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Effect of some locally available substrates on mass production of native *Trichoderma* spp.

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Biological control of plant disease is gaining momentum in recent years and assumes special significance in being eco-friendly cost effective strategy, which can be used in integration with other disease management systems to afford greater levels of protection and sustain crop yield. *Trichoderma* spp. is one of the most promising fungal bio-control agents and has created a new milestone in non-chemical disease management system and organic agriculture in particular. Variations in the bio-control potentiality of antagonistic mycoflora are bound to occur in different agro-climatic regions. Thus, in the present investigation emphasis was given for isolation and regional evaluation of native *Trichoderma* isolates along with their mass multiplication in locally available substrates. Tri Pun2 followed by Tri Pun inhibited maximum mean growth of soil-borne plant pathogens during dual culture method. In mass multiplication experiment, paddy grain and paddy husk was found to be the best locally available substrate for mass multiplication of native Tri Pun2 isolate. 81% spore viability was observed after 137 days at normal temperature in paddy grain substrate.

Key words : Trichoderma, mass production, competitive saprophytic ability.

INTRODUCTION

There is increasing public concern regarding the continued use of agrochemicals that are harmful to human health or the environment. Such concerns are driving the search for more environmentally friendly methods to control plant disease that will contribute to the goal of sustainability in agriculture. Therefore, disease containment through eco-friendly bio-control approaches ; using natural antagonistic micoflora is now becoming an inevitable component in the integrated management strategy of the disease. *Trichoderma* spp. is one of the most promising fungal biocontrol agents (Elad *et al.*, 1982) and has created a new milestone in non-chemical disease management system and organic agriculture in particular. *Trichoderma* is reported to be one of the most widely distributed soil fungi and effective biocontrol agent against many of the economically important plant-pathogenic fungi.

Antagonistic mycoflora are subjected to variation in soil characteristics, soil temperature. pH, rainfall pattern, affecting their adaptability to a specific environment and edaphic conditions. Physical factor such as soil moisture and pH influence the activity of biocontrol agents, unfavourable temperature may be an even more important limiting factor. Because of these limitations, variations in the biocontrol potentiality of antagonistic mycoflora are bound to occur in different agro-climatic regions. Use of location-specific strains needs to be emphasized so as to obtain an optimum benefit. Mass production of these fungi has become a focus of research in the search for alternatives to polluting chemicals for control of plant diseases. Keeping this background in mind the present work was undertaken with the objective to study the mass multiplication of most efficient competent indigenous antagonistic mycoflora through locally available and cheap carrier based formulation.

MATERIALS AND METHODS

Isolation of Trichoderma spp. Biocontrol agents (*Trichoderma* spp.) were isolated from soil of different ecological niches, using soil dilution plate method on TSM. The collected soil samples were air-dried, ground to powder using mortar and pestle. 10 g of powdered soil sample was mixed with 90 ml of sterile distilled water (SDW) to prepare 10^{-1} dilution. This suspension was used for serial dilutions up to 10^{-6}. One ml of the suspension from 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilution was plated separately on 20 ml of modified TSM previously poured in sterile Petri plates. The suspension was distributed uniformly on medium surface by horizontal shaking and incubated at 28 ± 1ºC for seven days. The green colonies of the antagonist isolates usually appeared 4 to 5 days after incubation. Each colony was studied separately under microscope using 0.1% lacto phenol—cotton blue (0.1 g cotton blue was mixed with 100 ml of standard lacto phenol preparation) and compared with Rifai's (1969) monograph on *Trichoderma*. The shape, size, aggregation of phialospores and phialides were considered as main criteria for identification.

In vitro screening of antagonistic potential of native Trichoderma isolates. Isolates of *Trichoderma* spp. were screened for their antagonistic activity by dual culture plate method (Dennis and Webster, 1971) on PDA. Both the antagonist and pathogen were inoculated at opposite ends in sterilized petri plates (90 mm diam.) containing 20 ml sterilized PDA medium. Inoculation was done in such a way (by staggering the day of inoculation) that the point of contact may take place at the centre of the plates. The plates were incubated at 28 ± 1ºC for 60 hr. Control plates were maintained by inoculating the pathogens i.e. *R. solani, S. rolfsii* and *S. sclerotiorum* in PDA at the same position as done in dual culture. Both the pathogen and antagonist grown towards each other and, after contact the extent of invasion by antagonist over pathogen was recorded. Antagonistic potential of the *Trichoderma* isolates against each test pathogens was determined by measuring the percent inhibition in growth over control. Rating of biocontrol potential of the *Trichoderma* spp. was done using modified Bell's scale (Bell *et al.*, 1982, Saha and Pan, 1997). The rating of the scale was as follows—

S_1 : When antagonist overlaps the total growth of pathogen.

