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ISOLATION AND EVALUATION OF ANTAGONISTIC POTENTIAL OF INDIGENOUS *TRICHODERMA* ISOLATES AGAINST SOIL BORNE PATHOGENS CAUSING RHIZOME ROT IN TURMERIC (*Curcuma longa* L.) AND GINGER (*Zingiber officinale* Rosc)

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ABSTRACT
Total of twenty five indigenous isolates of *Trichoderma* species were isolated from rhizosphere soils of turmeric and ginger grown in different locations of Belagavi and Bagalkot districts, Karnataka (India) using serial dilution pour plate method. These isolates were ascribed to two species namely *Trichoderma harzianum* (Th) and *Trichoderma viride* (Tv) based on cultural and morphological characterisation. The isolates were evaluated against major soil borne pathogens causing rhizome rot in turmeric and ginger *viz*., *Pythium, Fusarium, Rhizoctonia* and *Sclerotium* sp., by dual culture method. Isolate Tv-23 was recorded highest inhibition of *Pythium* (83.19 %). Isolate Tv-17 had shown maximum per cent inhibition of mycelia growth of *Fusarium* (90.92 %) and *Rhizoctonia* (89.56 %). Isolate Th-25 was most aggressive and able to inhibit 79.04 per cent of *Sclerotium* growth. Based on the antagonistic activity of all *Trichoderma* isolates tested against soil borne pathogens, eight isolates *viz*., Tv-1, Tv-2, Tv-8, Tv-14, Tv-17, Tv-23Th-18 and Th-25 were found to show highest per cent inhibition.

INTRODUCTION

Turmeric and ginger are the two major spices cultivated in India from ancient times. The crops are very succulent and their rhizomes succumb easily to different soil-borne diseases during cultivation such as rhizome rot, yellows and wilt. Among these, rhizome rot is the most destructive one which occurs in several parts of India wherever the crops are grown and causes economic damage. The disease is caused predominantly by *Pythium aphanidermatum*. But *Fusarium Rhizoctonia* and *Sclerotium* are the other organisms which are frequently associated with the infected rhizomes (Anoop *et al*., 2014) and causes huge crop loss up to 70% in a cropping season (Dohroo 2005). Many fungicides are reported to manage the disease (Sreeramansetty *et al*., 1996) but indiscriminate use and undesirable side effects of fungicides have increased the significance of alternative disease management methods like biological control. Recently, the application of biological control agents (BCAs) in agriculture has gained popularity as a way to reduce or eliminate the use of synthetic pesticides (Vinale *et al*., 2007). Biological control appears to be the best strategy for the long term sustainability and effective management of soil borne diseases. Among the BCAs, *Trichoderma* dominates the literature as successful antagonists and have gained wide acceptance as effective biocontrol agent against several soil borne plant pathogens due to their ability to successfully antagonize other fungi (Joshi *et al*., 2010; Hermosa *et al*., 2012).

Trichoderma species have evolved numerous mechanisms that are involved in enhancing plant growth and combating plant pathogens. These mechanisms include mycoparasitism, production of inhibitory compounds, competition for space and nutrients, inactivation of the pathogen's enzymes and induced resistance (Kapulnik and Chet, 2000; Roco and Perez, 2001; Srivastava, 2015).

With this background, the present study was conducted to identify the effective indigenous *Trichoderma* strains for the management of soil borne pathogens affecting turmeric and ginger. The main objective is to evaluate the antagonistic potential of indigenous isolates against major soil borne pathogens affecting turmeric and ginger.

MATERIALS AND METHODS

Soil sampling

Rhizospheric soil samples were collected from turmeric and ginger growing areas of Belagavi (Gokak & Raybag Taluks) and Bagalkot (Mudhol & Jamakhandi Taluks) districts of Karnataka. For rhizospheric soil, plants were gently uprooted, soil tightly adhering the roots was collected, mixed and composite mixture of soil of the region was obtained. The soil

samples were collected in plastic bags and labelled with information of collection sites and origin of samples. Then, the samples were transported to the laboratory and processed within 24 hours.

