



Research Note

Screening of *Trichoderma* species against major soil borne fungal pathogens

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ABSTRACT: The antagonistic potential of forty four isolates of *Trichoderma* were evaluated in vitro against the most widely occurring soil borne plant pathogens *viz., Macrophomina phaseolina, Rhizoctonia solani, Rhizoctonia bataticola* and *Sclerotium rolfsii* to identify the most potential *Trichoderma* isolate. Maximum growth inhibition of *M. phaseolina* (81.11 %), *R. solani* (82.59 %) and *S. rolfsii* (76.67 %) was recorded by *T. hamatum* where as *T. virens* was most aggressive against *R. bataticola* (68.15 %) in dual culture technique.

KEY WORDS: *Trichoderma, Rhizoctonia bataticola, Macrophomina phaseolina, Rhizoctonia solani, Sclerotium rolfsii*, biological control

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Genus Trichoderma consists of anamorphic free living fungi common in soil and root ecosystems that promote plant growth (Yedidia and Chet, 2001) and are effective in control of soil/seed-borne fungal diseases in several crop plants (Kubicek, 2001). Many Trichoderma species are known to produce hydrolytic enzymes viz., cellulases, chitinases and xylanases which are used in diverse fields such as food processing, pulp and paper industry and textile industry. In addition some species produce antibiotic products and hence have been successfully used as biological control agents against a range of phytopathogens. Hence, they are successfully used and commercialized to combat a broad range of phytopathogenic fungi such as Rhizoctonia bataticola (Taub.) Butler, Macrophomina phaseolina (Tassi) Goid, Rhizoctonia solani Kuhn and Sclerotium rolfsii Sacc. (Asran-Amal et al., 2005; Bosah et al., 2010 and Sreedevi et al., 2011). The objective of the present investigation is to isolate and screen effective Trichoderma spp. against the major soil borne pathogens.

Experiments were conducted in Biocontrol Laboratory (Plant Pathology), College of Agriculture, University of Agricultural Sciences, Raichur during 2014-15. Forty four isolates of *Trichoderma* spp. were included in this study. Isolation of the bioagent from soil was done by serial dilution method whereas isolation of the pathogenic fungus was done by following standard tissue isolation method under aseptic conditions.

Dual culture technique

The antagonistic potential of the Trichoderma isolates against soil borne pathogens viz., R. bataticola, M. phaseolina, R. solani and S. rolfsii was tested by dual culture method on potato dextrose agar medium. Five mm discs from actively growing colony of pathogen was cut with a sterile cork borer and placed near the periphery of PDA plate. Similarly, antagonistic fungi was placed on the other side *i.e.*, at an angle of 180°. Plates with no antagonists placed served as control for the pathogen. The plates were incubated at $28 \pm 1^{\circ}$ C for seven days. Each treatment was replicated thrice. The extent of antagonistic activity by Trichoderma isolates i.e., growth after contact with fungal plant pathogens was recorded after incubation period by measuring growth of fungal plant pathogens in dual culture plate and in control plate. The per cent inhibition of fungal plant pathogens was calculated using formula:

$$I = \frac{C - T}{C} x 100$$

Where,

- I = Per cent inhibition.
- C = Growth of fungal plant pathogens in control (mm).
- T = Growth of fungal plant pathogens in dual culture plate (mm).

The *Trichoderma* isolates screened for antagonistic action against *M. phaseolina* showed that the highest per

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cent inhibition (81.11 %) was recorded in isolate Tri-12 (T. hamatum) and the lowest inhibition of 48.15 per cent was recorded in case of Tri-27 (T. viride) whereas isolate Tri-8 (T. virens) was most aggressive against R. bataticola and was able to inhibit 68.15 per cent of pathogen growth and isolate Tri-37 (T. virens) recorded lowest inhibition of 37.78 per cent. Dual culture assay against R. solani revealed that among all the Trichoderma isolates tested, Tri-12 (T. hamatum) was significantly superior over the other isolates and showed 82.59 per cent inhibition of growth of R. solani whereas the lowest inhibition recorded was 32.22 per cent by Tri-21. Isolate Tri-12 (T. hamatum) showed maximum per cent inhibition of mycelial growth (76.67 %) of S. rolfsii and isolate Tri-2 (T. piluliferum) had shown minimum per cent inhibition of mycelial growth (47.78 %). The results on per cent inhibition of mycelial growth of pathogens have been summarized in Table 1.

Although, isolates of *T. harzianum* have received considerable attention because of their potential use as a biological control agent, in recent years several other *Trichoderma* spp. are being isolated and screened for their antifungal activity (Cigdem and Merih, 2005). Several researchers (Mahato, 2005; Zafari *et al.*, 2008 and Reddy *et al.*, 2014) reported *T. virens* effective against major pathogens. The *Trichoderma* isolate Tri-2 (*T. piluliferum*) was highly efficient in inhibiting three pathogens *viz.*, against *M. phaseolina, R. solani* and *R. bataticola* but showed least inhibition against *S. rolfsii*. This strong variability in bioefficacy may be due to the wide variety of mechanisms used by *Trichoderma* to antagonize other fungi (Mendoza-Mendoza *et al.*, 2003 and Mukherjee *et al.*, 2003).