S_2 : When antagonist overlaps 2/3 growth of the pathogen.

S_3 : When antagonist overlaps 1/2 of the pathogen growth.

S_4 : When pathogen growth is restricted at the point of contact.

S_5 : When pathogen starts overlapping the antagonist growth.

Mass multiplication of native Trichoderma isolates on locally available different substrates

The antagonist isolate was grown on PDA for 7 days, then the mycelial disc of 4 mm diam. are inoculated in 250 ml Ehrlenmeyer flask containing 100 ml PDB medium and incubated at 25 ± 1ºC for 12 days. The mycelial mat was harvested aseptically and spore suspension was prepared in 100 ml sterilized distilled water by crushing the mat. The substrates [Paddy grain (P.G.), rice husk (R.H.), wood dust (W.D.), sugarcane baggase (S.B.), paddy straw (P.S.) and farm yard manure (FYM)] pre-soaked and sterilized in autoclave at 21 lb/inch^2 for 1 hr. The flasks filled with over night soaked sterilized substrates (with or without 1% yeast-peptone-sucrose soln.) were inoculated with 10 ml of the spore suspension. Observation on spore concentration and spore viability were recorded in each substrate for 30, 60, 80 and 137 days after inoculation. Spore concentration was measured with haemocytometer whereas, spore viability were recorded by dilution plate method.

RESULTS AND DISCUSSION

In vitro antagonistic effect of different Trichoderma spp. agsinst soil borne plant pathogens

The results of (Table 1) indicated that the isolate of Tri Pun2 was the most effective against *R. solani* in dual culture technique in which maximum mycelial growth inhibition was recorded 55.55%. Tri Pun stood next best fungal bioagents and inhibited the mycelial growth of the pathogen by 52.96%. These observations were in accordance with the findings of Mathur and Gurjar (2002) who also recorded that *Trichoderma* isolates, copletely overgrew the mycelium of *R. solani* and stopped the formation of selerotia. Dath *et al.*, (1998) also

reported that several fungi especially *Trichoderma* spp. were found to be antagonistic to *R. solani* in *in vitro*. The perusal of the data in table indicated that Tri mnp followed by Tri Pun was found to be most effective in inhibiting the mycelial growth of *Sclerotinia sclerotiorum* by 57.08 and 55.33%, respectively. Among different isolates of *Trichoderma spp.* Tri Pun2 was found to be the most effective isolate and suppressed the mycelial growth of all the three soil-borne plant pathogenic fungi (mean % of growth inhibition 46.85%) followed by Tri Pun (mean % of growth inhibition 46.14%).

Table 1 : Crop rhizosphere and sources for different native isolates of *Trichoderma* spp.

Isolates	Name	Source	Locations
T. Pun	*Trichoderma* Pundibari	Turmeric	Cultivated land U.B.K.V.
T. Pun 2	*Trichoderma* Pundibari-2	Potato field	Cultivated land, U.B.K.V.
T. mnp	*Trichoderma* Midnapur		Contai, Midnapur
Thdl	*Trichoderma* Delhi		ITCC, IARI, New Delhi

Table-2 on antagonistic potential of different Trichoderma isolates by Bell's scale rating showed that Tri Pun2 was highly effective against *R. solani* and *S. folfsii* whereas T mnp was highly potential against *S. rolfsii* only. None of the isolates were found highly effective against *S. sclerotiorum*.

Table 2 : Antagonistic effect of different *Trichoderma* spp. on growth of different soil borne plant pathogens.

Isolates	Growth inhibition (%) of different pathogens			
	Rhizoctonia solani	*Sclerotium rolfsii*	*Sclerotinia sclerotiorum*	*Mean*
Tri Pun	52.96 (46.69)*	30.13 (33.29)	55.33 (48.05)	46.14
Tri Pun 2	55.55 (48.19)	35.34 (36.47)	49.67 (44.81)	46.85
T. mnp	49.63 (44.79)	23.72 (29.15)	57.08 (49.07)	43.47
Thdl	47.04 (43.30)	30.13 (33.29)	51.67 (45.96)	42.94
Mean	51.3	29.83	53.43	
SEm ±	1.34	1.45	1.36	
CD(P=0.05)	4.63	5.03	4.71	

* Figure in the parentheses represents angular transformed value

Mass production of Trichoderma spp. on some locally available substrates

The first step in the production of biocontrol agents in the development of a suitable medium, which is inexpensive and readily available agricultural bio product with appropriate nutrient balance. Substrates for mass multiplication is one of the most important key parameters, which has role in proper growth and sporulation. This factor was evaluated in the present study for achieving maximum growth and sporulation of Terai Agro Ecological Region specific sub tropical isolates of *Trichoderma* spp.

