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Article · January 2014

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Print ISSN 2277 - 3282

Journal of Science **Spices**

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EVALUATION OF ANTAGONISTIC POTENTIAL OF INDIGENOUS *TRICHODERMA* ISOLATES AGAINST *PYTHIUM APHANIDERMATUM* (EDSON) FITZ. CAUSING RHIZOME ROT IN TURMERIC (*CURCUMA LONGA* L.)

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ABSTRACT

Twenty two isolates of *Trichoderma* spp. isolated from the turmeric rhizosphere soil collected from major turmeric growing tracts of South India were evaluated for their antagonistic potential against *Pythium aphanidermatum* (Edson) Fitz. causing rhizome rot of turmeric (*Curcuma longa* L.). The isolates were evaluated based on the dual plating, growth rate and volatile and non-volatile metabolites production *in vitro*. Out of twenty two isolates, six isolates via, IISR CLT 102, IISR CLT 107, IISR CLT 110, IISR CLT 114, IISR CLT 118, and IISR CLT 121 showed mycelial inhibition of above 70% on dual plate assay. The isolates showed variability in the production of volatile and non volatile metabolites. The highest inhibition by volatile metabolites was shown by IISR CLT 118 (84.82%) and IISR CLT 121(82.22%). The non-volatile metabolites produced by IISR CLT 103 (37.78%), IISR CLT 107(38.52), IISR CLT 110 (46.30%) and IISR CLT 114 (42.22%) were comparatively effective against the pathogen. IISR CLT 114 from Bidar (Karnataka) and IISR CLT 102 from Vaithiri (Kerala) induced cytoplasmic coagulation of the pathogen on dual culture technique. A total of six isolates were shortlisted for further evaluation in pot culture and field evaluation against rhizome rot of turmeric considering their above listed potentialities.

Keywords: *Trichoderma*, *Pythium aphanidermatum*, Rhizome rot, Turmeric.

INTRODUCTION

In turmeric, rhizome rot is the most destructive disease that causes economic damage to the crop. The disease survey conducted in various turmeric growing tracts of South India revealed that the disease is caused predominantly by *Pythium aphanidermatum* (Edson.) Fitz.[1]. Fungicides like metalaxyl and various organic amendments are reported to manage the disease [2]. The indiscriminate use and undesirable side effects of fungicides have increased the significance of alternative disease management methods like biological control. Biological control appears to be the best strategy for the long term sustainability and effective management of soil borne diseases. *Trichoderma* strains have long been recognized as biological agents, for the control of plant diseases and also for their ability to increase root growth, crop productivity and uptake of nutrients. The most common targeted pathogens are the species of *Pythium*, *Fusarium*, *Phytophthora* and *Rhizoctonia* reflecting its worldwide importance. The basic mechanisms responsible for their ability to control pathogens include antibiosis, mycoparasitism and competition for nutrients. Several species of *Trichoderma* have been reported for their ability to produce antifungal metabolites [3] and lytic enzymes [4]. There are numerous reports of compounds derived from *Trichoderma* species with volatile and non volatile activities. The synergistic effect of many compounds and enzymes appears to be significant in the management of the disease [3]. Many species of this genus can be characterized as opportunistic avirulent plant symbionts with high resistant to a range of toxicants

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[5, 6] and also have the ability to promote growth and induce resistance in plants [7]. *Trichoderma* spp. are known to be the most commonly used antagonists against *P. aphanidermatum*[8-10]. Since the indigenous isolates are more adaptable to the rhizosphere soil, an attempt was made to evaluate the antagonistic potential of indigenous *Trichoderma* isolates from rhizosphere soil of major turmeric growing tracts of South India, against *P. aphanidermatum*, the predominant species causing rhizome rot in turmeric.

MATERIALS AND METHODS

Isolation of *Trichoderma* from turmeric rhizosphere

The rhizosphere soil samples were collected from major healthy turmeric growing tracts of South India during the survey conducted in 2005-07 seasons and was used for the isolation of *Trichoderma* spp. by dilution plate technique using *Trichoderma* Selective Medium (TSM) [11]. 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 and1 ml each of this dilution was poured in to petri dishes and 20 ml of TSM was added. The plates were incubated at 24±1ºC. The isolates of *Trichoderma* obtained were purified and maintained on PDA slants at 4^{o}C for further studies.

