 T2= Hing-1
T3= Ltr-1 T4= Nand-1
T5= Osm-1 T6= Jal-1
T7= Pbn-1 T6= A. bad-1
T9= Control

Fig.1. *In vitro* evaluation of *Trichoderma* spp. against *Sclerotium rolfsii*.

Table.1. *In vitro* evaluation of *Trichoderma* spp. against *Sclerotium rolfsii*.

Treatment	Isolates	Avg. colony diameter (mm)	Per cent inhibition of mycelial growth	Avg. colony diameter (mm	Per cent inhibition of mycelial growt	Avg. colony diameter (mm))	Per cent inhibition of mycelial growth
		48 hrs		72 hrs		96 hrs	
T1	*T. viride* (Tv-B)	6.20	6.86 (15.18)	29.89	56.20 (48.56)	47.02	40.55 (39.55)
T2	*T. viride* (Tv-H)	3.76	8.11 (16.54)	26.52	60.11 (50.83)	38.72	56.06 (48.48)
T3	*T. hamatum* (Thm-L)	3.33	8.33 (16.77)	25.67	61.78 (51.81)	40.79	62.21 (52.06)
T4	*T. hamatum* (Nand-1)	3.13	8.43 (16.87)	26.53	59.72 (50.60)	42.54	49.14 (44.50)
T5	*T. harzianum* (Thr-O)	3.76	8.11 (16.54)	25.89	60.82 (51.24)	38.78	56.33 (48.63)

T6	*T. harzianum* (Thr-J)	6.83	6.58 (14.86)	25.51	61.22 (51.48)	40.35	53.36 (46.92)
T7	*T. harzianum* (Thr-P)	6.96	6.46 (14.70)	31.38	54.47 (47.53)	44.98	44.44 (41.80)
T8	*T. harzianum* (Thr-Ab)	4.73	7.63 (16.03)	28.18	58.15 (49.69)	40.36	53.36 (46.92)
T9	Control	20	20	80.50	80.5	90	90
SE±		0.73	0.37	1.40	1.62	1.62	1.62
CD at 1%		2.19	1.10	4.20	4.86	4.87	4.82

*-Mean of two replications, Dia.: Diameter

Figure in Parentheses are transformed in arc sine values.

Results of the present study on effect of bio-agents viz., *Trichoderma viride*, *T. harzianum*, and T. hamatum on mycelial growth of *Sclerotium rolfsii* were in consonance with those reported earlier by several workers Das et al., 2000 evaluated in vitro and in vivo the antagonistic potential of *Trichoderma harzianum*, *T. viride* and *T. koningii* against *Sclerotium rolfsii* causing collar rot of tomato and reported that, *T. harzianum* significantly inhibited mycelial growth of the test pathogen as well as, it also caused highest reduction in the disease with increase in fruit yield under field conditions. Result on bioagent viz., *Trichoderma viride*, *Trichoderma harzianum* and *Trichoderma hamatum* on mycelial growth of *Sclerotium rolfsii* were also reported by several workers Bosah *et al.*, 2010; Belete *et al.*, 2015; Chandulal *et al.*, 2015; Akrami and Yousefi 2015; Manoranjitham *et al.*, 2015 and Ambuse *et al.*, 2016), respectively.

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