Isolation and Identification of *Trichoderma* sp.

Collected rhizosphere soil samples were air dried for 4 h and used for the isolation of *Trichoderma* spp. by dilution plate technique using *Trichoderma* Selective Medium (TSM) (Elad and Chet, 1983). Serial dilution was done by pour plate method. One gram of the air dried soil was taken in 9 ml of sterile distilled water and serially diluted. Serial dilution up to 10^{-4} was made and 1 ml each of this dilution was poured in to petri dishes and 20 ml of TSM was added. The plates were incubated at 26 ± 1^{o}C for 7 days. Observation on the appearance of the colonies was recorded from 3rd to 7th day. Individual colonies were picked up and maintained in pure culture for further study. *Trichoderma* species were identified and examined under compound microscope on the basis of their cultural and morphological character (Rifai, 1969) and were maintained on PDA slants at 4^{o}C for subsequent studies.

Isolation and identification of fungal pathogens associated with rhizome rot disease

The infected samples collected during soil sampling were used for isolation. Isolation has done according to the procedure followed by Anoop *et al*., 2014. The infected parts were washed thoroughly with tap water to remove the adhered soil. Small bits cut from the diseased portions along with some healthy portions were surface sterilized with 1% Sodium hypo chloride (NaOCl) for 30 seconds and then washed in three changes of sterile distilled water each of 30 seconds and transferred onto Potato Dextrose Agar (PDA) in 90 mm petri plate. The hyphal tips growing out from the tissue were excised and transferred onto Petri plate containing PDA for further growth and identification. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture or single spore isolation under aseptic conditions. The cultures were grown on PDA slants at 28 ± 1^{0}C and preserved in a refrigerator at 4^{0} C and used for further studies.

Fungal pathogens associated with rhizome rot samples were identified as *viz*., *Pythium, Fusarium, Rhizoctonia* and *Sclerotium* based on morphological and other characters.

***In vitro* evaluation of antagonistic potential of indigenous *Trichoderma* isolates**

The antagonistic potential of the *Trichoderma* isolates against soil borne pathogens *viz*., *Pythium*, *Fusarium*, *Rhizoctonia* and *Sclerotium* sp. was tested *in vitro* by dual culture technique (Morton and Stroube, 1955). Five mm disc cut from the actively growing margins of 72 hrs old culture was placed at the margin of the 90 mm petri plates containing 20 ml Potato Dextrose Agar (PDA). Disc of 5 mm size of 72 hrs old culture of test pathogen was placed opposite to the antagonist. The plates were incubated at 26 ± 1^{o}C for seven days. Each treatment was replicated thrice. A petri plate inoculated with pathogen alone served as the control. Periodical observations on the growth of biocontrol agents and their ability to colonize the pathogen were recorded. Per cent inhibition of mycelial growth of pathogen was calculated by using following formula

$$PI = \frac{C - T}{C} X\,100$$

PI = Percentage inhibition

C = Radial growth of the pathogen in control plate (cm)

T = Radial growth of the pathogen in dual culture (cm)

Statistical analysis

The data obtained in the experiment was statistically analysed by using completely randomized design (CRD). The data pertaining to percentages were arc sin transformed using Web Agri. Stat. Package 2 developed by ICAR research complex, Goa. The significance of effect of *Trichoderma* on growth characteristics was determined by the magnitude of the F value ($P = 0.01$). Results of the experiment was analysed following appropriate statistical methods as per the procedure suggested by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Isolation of indigenous *Trichoderma* sp.