Dual culture

The potential of *Trichoderma* spp. was evaluated *in vitro* using dual culture technique [12]. 5 mm disc cut from the actively growing margins of 72h old culture was placed at the margin of the 90mm petri plates containing 20 ml Potato Dextrose Agar (PDA). Disc of 5 mm size of 72h old culture of *P. aphanidermatum* was placed opposite to the antagonist. The plates were incubated at 25^{o}C for five days. Each treatment was replicated thrice. A petri plate inoculated with pathogen alone served as the control. The plates were observed regularly for their action over pathogen. Antagonistic activity was scored using Bell's Scale [13]. The percentage inhibition was calculated by the formula

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

PI = Percentage inhibition

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

T= Radial growth of the pathogen in Dual culture (mm)

Effect of non-volatile metabolites

The activity of non volatile metabolites of *Trichoderma* isolates was studied using the culture filtrate of the antagonist [14]. Mycelial discs of 5 mm size from 96h old culture of *Trichoderma* isolates were grown in Roux Bottles containing 150 ml Potato Dextrose Broth(PDB) and incubated at 25^{o}C for 10 days. The culture medium was filtered using Whatman No.1 filter paper after removing the mycelial mats. The filtrate was centrifuged at 9000 rpm for 10 min. The supernatant was filter sterilized using Millipore membrane filter paper (0.22µm). The filtrate at a concentration of 10% was added to Potato Dextrose Agar (PDA) before pouring in to petri dishes. Mycelial discs of the pathogen were inoculated at the centre of the petri plates. The plates were incubated at 25^{o}C for five days. The pathogen alone inoculated on to PDA was kept as control. The radial growth was recorded and the percentage of inhibition was calculated as above.

Effect of volatile metabolites

The effect of volatile metabolites was studied by the method followed by Dennis and Webster [14]. The antagonists were grown by inoculating centrally on 90mm petri dishes containing 20 ml PDA and incubated for 5 days at room temperature. The upper lid of the each petri plate was removed and the lower lid was sealed with lower lid of another petri plate containing PDA inoculated with the mycelial disc of pathogen. Petri plates with pathogen inverted over the plate containing only PDA served as control. The pair of each plate was sealed with adhesive tape and incubated at 25^{o}C for 5 days. The radial growth was recorded and the percentage of inhibition was calculated as above.

RESULTS

Isolation of *Trichoderma* spp. from turmeric rhizosphere

A total of 22 isolates of *Trichoderma* were isolated including four isolates from Andhra Pradesh, four from Karnataka, six from Kerala and eight from Tamil Nadu representing nineteen locations of South India (Table 1).

Dual culture assay

Trichoderma spp. isolated from rhizosphere soil were used to evaluate their antagonism against *P. aphanidermatum* by dual culture technique and showed variation in their antagonistic potential. The isolates showed percentage of inhibition (PI) ranging from 32.37 – 72.15(Table 2). The highest PI was shown by the isolate IISR CLT 107 (72.15) collected from Kunjanapalle, Guntur district of Andhra Pradesh followed by IISR CLT 102 (71.78) from Vaithiri, Wayanad district of Kerala. Isolate IISR CLT 115 showed comparatively slow growth. Six isolates *viz*., IISR CLT 102, IISR CLT 107, IISR CLT 110, IISR CLT 114, IISR CLT 118 and IISR CLT 121 showed more than 70% inhibition. These isolates also showed fast growth rate in comparison with others showing their potential to overgrow the pathogen. All isolates showed 100% overgrowth on the mycelium of pathogen within 7 days of incubation. The growth of the antagonist over pathogen was scored using modified

Bell's Scale[13] (Table 3). IISR CLT 102, IISR CLT 107, IISR CLT 110, IISR CLT 114 showed high growth rate with a rating scale of S1 i.e., 100% growth within 72 h. IISR CLT 118 and IISR CLT 121 also showed fast growth and scored S1 after 96 h of incubation. All other isolates took more than 96 h to grow fully inside the petri dishes.

The microscopic examination of the meeting point of the pathogen and antagonist showed the cytoplasmic coagulation of *P. aphanidermatum* against the isolates IISR CLT 114, IISR CLT 110 and IISR CLT 102. The isolate IISR CLT 110 also showed a yellowish brown pigmentation at the reverse of the petri dish and at the point of meeting the pathogen. The cytoplasmic coagulation was also noticed in IISR CLT 110 and IISR CLT 102 (Fig.1 a & b). These isolates also showed hyphal evacuation and deformation during dual culture (Fig.1 c & e). Variations in the size of hyphae of the pathogen was also noticed in some parts of the petri dishes (Fig.1 f)

