

Agro-based Waste Products as a Substrate for Mass Production of *Trichoderma* spp.

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Abstract

Various agricultural by products such as vegetable wastes, fruit wastes, crop wastes, FYM and Poultry manure were evaluated for mass production of *Trichoderma viride* and *Trichoderma harzianum*. Among the substrates used wastes of brinjal, banana, papaya, guava, spinach, sugarcane, tealeaves and pea husk of solid and liquid was found supported maximum spore production in solid and liquid for the growth of *Trichoderma harzianum* and *Trichoderma viride* as compared with wastes of carrot, cucumber, potato, radish, cabbage, orange peel, wheat bran, FYM and Poultry manure.

Keywords: Mass production, Agricultural wastes products, *Trichoderma* spp.

1. Introduction

Trichoderma is widely used as bio-control agent against several root pathogenic fungi throughout the world (Chet et al., (1979) Elad et al. (1980), Sivan et al. 1984). *Trichoderma* spp. is free living fungi that are highly interactive in root, soil foliar and environments. It has been known for many years that they produce a wide range of antibiotic substances and they paralyze other fungi (Tayler and Farncis, 1998). They can also compete for key exudates from seeds that stimulates the germination of propagules of plant pathogenic fungi in soil (Howel 2002) and more generally compete with soil micro-organisms for nutrient and space (Elad 1996). *Trichoderma* spp. was identified as potential antagonists of *Rhizoctonia solani* and *Macrophomina phaseolina* (Singh 1994).

Antifungal metabolites of *Trichoderma* have been grouped by Ghisalberti and Sivasithamparam, 1991. *Trichoderma* spp. are known to produce mycolytic enzymes such as β -1, 3, glucanase, β -1, 4 endo-glucanase, chitinase and protease. These enzymes play an important role in the degradation of chitin which is the structural component of the target pathogens and herbivorous insects and consequent myco-parasitism (Harman et al., 1993). Baker and Dickman (1993) found high enzyme activity in susceptible pathogen. Genetic improvement of *Trichoderma* as a biological control agent using induced mutagenesis has been successfully attempted for fungicidal resistance (Papavizas et al., 1982), antibiotic production (Faull et al, 1994) and enzyme secretion (Witkowska and Bien 1991). Metcalf et al., (2001) studied the disease control potential of *Trichoderma* spp. For biological control of onion white root-rot disease caused by *Sclerotium capivorum*. The mode of action in environmental condition of favourable growth and establishment of *Trichoderma* spp. and mechanism of antagonize and parasitize to other fungi was reported by Kulling et al., (2000). Howell et al., (2000) examined

that seed treatment with *Trichoderma virens* stimulates defense response, as indicated by the synthesis of terpenoids in cotton roots and the role of terpenoid compounds is in disease control.

Lin et al., (1994) reported that Tricholin, a ribosome inactivating protein isolated from the culture broth of *T. viride* and they concluded Tricholin is an effective inhibitor of protein synthesis. Cherif and Benhamou (1980) reported a strain of *Trichoderma* has the ability to reproduce chitinases and inhibits growth of *F. oxysporum*.

Mass production of *Trichoderma* required to find out suitable media on large amount of *Trichoderma* biomass is required therefore, the first step for the mass production of any bio-control agent is to identify the suitable substrates, which should be comparatively cheap, stable and easily available within a short period of time. The type and form of substrate i.e. broth and solid may also vary according to the specific purpose for which bio-control agent biomass is required.

The quality of a microbial bio-protectant is dependent on the propagate density in the biomass and its ability to survive processing (Harman et al, 1991). Production of adequate quantities of good quality inoculum is an essential component of the biocontrol programme. The production of *Trichoderma* may be taken up by the labour intensive and economically viable methods for relatively progressive farmers. Development of simple and reliable production system of submerged liquid fermentation for the production of blastospores, which are short lived and hydrophilic (Ramback 1989) or solid state fermentation (Roussan et al; 1988) for the production of aerial conidia. Therefore the present study was aimed to find out the suitability of various semi solid substrates and liquid media for biomass production of selected *Trichoderma* species.

2. Materials and Methods

Semisolid substrates of 50g each eighteen different agro-wastes products viz. Carrot, Cucumber, Potato, Brinjal, Banana, Papaya, Radish, Cabbage, FYM, Poultry, Guava, Spinach, Orange, Sugarcane, Tea leaves, Pea husk, Rice husk, Wheat bran were selected and wetted with 2% molasses. Each substrate was transferred to 250 ml conical flask and put cotton cork. For broth substrate the above substrates were used @ 50g/150ml distilled water, boiled for 10 minutes and filter through mashlin cloth. The supernatant solution was collected in 250 ml of conical flask. Further solid and liquid substrates were sterilized in autoclave at 15 psi pressure for 20 minutes. After proper sterilization both the substrates (solid and liquid) were inoculated with *Trichoderma viride* and *Trichoderma harzianum* and incubated at $27 \pm 1^\circ\text{C}$. Observation were recorded on growth rate and colony colour at 20 days after incubation.

