Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 854-856
Received: 19-01-2019
Accepted: 21-02-2019

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Isolation and identification of local antagonistic *Trichoderma harzianum* from rhizosphere of Garlic, redgram and potato

Manisha Pandey and Brajesh Mishra

Abstract
A total of 18 rhizosphere soil samples were collected from healthy plants present in garlic, Red gram and potato fields, Piprauli block of Gorakhpur district. Five *Trichoderma* spp. were obtained from soil samples on the basis of morphological identification such as shape of phialides, conidia and growth characters. Further on the basis of farmer's response two isolates were highly effective as compare to others *viz*; TCIPMCG-1 and TCIPMCP-2 and were identified as *Trichoderma harzianum*, morphologically and molecularly from ITCC, New Delhi.

Keywords: Rhizosphere, *Trichoderma harzianum*, antagonistic, native strain

Introduction
The species of *Trichoderma* are well known for their biocontrol activity against many plant pathogens that cause major problems in the current agricultural scenario. *Trichoderma* spp. usually available in soil and root ecosystems has gained huge significance since last few decades due to its biological control ability against soil born plant pathogens. *Trichoderma* in managing soil borne plant pathogens are reported by different workers such as, eco friendly (Gaur *et al*., 2005) [5], effective in managing diseases caused by soil borne plant pathogens which cannot be easily controlled by chemicals (Manoranjitham *et al*., 2001) [7], ease and cost effective mass culturing of antagonists (Das *et al*., 2006) [3], growth promoting effect (Pan and Bhagat, 2007) [9] and long lasting effective disease management (Sarojini *et al*., 2007) [10]. The potential of *Trichoderma* spp. as biocontrol agents of plant diseases was first recognised in early 1930's. *Trichoderma* is characterized as rapidly growing colonies bearing tufted or postulate, repeatedly branched conidiophores with lageniform phialides and hyaline or green conidia born in slimy heads (Shalini and Kotasthene, 2007) [11]. Several strains of *T. viride* and *Trichoderma harzianum* had a significant reducing effect on plant diseases caused by pathogens such as *Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium aphanidermatum*, *Fusarium oxysporum*, and other soil borne pathogens under green house and field conditions (Bassim *et al*., 1999). Soil borne pathogens causing diseases like; collar rot, stem rot, root rot, damping-off and wilt in crops like brinjal, Papaya, Redgram and Tomato. Root colonization ability of *Trichoderma* spp. is another important property which make them strong competitive colonizer in the rhizosphere of plant and protect the root infection by pathogens. Thus in present study we concentrated on isolation, identification of *Trichoderma* isolates existing in rhizosphere of Garlic, Redgram and Potato fields in Piprauli block of Gorakhpur district.

Materials and methods
Isolation and Identification of *Trichoderma* species
Soil samples were collected from rhizosphere of 18 different agricultural fields such as garlic, redgram and potato fields in Gorakhpur district, Uttar Pradesh. Among 18 soil samples, 6 samples from Garlic, 6 samples from Redgram and 6 samples from potato rhizospheric region. These soil samples were collected in clean poly bags and brought to laboratory and kept at 4ºC until used. Dilution plate method was used for the isolation of antagonists from soil, for that serial dilutions of each soil samples was done with sterilized distilled water up to 10^{-6} and 0.1 ml diluted sample was poured on the surface of Trichoderma Specific Medium (TSM) (Elad and Chet, 1983). These plates were incubated at 25±2ºC and after 4 to 6 days of incubation, the fungal growths on petri plates were screened for *Trichoderma* fungus. Selected fungal isolates were purified through subcultures from single spores. The cultures were identified using the available literature Kiffer and Morelet (2000) [6] and monographic contribution provided by Bissett (1991 a, b) [1, 2].

Isolates were confirmed by morphological characters on the basis of colony colour and growth; size and shape of conidiophore, phialides and conidia and at species level by ITCC, Division of Plant Pathology IARI, New Delhi. *Trichoderma* spp. was maintained on PDA slants at 4ºC for further studies.