Effect of Non Volatile Metabolites

The *Trichoderma* isolates showed variations in the PI of non volatile metabolites ranging from 0.00 – 46.30% (Table 2). The highest inhibition was shown by IISR CLT 110 isolated from Chittoor of Palakkad district, Kerala followed by IISR CLT 114 (42.22%) isolated from Bidar district, Karnataka. The highest inhibitions showed by other isolates were IISR CLT 103(37.78%), IISR CLT 107(38.52%), and IISR CLT 118 (34.44%) (Table 2). However some isolates reduced the profuse growth of the pathogen in comparison with its control showing the efficacy of metabolites to reduce the growth of *P. aphanidermatum*. Malformation of the pathogen mycelium was also noted in plates of IISR CLT 110 (Fig 1d). The size of the mycelium was found to be reduced in the plates of IISR CLT 103, IISR CLT 107, and IISR CLT 118 in comparison with control. IISR CLT 109, IISR CLT 115, IISR CLT 116, IISR CLT 120 and IISR CLT 122 showed no inhibition against the pathogen. The aerial mycelia of pathogen in these plates showed no variation with the control. IISR CLT 102 and IISR CLT 121 that showed high percentage inhibition on dual plating were found to be less effective by this method.

Effect of Volatile Metabolites

All the isolates showed variation in the production of volatile metabolites and PI ranged from 0.00 – 84.82 (Table 2). The highest inhibition was shown by the isolate IISR CLT 118 (84.82%) collected from Settiputhoor, Coimbatore District, Tamil Nadu followed by IISR CLT 121 (82.22%) from Chincholi, Gulbarga District, Karnataka. The growth of the mycelia was arrested in the plates with higher PI after 24h of incubation. These isolates were comparatively fast growing and showed high inhibition in dual plating. IISR CLT 102 also produced volatile metabolites that inhibited 60% growth of the pathogen. IISR CLT 103, IISR CLT 108, IISR CLT 116, IISR CLT 117 and IISR CLT 122 showed no inhibition against the pathogen (Table 2). All other isolates showed PI of 4.81 - 49.63 with reduced growth of aerial mycelia.

The overall results showed that IISR CLT 102, IISR CLT 118 and IISR CLT 121 are capable of producing volatile metabolites that can check the growth of *P. aphanidermatum*. The overall results shows that the six isolates *viz*., IISR CLT 102, IISR CLT 107, IISR CLT 110, IISR CLT 114, IISR CLT 118 and IISR CLT 121 are promising isolates of *Trichoderma* and can be used for their further evaluation under pot culture and field conditions.

Table 1. Details of *Trichoderma* isolates collected

Sl No.	Isolate No.	Location		Name of the isolate	State
1	IISR CLT 101	Calicut	75° 49'E 11° 36'N	*T. harzianum*	Kerala
2	IISR CLT 102	Vaithiri	76° 02'E 11° 32'N	*T. harzianum*	Kerala
3	IISR CLT 103	Vaithiri	76° 02'E 11° 32'N	*Trichoderma sp.*	Kerala
4	IISR CLT 104	Kodumudi	75° 51'E 11° 04'N	*T. harzianum*	Tamil Nadu
5	IISR CLT 105	Thondamuthoor	76° 50'E 10° 58'N	*Trichoderma sp.*	Tamil Nadu
6	IISR CLT 106	Thondamuthoor	76° 50'E 10° 58'N	*Trichoderma sp.*	Tamil Nadu
7	IISR CLT 107	Kunjanapalle	80° 37'E 16° 27'N	*T. harzianum*	Andhra Pradesh
8	IISR CLT 108	Vallabapuram	80° 43'E 16° 21'N	*T. viride*	Andhra Pradesh
9	IISR CLT 109	Cheraooru	80° 16'E 16° 19'N	*Trichoderma sp.*	Andhra Pradesh
10	IISR CLT 110	Chittoor	76° 44'E 10° 42'N	*T. longibrachiatum*	Kerala
11	IISR CLT 111	Kalpetta	76° 10'E 11° 36'N	*Trichoderma sp.*	Kerala
12	IISR CLT 112	Nathamkuni	76° 10'E 11° 36'N	*T. harzianum*	Kerala
13	IISR CLT 113	Jagityal	78° 55'E 18° 47'N	*Trichoderma sp.*	Andhra Pradesh
14	IISR CLT 114	Bidar	77° 31'E 17° 54'N	*Trichoderma sp.*	Karnataka
15	IISR CLT 115	Annur	77° 06'E 11° 03'N	*T. harzianum*	Tamil Nadu
16	IISR CLT 116	Kolathoor	77° 44'E 11° 51'N	*Trichoderma sp.*	Tamil Nadu
17	IISR CLT 117	Mettoor	77° 48'E 11° 47'N	*T. harzianum*	Tamil Nadu