Table 1. In vitro evaluation of Trichoderma isolates ag	gainst major soil born	e pathogens usin	g dual culture method

Sl. No. Isolate	Isolate	e Identification	Per cent inhibition of mycelial growth over control			
			M. phaseolina	R. bataticola	R. solani	S. rolfsii
1.	Tri-1	T. viride	60.37 (50.99)	47.41 (43.51)	55.55 (48.19)	64.07 (53.17)
2.	Tri-2	T. piluliferum	75.93 (60.62)	55.19 (47.98)	75.55 (60.37)	47.78 (43.73)
3.	Tri-3	Trichoderma sp.	61.85 (51.86)	49.26 (44.58)	42.22 (40.52)	61.85 (51.86)
4.	Tri-4	T. viride	54.81 (47.76)	53.70 (47.12)	40.00 (39.23)	52.96 (46.70)
5.	Tri-5	T. harzianum	59.63 (50.55)	51.48 (45.85)	50.74 (45.42)	62.96 (52.51)
6.	Tri-6	Trichoderma sp.	62.22 (52.07)	52.22 (46.27)	39.63 (39.01)	59.26 (50.34)
7.	Tri-7	T. viride	53.33 (46.91)	39.26 (38.80)	34.44 (35.93)	54.81 (47.76)
8.	Tri-8	T. virens	61.48 (51.64)	68.15 (55.64)	81.11 (64.24)	69.63 (56.56)
9.	Tri-9	T. viride	72.22 (58.19)	57.04 (49.05)	80.00 (63.43)	67.04 (54.96)
10.	Tri-10	Trichoderma sp.	55.56 (48.19)	44.07 (41.60)	71.11 (57.49)	59.63 (50.55)
11.	Tri-11	T. virens	63.70 (52.95)	49.63 (44.79)	38.89 (38.58)	57.41 (49.26)
12.	Tri-12	T. hamatum	81.11 (64.24)	57.41 (49.26)	82.59 (65.34)	76.67 (61.12)
13.	Tri-13	T. virens	57.04 (49.05)	50.00 (45.00)	54.81 (47.76)	55.93 (48.40)
14.	Tri-14	Trichoderma sp.	60.00 (50.77)	48.52 (44.15)	40.74 (39.66)	58.52 (49.90)
15.	Tri-15	T. viride	62.59 (52.29)	47.41 (43.51)	47.40 (43.51)	57.04 (49.05)
16.	Tri-16	Trichoderma sp.	59.26 (50.34)	48.15 (43.94)	50.00 (45.00)	58.89 (50.12)
17.	Tri-17	Trichoderma sp.	62.96 (52.51)	52.59 (46.49)	55.92 (48.40)	63.70 (52.95)
18.	Tri-18	T. virens	59.63 (50.55)	51.85 (46.06)	39.25 (38.79)	55.93 (48.40)
19.	Tri-19	T. viride	61.85 (51.86)	49.63 (44.79)	40.00 (39.23)	60.74 (51.20)
20.	Tri-20	T. viride	57.78 (49.47)	54.07 (47.34)	43.33 (41.17)	55.19 (47.98)
21.	Tri-21	Trichoderma sp.	71.11 (57.49)	50.37 (45.21)	32.22 (34.58)	59.26 (50.34)
22.	Tri-22	Trichoderma sp.	58.15 (49.69)	51.85 (46.06)	35.55 (36.60)	57.04 (49.05)
23.	Tri-23	Trichoderma sp.	58.89 (50.12)	50.74 (45.42)	46.29 (42.87)	60.74 (51.20)
24.	Tri-24	Trichoderma sp.	66.67 (54.74)	47.04 (43.30)	44.07 (41.59)	67.41 (55.19)
25.	Tri-25	T. piluliferum	63.33 (52.73)	51.11 (45.64)	58.51 (49.90)	61.11 (51.42)
26.	Tri-26	T. virens	55.19 (47.98)	52.96 (46.70)	49.25 (44.57)	53.33 (46.91)
27.	Tri-27	T. viride	48.15 (43.94)	50.74 (45.42)	55.18 (47.97)	49.63 (44.79)
28.	Tri-28	Trichoderma sp.	60.74 (51.20)	49.26 (44.58)	44.43 (41.80)	58.15 (49.69)
29.	Tri-29	Trichoderma sp.	69.63 (56.56)	50.00 (45.00)	49.63 (44.79)	64.44 (53.40)