Results and Discussion

Among 18 soil samples (6 samples from garlic, 6 samples from redgram and 6 samples from potato rhizosphere region) 5 *Trichoderma* spp. were isolated. From these isolates, 2 were from garlic, 2 from redgram and 1 from potato rhizosphere soil (Table 1 and 2). They were designated as TCIPMCG-1, TCIPMCP-2, TRGRH, TPRB and TRGRJ. In similar study several scientists reported the isolation of *Trichoderma* spp. from rhizosphere soils of different crops. Gajera *et al.* (2011) [4] isolated several *Trichoderma* from groundnut crop. Sundaramoorthy and Balabaskar (2013) [13] isolated fungal native isolates of *Trichoderma* from rhizosphere of Tomato. According to growth pattern 5 *Trichoderma* spp. were selected for identification. Out of them two species, TCIPMCG-1 and TCIPMCP-2 were very fast growing. According to farmer's response on effectiveness of these two species were identified as *Trichoderma harzianum* on the basis morphological and of molecular characterization from ITCC (Indian Type of Culture Collection), New Delhi. Similarly, Singh., (2006) [12] obtained twenty seven isolates of *T. harzianum* from soil samples collected randomly from fallow agricultural fields throughout the Punjab and studied their growth rate on PDA medium and were categorised as medium, fast and very fast growing and Muthukumar and Pratibha Sharma (2011) [8] used morphological description for characterization and grouping of *Trichoderma* isolates.

Table 1: Soil Samples collected from rhizosphere region of garlic, redgram and potato fields from different villages of Piprauli block in Gorakhpur district

S. No	Name of the District	Name of the Block	Name of the village	Crops	No of soil samples
1.	Gorakhpur	Piprauli	Chhapia	Garlic (Farm sample)	2
2.	-do-	-do-		Red gram	1
3.	do	do		Potato	1
4.	do	do	Jaitpur	Garlic	1
5.	do	do		Red gram	2
6.	do	do		Potato	1
7.	do	do	Bargahan	Garlic	1
8.	do	do		Red gram	1
9.	do	do		Potato	1
10.	do	do	Hardia	Garlic	1
11.	do	do		Red gram	1
12.	do	do		Potato	2
13.	do	do	Haraya	Garlic	1
14.	do	do		Red gram	1
15	do	do		Potato	1
Total					18

Table 2: List of *Trichoderma* spp. isolated from soil samples collected from different villages in Gorakhpur Dist.

S. No.	Isolate No	Name of village
1.	TCIPMCG-1	Chhapia (Farm sample)
2.	TCIPMCP-2	Hardia
3.	TRGRH	Haraya
4.	TPRB	Bargahan
5.	TRGRJ	Jaitpur

TCIPMCG = *Trichoderma* Central IPM Centre Garlic, TCIPMCP = *Trichoderma* Central IPM Centre Potato TRGRH = *Trichoderma* Red gram Rhizosphere Haraya, TPRB= *Trichoderma* Potato Rhizosphere Bargahan, TRGRJ = *Trichoderma* Red gram Rhizosphere Jaitpur.

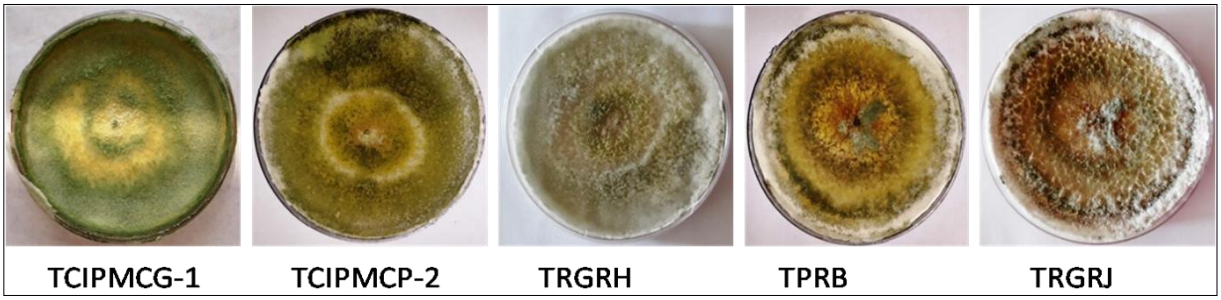

Fig 1: Pure cultures of *Trichoderma* isolates from rhizosphere region of Garlic, Potato and redgram.

Conclusion

Result of above finding's reveals that the TCIPMCG -1 and TCIPMCP-2 isolated from the rhizospheric soil of healthy garlic and potato plants, have strong biocontrol activity against several plant diseases in field conditions. Therefore, the application of indigenous *Trichoderma harzianum* in the field tended to reduce the soil and seed borne diseases of several crops.

Acknowledgement
The authors express their gratitude to the Plant Protection Adviser and Joint Director (IPM), Directorate of Plant Protection Quarantine & Storage, N.H.IV, Faridabad-121001 (Haryana) for their encouragement and facilities provided during the work.

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