Table 3 : Different phases of *Trichoderma* spp. against different pathogens

Isolates	Phases		
	Rhizoctonia solani	*Sclerotium rolfsii*	*Sclerotinia sclerotiorum*
Tri Pun	S_2-S_1	S_2	S_2
Tri Pun 2	S_1	S_1	S_3-S_2
T. mnp	S_2-S_1	S_1	S_3-S_2
Thdl	S_3-S_2	S_2	S_3-S_2

Among various substrates tried the antagonist grew profusely on paddy grain (P.G.), rice husk (R.H.), however comparatively less colonization was observed on wood dust (W.D.), sugarcane baggase (S.B.), paddy straw (P.S.) and farm yard manure (FYM). Maximum spore concentration was observed in P.G. (N) (24×10^8/g) of preparation) followed by R.H., (21.6×10^8/g) of preparation) [Table 3]. In all the cases significantly more spore concentration were observed when substrates were pre treated with nutrient solution. In all the cases maximum spore concentration was recorded between 19^{th} to 30^{th} days after inoculation. Among various substrates tried, P.G and R. H was formed to be the best for mass multiplication of different *Trichoderma* isolates of this region. However, Singh *et al.* (2001) using sorghum grain as substrates had recorded maximum spore concentration in 30 days old *Trichoderma virens* culture. The laboratory studies conducted during the present investigation clearly showed that locally available substrates P.G. and R. H. could be used for mass multiplication of regional specific native Tri Pun 2 isolate. Sangle *et al.* (2003) also encouraged the use of locally available substrates for mass multiplication of *Trichoderma* isolates. Among various substrates

Table 4 : Spore count of *Trichoderma* spp. on different locally available substrates

Sample	Spores in 10^6/g of substrates				
	10th days	13th days	16th days	19th days	30th days
Paddy Grain (N)	483.33	555.33	973.33	1540.33	2403.33
Paddy Grain (WON)	223.33	451.33	781.33	1181.66	1700.66
Rice Husk (N)297.33	639.66	1100.00	1972.33	2165.66	
Rice Husk (WON)	282.00	584.66	891.00	1041.33	1400.66
Wood Dust (N)	258.00	300.00	353.66	418.66	441.66
Wood Dust (WON)	19.66	21.66	31.66	40.00	42.00
Sugarcane Baggase (N)	171.00	347.00	371.66	505.33	551.66
Sugarcane Baggase (WON)	120.33	228.00	331.33	443.00	493.33
Paddy Straw (N)	161.00	223.33	282.33	313.33	361.33
Paddy Straw (WON)	11.66	17.66	21.66	26.66	25.33
Farmyard Manure (N)	243.33	281.66	440.66	672.33	550.66
Farmyard Manure (WON)	101.33	163.33	260.33	383.33	323.33
For substrates	SEm ±	7.62			
	CD (p=0.05)	21.36			
For nutrient status	SEm ±	4.40			
	CD (p=0.05)	12.33			
Substrates × nutrient status	SEm ±	10.79			
	CD (P=0.05)	30.21			

tried paddy grain with nutrient was found to be the best for mass multiplication of native *Trichoderma* isolate (Tri Pun2) as it provided more than 80% spore viability even after four and half months at room temperature storage condition (Table 4). Thus, from the above experiment it can be concluded that this mass multiplication technology may be applied for other antagonistic fungal bioagents for this region.

Table 5 : The shelf life of native *Trichoderma* isolate (Tri Pun 2) on different substrates at room remperature.

Substrates	Viability (%)			
	30 days	60 days	80 days	137 days
Paddy Grain (N)*	91.2	89.6	88.7	81.3
Paddy Grain (WON)	88.7	87.2	84.2	72.6
Rice Husk (N)	89.6	87.8	85.2	74.2
Rice Husk (WON)	87.2	84.2	81.3	70.4
Wood Dust (N)	84.7	81.6	77.4	66.4
Wood Dust (WON)	83.4	80.2	73.8	60.5
Sugarcane Baggase (N)	87.6	86.6	84.3	79.8
Sugarcane Baggase (WON)	87.4	86.9	82.1	74.6
Paddy Straw (N)	85.2	83.2	79.3	70.4
Paddy Straw (WON)	83.8	81.6	78.7	66.8
Farmyard Manure (N)	87.2	81.3	77.4	67.4
Farmyard Manure (WON)	85.1	80.1	75.2	61.3

* N = with nutrient ; WON = Without nutrient

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