4. Results and Discussion

The data regarding growth rate and sporulation pattern of *Trichoderma viride* and *Trichoderma harzianum* are summarized in table no. 1. In case of *Trichoderma viride* in solid and liquid media in different substrate, it was observed that *T. viride* produced hyaline colony at first, which gradually changed to yellowish green colour in latter stage. The yellowish green colour was more prominent at later stage, *Trichoderma harzianum* initially produced light green colour colony further with in 24 hrs the mycelium spread over the surface of the substrates which become dark in colour due to abundant sporulation.

The moderate sporulation was also obtained in the substrates of wheat bran was 0.79(s), 0.83(L), in radish 0.68(S); 0.84(L) $\times 10^6$ CFU/ml for *T. harzianum*. In case of *T. viride* the sporulation was noted in wheat bran 0.81(s); 0.84 (L) and in radish 0.46(s); 0.69(L) $\times 10^6$ cfu/ml. Rama et al., (2001) also reported the best substrate of *T. harzianum*, *T. reesei* grow better in sugarcane waste and used tea leaves, where as *T. viride* and *T. Kaningii* showed maximum growth in tea leaves + wheat bran substrate. The result of table no.1 shows that *T. harzianum* sporulation shows slow in FYM 0.27(S), 0.32(L) and rice husk 0.20(S), 0.41(L), Poultry manure 0.26(S); 0.27(L) $\times 10^6$ cfu/ml. In case of *T. viride* the sporulation rate in FYM 0.16(S); 0.19(L); rice Rusk 0.23(S); 0.37(L) and in poultry manure 0.13(S); 0.16(L) $\times 10^6$ cfu/ml respectively.

In the present study wastage of Potato peel, Brinjal, Banana, Papaya, Guava, Spinach, Sugarcane, used Tea leaves and Pea husk medium of solid and liquid was found best for the growth of *Trichoderma harzianum* and *Trichoderma viride* isolates. Suitability of the wheat bran for the growth of *Trichoderma* has been reported by Lumsden and Lewis (1988) and Singh (1994) and Rama et al, (2001). From the study it was clear that *Trichoderma* are able to grow on a wide variety of agriculture by products on both solid and liquid state and this can be useful to farmers to cultivate these fungi very easily.

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Table 1. Growth and sporulation of *Trichoderma* isolates on agro-wasted solid and liquid at 20 days after inoculation

Substrate	<i>T. harzianum</i>				<i>T. viride</i>			
	GR	SP	Spore density (1x10 ⁶ CFU/g)		GR	SP	Spore density (1x10 ⁶ CFU/g)	
			Solid	Liquid			Solid	Liquid
Carrot peel	F	+++	1.31	1.93	F	+++	1.24	1.78
Cucumber peel	F	++	0.67	1.03	F	++	0.70	0.78
Potato peel	F	++	1.16	1.37	F	+++	1.05	1.21
Brinjal peel	F	+++	1.05	1.26	F	+++	0.91	1.11
Banana peel	F	+++	1.22	1.82	F	+++	1.19	1.78
Papaya Peel	F	+++	1.34	1.97	F	+++	1.30	1.79
Radish waste	M	++	0.68	0.84	M	++	0.46	0.69
Cabbage waste	F	+++	0.98	1.31	F	+++	1.09	1.11
FYM	S	+	0.27	0.32	S	+	0.16	0.19
Poultry	S	+	0.26	0.27	S	+	0.13	0.16
Guava waste	F	+++	1.08	1.97	F	+++	1.05	1.09
Spinach waste	F	+++	1.23	1.24	F	+++	1.06	1.07
Orange peel	F	+++	0.93	1.04	F	+++	0.72	0.81
Sugarcane waste	F	+++	1.21	1.67	F	+++	1.21	1.87
Used tea leaves	F	+++	1.01	1.54	F	+++	0.91	0.97
Pea husk	F	+++	1.82	1.89	F	+++	0.83	0.84
Rice husk	S	+	0.20	0.41	S	+	0.23	0.37
Wheat bran	M	++	0.79	0.83	M	++	0.81	0.84

+ = Slow growth

++ = Moderate (M)

+++ = Abundant (Fast)

F = Fast

M = Mediation

GR = Growth rate

SP = Sporulation pattern