18	IISR CLT 118	Settiputhoor	77° 06'E 11° 03'N	*Trichoderma sp.*	Tamil Nadu
19	IISR CLT 119	Settiputhoor	77° 06'E 11° 03'N	*T. harzianum*	Tamil Nadu
20	IISR CLT 120	Ainoli	77° 44'E 17° 49'N	*Trichoderma sp.*	Karnataka
21	IISR CLT 121	Chincholi	77° 25'E 17° 28'N	*Trichoderma sp.*	Karnataka
22	IISR CLT 122	Bidar	77° 31'E 17° 54'N	*T. harzianum*	Karnataka

Table 2. Evaluation of the antagonistic effect of *Trichoderma* against *P. aphanidermatum* (*in vitro*)

Isolate No.	Inhibition (%)		
	Dual	**Volatiles**	**Non volatiles**
IISR CLT 101	47.78 * (43.68)**	27.41 (31.56)	12.22 (20.44)
IISR CLT 102	**71.78 (57.86)**	**60.00 (50.77)**	21.11 (27.35)
IISR CLT 103	45.33 (42.3)	0.00 (0.00)	**37.78** (37.88)
IISR CLT 104	43.56 (41.27)	5.74 (13.81)	16.67 (24.04)
IISR CLT 105	59.11 (50.18)	17.41 (24.65)	21.11 (27.35)
IISR CLT 106	54.89 (47.75)	4.81 (12.66)	5.56 (13.56)
IISR CLT 107	**72.15 (58.12)**	41.11 (39.87)	**38.52** (38.35)
IISR CLT 108	49.27 (44.14)	0.00 (0.00)	18.14 (25.18)
IISR CLT 109	38.89 (38.53)	5.56 (13.56)	0.00 (0.00)
IISR CLT 110	**71.26 (57.48)**	49.63 (44.77)	**46.30 (42.88)**
IISR CLT 111	45.93 (42.65)	24.04 (29.33)	24.07 (29.33)
IISR CLT 112	38.44 (38.29)	13.33 (21.39)	27.78 (31.76)
IISR CLT 113	44.00 (41.55)	10.74 (19.09)	29.63 (32.96)
IISR CLT 114	**70.98 (57.35)**	38.15 (38.12)	**42.22 (40.51)**
IISR CLT 115	32.37 (34.63)	29.63 (32.96)	0.00 (0.00)
IISR CLT 116	35.64 (36.63)	0.00 (0.00)	0.00 (0.00)
IISR CLT 117	40.00 (39.23)	0.00 (0.00)	25.56 (31.33)
IISR CLT 118	**70.37 (57.04)**	**84.82 (67.05)**	**34.44 (35.97)**
IISR CLT 119	45.93 (42.65)	12.22 (20.44)	12.22 (20.44)
IISR CLT 120	47.04 (43.28)	8.89 (17.6)	0.00 (0.00)
IISR CLT 121	**70.37 (56.98)**	**82.22 (65.05)**	20.00 (26.56)
IISR CLT 122	46.96 (43.22)	0.00 (0.00)	0.00 (0.00)
LSD	**0.053**	**0.120**	**0.246**

*Transformed arc sine values **Original values in parantheses

Table 3.**Ratings of selected isolates of *Trichoderma* spp. on the inhibition of *P. phanidermatum* (Bell's Scale, 1981)**

Isolate No.	Days			
	72h	**96 h**	**120 h**	**144 h**
IISR CLT 101	S3	S3-S2	S2	**S1**
IISR CLT 102	**S1**			
IISR CLT 103	S3	S2	**S1**	
IISR CLT 104	S2	S2-S1	**S1**	
IISR CLT 105	S3	S3-S2	S2-S1	**S1**
IISR CLT 106	S3	S3-S2	S2-S1	**S1**
IISR CLT 107	**S1**			
IISR CLT 108	S3	S3-S2	S2	**S1**
IISR CLT 109	S3	S3-S2	S2	**S1**
IISR CLT 110	**S1**			
IISR CLT 111	S3	S3-S2	**S1**	
IISR CLT 112	S3	S3-S2	S2-S1	**S1**
IISR CLT 113	S2	S2-S1	**S1**	
IISR CLT 114	**S1**			
IISR CLT 115	S3	S3-S2	S2	**S1**
IISR CLT 116	S2	S2-S1	**S1**	
IISR CLT 117	S2	S2-S1	**S1**	
IISRCLT 118	S2	**S1**		
IISR CLT 119	S3	S2	**S1**	
IISR CLT 120	S3	S2	**S1**	
IISR CLT 121	S2	**S1**		
IISR CLT 122	S3	S2	**S1**	