Soil samples were collected from major turmeric and ginger growing areas of Belagavi (Gokak, Raybag) and Bagalkot

Figure 1: Antagonistic activity of *Trichoderma* isolate (Tv-23) against *Pythium* in dual culture technique

Figure 2: Antagonistic activity of *Trichoderma* isolate (Tv-17) against *Fusarium* in dual culture technique

Figure 3a: Antagonistic activity of *Trichoderma* isolate (Tv-2) against *Rhizoctonia* in dual culture technique

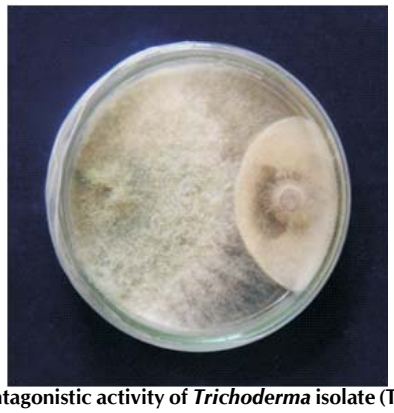

Figure 3b: Antagonistic activity of *Trichoderma* isolate (Tv-17) against *Rhizoctonia* in dual culture technique

Table 1: Details of *Trichoderma* isolates collected from different locations

Sl. No.	District	Taluk	Location	Crop	Isolate No.
1	Belagavi	Gokak	Sangankere	Turmeric	Tv-1
2			KRCCH, Arabhavi	Turmeric	Tv-2
3			Kalloli	Ginger	Th-7
4			Sangankere	Ginger	Tv-8
5			Hallur	Turmeric	Th-12
6			Gurlapur	Ginger	Th-13
7			Tukanatti	Turmeric	Tv-22
8			Naganur	Turmeric	Tv-23
9		Raybag	Kaladal	Turmeric	Th-10
10			Itnal	Ginger	Tv-11
11			Kankanwadi	Turmeric	Tv-14
12			Kaladal	Ginger	Th-16
13			Devapur	Ginger	Tv-17
14			Halagawadi	Ginger	Tv-20
15			Hastuwada	Turmeric	Th-21
16	Bagalkot	Mudhol	Shirol	Turmeric	Tv-5
17			Mareguddhi	Turmeric	Tv-6
18			Siddapur	Turmeric	Tv-15
19			Mallapur	Turmeric	Th-24
20			Mudhol	Turmeric	Th-25
21		Jamakhandi	Rabakavi	Ginger	Th-3
22			Sasalatti	Turmeric	Tv-4
23			Bandigani	Ginger	Th-9
24			Madarakandi	Turmeric	Th-18
25			Aasangi	Turmeric	Th-19

(Mudhol, Jamakhandi) districts, Karnataka and were used for isolation of *Trichoderma* sp. Details of the *Trichoderma* isolates collected from different locations were presented in Table 1.

A total of 25 *Trichoderma* isolates were collected from different rhizosphere soils. Among them, eight isolates *viz*., Tv-1, Tv-2, Th-7, Tv-8, Th-12, Th-13, Tv-22 and Tv-23 were collected from Gokak taluk, seven isolates *viz*., Th-10, Tv-11, Tv-14, Th-16, Tv-17, Tv-20 and Th-21 from Raybag taluk while five isolates namely Tv-5, Tv-6, Tv-15, Th-24 and Th-25 from Mudhol taluk and five isolates *viz*., Th-3, Tv-4, Th-9, Th-18 and Th19 from Jamakhandi taluk. A total of 25 indigenous *Trichoderma* isolates have shown in the Plate 2.

Species identification

Twenty five *Trichoderma* isolates were identified according to the identification key (Rifai 1969) based on cultural (linear growth, growth patterns, colour of colony etc.) and morphological (Structure, shape and arrangement of conidiophore, phialide and conidia) characters. These isolates were identified into two species, *viz*., *T. harzianum* (12 isolates) and 13 isolates of *T. viride* (Table 2).

***In vitro* evaluation of antagonistic potential of *Trichoderma* isolates against major soil borne pathogens**

Total of 25 *Trichoderma* isolates were studied for their antagonistic activity against four major soil borne pathogens *viz*., *Pythium*, *Fusarium*, *Rhizoctonia* and *Sclerotium* sp. in order to identify the potential isolates (Table 3).