Sl. No.	Isolate	Identification	Per cent inhibition of mycelial growth over control			
			M. phaseolina	R. bataticola	R. solani	S. rolfsii
30.	Tri-30	Trichoderma sp.	68.52 (55.87)	54.07 (47.34)	38.88 (38.58)	65.93 (54.29)
31.	Tri-31	Trichoderma sp.	64.81 (53.62)	47.04 (43.30)	38.14 (38.14)	61.48 (51.64)
32.	Tri-32	Trichoderma sp.	70.00 (56.79)	57.41 (49.26)	40.74 (39.66)	69.63 (56.56)
33.	Tri-33	Trichoderma sp.	62.22 (52.07)	51.85 (46.06)	47.40 (43.51)	64.81 (53.62)
34.	Tri-34	Trichoderma sp.	57.41 (49.26)	48.15 (43.94)	35.55 (36.60)	65.93 (54.29)
35.	Tri-35	Trichoderma sp.	61.11 (51.42)	50.37 (45.21)	44.07 (41.59)	59.63 (50.55)
36.	Tri-36	Trichoderma sp.	50.37 (45.21)	53.70 (47.12)	73.33 (58.91)	72.96 (58.67)
37.	Tri-37	T. virens	56.67 (48.83)	37.78 (37.93)	50.73 (45.42)	58.15 (49.69)
39.	Tri-39	T. viride	60.37 (50.99)	44.44 (41.81)	49.25 (44.57)	54.81 (47.76)
ł0.	Tri-40	T. harzianum	61.11 (51.42)	47.04 (43.30)	50.74 (45.42)	55.93 (48.40)
41.	Tri-41	Trichoderma sp.	54.81 (47.76)	45.93 (42.66)	74.07 (59.39)	51.85 (46.06)
42.	Tri-42	Trichoderma sp.	60.00 (50.77)	38.89 (38.58)	53.33 (46.91)	59.26 (50.34)
13.	Tri-43	Trichoderma sp.	58.15 (49.69)	53.33 (46.91)	42.96 (40.95)	55.93 (48.40)
14.	Tri-44	Trichoderma sp.	54.07 (47.34)	55.19 (47.98)	41.11 (39.88)	54.07 (47.34)
45.	Control	-	-	-	-	-
S.Em±		-	00.86	00.88	00.23	00.80
CD (P=0	0.01)	-	03.21	03.27	00.89	02.99

Figures in parenthesis are arcsine transformed value

REFERENCES

- Asran-Amal A, Abd-Elsalam KA, Omar MR, Aly AA. 2005. Antagonistic potential of *Trichoderma* spp. against *Rhizoctonia solani* and use of M13 microsatellite-primed PCR to evaluate the antagonist genetic variation. *J Plant Dis Prot.* **112**: 550–561.
- Bosah O, Igeleke CA, Omorusi VI. 2010. *In vitro* microbial control of pathogenic *Sclerotium rolfsii*. *Int J Agr Biol*. **12**: 474–476.
- Cigdem K, Merih K. 2005. Effect of formulation on the viability of biocontrol agent, *Truchoderma harzianum* conidia. *Afr J Biotechnol.* **4**(5): 483–486.
- Kubicek CP. 2001. *Trichoderma*: from genes to biocontrol. *J Plant Pathol.* **83**: 11–23.
- Mahato U. 2005. Characterization of native isolates of *Trichoderma* spp. and cloning of endochitinase gene. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Dharwad, 27pp.
- Mendoza-Mendoza A, Pozo MJ, Grzegorski D, Martinez P, Garcia JM, Olmedo-Monfil V, Cortes C, Kenerley C, Herrera-Estrella A. 2003. Enhanced biocontrol activity of *Trichoderma* through inactivation of a mitogen-activated protein kinase. *Proc Natl Acad Sci.* **100**: 15965–15970.

- Mukherjee PK, Latha J, Hadar R, Horwitz BA. 2003. TmkA, a mitogen-activated protein kinase of *Trichoderma virens*, is involved in biocontrol properties and repression of conidiation in the dark. *Eukaryot Cell* **2**: 446–455.
- Reddy BN, Saritha KV, Hindumathi A. 2014. In vitro screening for antagonistic potential of seven species of Trichoderma against different plant pathogenic fungi. Res J Biol. 2: 29–36.
- Sivan A, Chet I. 1989. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. *Phytopathol* **79**: 198–203.
- Sreedevi B, Devi CM, Saigopal DVR. 2011. Isolation and screening of effective *Trichoderma* spp. against the root rot pathogen *Macrophomina phaseolina*. J Agr Technol. 7: 623–635.
- Yedidia I, Chet I. 2001. Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant and Soil.* **235**: 235–242.
- Zafari D, Mehdi MK, Eidi B. 2008. Biocontrol evaluation of wheat take-all disease by *Trichoderma* screened isolates. *Afr J Biotechnol.* **7**: 3653–3659.