S1- 100% overgrowth, **S2**-75% overgrowth,
S3- 50% overgrowth, **S4**-locked at the point of contact

DISCUSSION

In the present study, indigenous *Trichoderma* isolates from different parts of the turmeric cultivating areas of south India were tested for their efficacy against *P. aphanidermatum* for the management of rhizome rot disease. Among the fungal antagonists, *Trichoderma* dominates the literature as successful antagonistic fungiandhave gained wide acceptance as effective biocontrol agent against several commercial plant pathogens. The main objective was to shortlist promising *Trichoderma* isolates for the management of rhizome rot. The present study involved preliminary screening of the isolates by the three methods i.e. dual plating, activity of volatile metabolites and non-volatile metabolites. The promising antagonistic activity of the isolates IISR CLT 102, IISR CLT 107, IISR CLT 110, IISR CLT 114, IISR CLT 118 and IISR CLT 121 on dual culture may be due to mycoparasitism. The ability of *Trichoderma* to inhibit the growth of plant pathogens like *Pythium* spp.has been reported by several authors [16-19]. Hyphal parasitism of *Pythium* spp. by *Trichoderma* was also observed *in vitro* by many workers [8, 14, 21]. Among them Chet *et al.*, [8] reported coiling and puncturing of the mycelium of *Pythium* by *Trichoderma* spp. during hyphal interactions. Since the nature of interaction between the antagonist and pathogen is important in biocontrol approach, the mycoparasitism and competition showed by these isolates *in vitro*, shows the potential of these *Trichoderma* spp. to be used as a biocontrol agent for the management of the disease. A similar study conducted by Balakrishnan*et al* [22] on *Pythium* spp. causing rhizome rot in ginger reported parasitism on sporangia of *Pythium* by *T. harzianum* on dual plating.

The mechanism for inhibition such as antibiosis has been explained by several authors [14, 15]. The result of the present study also supports the antagonistic activity of *Trichoderma* against *P.aphanidermatum*. The hyphal coagulation and evacuation may be due to antibiosis as reported by several authors. Howel and Stipanovic[20] reported an antibiotic Gliovirin as active against *Pythium* spp. *Trichoderma* spp. are known to produce several hundreds of antimicrobial compounds and the synergistic actions of such compounds are responsible for their anti microbial activity. These metabolites produced by *Trichoderma* are also known to enhance competitiveness and thereby control the disease [23,24]. The crude filtrates of IISR CLT 102 and IISR CLT 110 were characteristically dark brown in colour and that of IISR CLT 107 was pale green. These isolates may have the ability to produce secondary metabolites and extracellular enzymes as reported by Cigdem and Merih[25] and Vizcaino *et al*., [26]. Out of the 22 isolates screened, three isolates *viz*., IISR CLT 103, IISR CLT 118 and IISR CLT

121 were found to produce volatile metabolites in sufficient quantities to inhibit the growth of the pathogen. The lowest level of inhibition may either be due to the lack of production of active volatile metabolites or due to the production of volatile metabolites in quantity which is not sufficient to check the growth of the pathogen. IISR CLT 121 which showed highest inhibition produced a characteristic odor after 24 h of normal incubation. Claydon*et al*., [27] reported that the volatile metabolites of *T.harzianum* are alkyl pyrones and also reported that these compounds are fungistatic. Rathore*et al*., [28] have reported the activity of volatile metabolites against the pathogens causing rhizome rot of ginger *in vitro*. They also reported the formation of thin mycelia of pathogen when exposed to these volatile metabolites which was observed in the present study also. Similarly the effect of volatile compounds from *T. harzianum* and *T. viride* was reported by Pandey and Uapadhyay [29]. The production of these volatile metabolites in the soil may play an important role in the suppression of the pathogen and thereby reducing the load of inoculum and its further spread. Thus the shortlisted six indigenous isolates of *Trichoderma* are found to be promising against *P. aphanidermatum*, the rhizome rot pathogen of turmeric. Hence, these isolates can be considered for the pot culture and field evaluation for the management of rhizome rot disease in turmeric.

AKNOWLEDGEMENT

The authors acknowledge the financial support provided by University Grants Commission (UGC) New Delhi, India awarding Junior Research Fellowship to Anoop K. during the research period.

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