Antagonistic effect of the isolates on *Pythium* sp. revealed that per cent inhibition of the pathogen was ranged from 32.89 to 83.19 per cent. The results indicated that among all the *Trichoderma* isolates tested, isolate Tv-23 was significantly

Table 2: Identification of indigenous *Trichoderma* isolates

Sl. No.	*Trichoderma* species	*Trichoderma* isolates
1	*T. harzianum*	Th-3, Th-7, Th-9, Th-10, Th-12, Th-13, Th-16, Th-18, Th-19, Th-21, Th-24 and Th-25
2	*T. viride*	Tv-1, Tv-2, Tv-4, Tv-5, Tv-6, Tv-8, Tv-11, Tv-14, Tv-15, Tv-17,Tv-20, Tv-22 and Tv-23

Table 3: *In vitro* evaluation of *Trichoderma* isolates against soil borne pathogens using dual culture method

Sl. No.	Isolate No.	Percent inhibition of mycelial growth over control			
		Pythium sp.	*Fusarium* sp.	*Rhizoctonia* sp.	*Sclerotium* sp.
1	Tv-1	65.78*(54.23)**	84.00(66.42)	84.37(66.71)	65.78(54.19)
2	Tv-2	60.37(50.98)	77.00(61.35)	89.70(71.30)	73.78(59.20)
3	Th-3	49.04(44.44)	45.83(42.60)	67.56(55.28)	40.67(39.60)
4	Tv-4	49.11(44.49)	47.50(43.56)	67.93(55.50)	36.81(37.35)
5	Tv-5	56.89(48.95)	55.33(48.06)	56.89(48.96)	63.41(52.78)
6	Tv-6	40.15(39.31)	51.00(45.57)	66.52(54.65)	54.52(47.59)
7	Th-7	52.30(46.31)	57.25(49.16)	47.93(43.80)	53.48(46.99)
8	Tv-8	83.19(65.80)	77.25(61.52)	69.04(56.20)	69.33(56.37)
9	Th-9	48.74(44.27)	54.58(47.63)	42.00(40.39)	59.04(50.20)
10	Th-10	32.89(34.98)	43.83(41.45)	48.22(43.97)	37.78(37.88)
11	Tv-11	56.00(48.44)	61.67(51.75)	40.15(39.31)	55.93(48.40)
12	Th-12	42.07(40.44)	59.33(50.38)	51.48(45.84)	48.81(44.31)
13	Th-13	35.56(36.59)	46.75(43.13)	52.22(46.27)	40.00(39.20)
14	Tv-14	48.22(43.98)	83.17(65.77)	67.33(55.14)	55.93(48.40)
15	Tv-15	47.41(43.50)	62.08(51.99)	55.11(47.93)	49.11(44.48)
16	Th-16	44.89(42.06)	56.58(48.78)	64.22(53.26)	50.22(45.12)
17	Tv-17	50.67(45.38)	90.92(72.46)	89.56(71.19)	64.89(53.66)
18	Th-18	68.59(55.91)	58.08(49.65)	64.22(53.26)	59.19(50.29)
19	Th-19	62.30(52.11)	55.08(47.91)	54.30(47.46)	46.81(43.17)
20	Tv-20	54.00(47.29)	54.75(47.72)	48.89(44.36)	45.48(42.40)
21	Th-21	56.67(48.83)	58.08(49.65)	52.30(46.31)	58.30(49.78)
22	Tv-22	48.22(43.98)	63.92(53.08)	52.81(46.61)	50.89(45.51)
23	Tv-23	62.52(52.25)	71.67(57.84)	53.85(47.20)	73.93(59.29)
24	Th-24	58.52(49.90)	57.17(49.12)	56.44(48.70)	61.48(51.72)
25	Th-25	66.52(54.64)	64.00(53.23)	41.78(40.26)	79.04(62.76)
26	Control	0.00(0.32)	0.00(0.32)	0.00(0.32)	0.00(0.32)
S. Em ±		1.14	1.40	1.07	1.70
CD(0.01)		4.31	5.31	4.04	6.44

* Original values; **Figures in parenthesis are arcsine transformed values

Figure 4: Antagonistic activity of *Trichoderma* isolate (Th-25) against *Sclerotium* in dual culture technique

superior and showed 83.19 per cent inhibition followed by isolates Th-18 (68.59%), Th-25 (66.52%) and Tv-1 (65.78%) which were on par with each other. (Table 3, Figure 2).

The results were supported by Singh *et al*. (2014) who evaluated the antagonistic efficacy of four isolates of *Trichoderma* sp. against *Pythium aphanidermatum*. Among them, *T. harzianum* was recorded maximum growth inhibition (60.38%).

Trichoderma isolates tested against *Fusarium* sp., isolate Tv-17 recorded maximum per cent inhibition of mycelial growth (90.92 %) followed by isolates Tv-1 (84.00%) and Tv-14 (83.17%) which showed on par results. (Table 3, Figure 2). The results were in accordance with the findings of Sundaramoorthy and Balabaskar (2013). Similarly Javaid *et al*. (2014) evaluated antagonistic behaviour of *Trichoderma* sp. against *F. oxysporum* which exhibited reduction of radial growth of *F. oxysporum* (59-74%).

Antagonistic activity of *Trichoderma* isolates were evaluated against *Rhizoctonia* sp. and inhibition percentage was ranged from 40.15 to 89.70 per cent. Highest per cent inhibition was recorded by isolates Tv-2 (89.70%) and Tv-17 (89.56%) which were on par with each other followed by isolate Tv-1 (84.37%) (Table 3, Figure 4a and 4b). Similar results were observed by Gaikwad and Nimbalkar (2003) and Kumar *et al*. (2008) who reported that *T. viride* showed inhibitory effect under *in vitro* condition against *R. solani*.

The data on per cent inhibition of *Sclerotium* sp. was indicated that isolates Th-25 (79.04%), Tv-23 (73.93%) and Tv-2 (73.78%) had shown maximum per cent inhibition of

pathogen. It was followed by isolates and which showed on par results whereas lowest per cent inhibition (36.81 %) was recorded by isolate Th-10 (Table 3, Figure 5). The potentiality of *Trichoderma* sp. against *S. rolfsii* was also reported by Kumar *et al*. (2012), Lopez *et al*. (2015) and Sangle *et al*. (2012).

Based on the antagonistic activity of all *Trichoderma* isolates tested against soil borne pathogens, eight isolates were found to show highest per cent inhibition and were listed below

Sl. No.	*Trichoderma* species	*Trichoderma* isolates
1	*Trichoderma harzianum*	Th-18 and Th-25
2	*Trichoderma viride*	Tv-1, Tv-2, Tv-8, Tv-14, Tv-17 and Tv-23

In the present investigation, the antagonistic activity of indigenous isolates varied among the isolates. i.e., one particular isolate was not always effective against all the pathogens. Isolate Th-25 was highly efficient in inhibiting the pathogens *Pythium* and *Sclerotium* but poor in the inhibition of *Rhizoctonia*. Similar observations were also made by Patil (2011) and reason could be due to the wide variety of mechanisms used by *Trichoderma* to antagonize other fungi as reported by Mendoza *et al*. (2003) and Mukherjee *et al*. (2003).

It was also observed that, the antagonism of different isolates that belong to the same species varied. Isolates Tv-23 and Tv-6 which were both identified as *T. viride* showed big difference in their inhibition percentage against *Pythium* (83.19 and 40.15% respectively). Similarly, in the isolates Th-25 and Th-10 which were identified as *T. harzianum* showed difference in their inhibition percentage against *Sclerotium* (79.04 and 36.81% respectively). Results were in accordance with Anees *et al*. (2010) and who quoted a reason as different isolates act by different biocontrol mechanisms.

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