Antagonistic effect of Trichoderma viride and Trichoderma harzianum against plant pathogenic fungi and its growth on different agro-waste substrates

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***Abstract*— *Trichoderma* comprises of a number of fungal strains that act as biological agent. Genus *Trichoderma* is effective as biocontrol agent against fungal and bacterial pathogen. *Trichoderma viride and Trichoderma harzianum* grows rapidly in culture and easily isolated. In present study various agro-waste substrates medium were used to check growth of *Trichoderma* spp. Among all these substrates medium, maximum growth of *T. viride* was found on sugarcane bagasse and *T. harzianum* exhibited maximum growth on wheat medium. *T. viride* inhibited 75 % mycelial growth of *Fusarium moniliforme* and 45 % of *Fusarium sacchari*. *T.harzianum* inhibited 70% growth of *Fusarium moniliforme* and 55% of *Fusarium sacchari.* Use of *Trichoderma* spp as a biocontrol agent is not only safe for environment and farmers as well.**

***Index Terms*— Growth, Trichoderma, biocontrol, antagonistic, pathogenic .**

I. INTRODUCTION

Genus *Trichoderma* has high ability to inhibit the growth of pathogenic fungus by serecting some enzymes. *Trichoderma harzianum* is an asexually reproducing filamentous fungus and a species aggregate. It is grouped on the basis of conidiophores branching patterns with short side branches, short inflated phialides, smooth and small conidia (Rifai 1969). *Trichoderma viride* and *Trichoderma harzianum* is mainly used as biocontrol agent due to its high antagonistic activity against various pathogenic fungi. *T.viride* and *T.harzianum* isolated from soil and show antagonistic activity against *Colletotrichum gliosporioides* and *Fusarium* by dual culture. (Lal sahib yadav ,2012)

After green revolution people used many chemical fertilizers which are not good for environment and long term use of these fertilizer effected the soil fertility and crop products. These days many non chemical and eco-friendly fertilizers are in use for a better and healthy crop. Many bacteria, algae, fungi used as bio control agent play a significant role in the field of plant disease control, thereby increasing plant productivity. The potential efficacy of *Trichoderma harzianum* against the pathogenic fungi like *Cladosporium spherospermum*, *Aspergillus niger* and *Fusarium oxysporum* was evaluated on the fungal growth by culture pattern (Mansoor Ahmad Lone, 2012) For sustainable crop production, eco-friendly pest management is essentially required. To fulfill these requirement mass production of *Trichoderma* spp as a biocontrol agent is becoming a very popular and useful research tool so that farmers can replace chemical fungicides with *Trichoderma* spp. The genus *Trichoderma* is present in all soils and can be easily isolated, growing rapidly on different culture medium. Mass production of *Trichoderma viride* and *Trichoderma harzianum* can also be achieved on different agro waste substrates used as medium for its growth. In present study to check the growth of *Trichoderma* spp different agrowastes viz., wheat grain, mustard, apple peel, coriander ,sugarcane bagasse ,and dry leaves of crop plant, as substrate medium were used. *Trichoderma* is a versatile fungi, used commercially in a number of ways. It is used for production of cellulases and other enzymes that degrade complex polysaccharides and also used in poultry feed to increase the digestibility of hemicelluloses from other crops and its enzyme activity have ability to increase the rate of plant growth and development.

II. MATERIALS AND METHODS

A. Growth of Trichoderma spp on different agro-waste substrate medium.

For the study of growth of *Trichoderma* spp, 50g of different substrates in 150 ml distilled water were boiled for 10 minutes and filtered through muslin cloth. The supernatant solution was collected in 250 ml of conical flask. These substrates were sterilized in autoclave at 15 lb pressure for 20 minutes. After proper sterilization the substrates were inoculated with *Trichoderma* spp and incubated at 27^{o}C for 10 days. Observations were recorded on growth rate, colony colour and characters.

B. To evaluate the antagonistic activity of Trichoderma spp

a) Dual culture plate technique

Cultures of two different spp of *Fusarium moniliforme* and *Fusarium sacchari* were inoculated on PDA medium on two separate petriplate 2mm away from the center, and were

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incubated at 28^0 C for four days After four days same plates were inoculated with *Trichoderma* spp 2mm away from previous inoculums of plant pathogenic fungi and again kept for incubation at 28^0 C for four days. The antagonistic activity of *Trichoderma* sp. was observed. The percent inhibition of mycelial growth of pathogens was calculated by using the formula:

$$I = (C - T) / C \times 100$$

Where

I = Per-cent inhibition in mycelia growth

C = Growth of pathogen in control plates

T = Growth of pathogen in dual culture plates

b) Pot culture technique

Pot culture technique was used for measurement of antifungal activity of *Trichoderma* spp against fungal pathogen by sowing seeds. For this seeds were treated with pathogenic fungus ; with both pathogenic fungus and *Trichoderma* spp; with *Trichoderma* spp only. Control was untreated seeds .These treated seeds were sown in pots in 200 gm soil subsequently, as experimental and control pots respectively and observed for antifungal activity, seed germination rate.

III. RESULTS AND DISCUSSION

A. Growth of Trichoderma of different substrate medium

Among the six different substrate used as growth medium for *T.harzianum*, wheat exhibited maximum growth rate. Growth on apple peel and sugarcane medium was identical. Growth of *T. harzianum* on dry leaves and coriander medium was very scanty rated as poor growth after 7 days of inoculation. There was absolutly no growth on mustard medium. According to P.J Chaudhari et.al (2011) sugarcane bagasse media produced high amount of mycelia. In present study growth of *Trichoderma* spp on different substrate medium was not same. Growth of *T.viride* on sugarcane bagasse media was best and minimum growth was observed on mustard medium**.** Maximum growth for *Trichoderma viride* was on sugarcane bagasse. After 4 days of inoculation white mycelium was observed on the surface of medium and green coloured conidia covered the surface of medium after 9 days. Growth rate on apple peel and wheat was rated as good growth. Growth on coriander was medium on the growth scale. Growth of *T.viride* on dry leaves was poor.(Table1). Sobita simon (2011) reported that 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.

B. To evaluate the antagonistic activity of Trichoderma spp

a) Dual culture technique

The antagonistic activity of *Trichoderma* sp. was observed .The percent inhibition of mycelial growth of pathogens was calculated by using formula:

$$\mathbf{I = (C - T) / C \times 100}$$

Evaluation for antagonistic potential against two fungal plant pathogen viz. *Fusarium moniliforme* and *Fusarium sacchari* was done by dual culture techniques. *T. viride* and *T. harzianum* inhibited mycelial growth of both pathogens which were well stabilized in plates. In dual culture plates initially growth of *Fusarium* spp (*F.moniliforme* and *F.sacchari*) was normal but it completely restricted growth of the mycelium in plates in the presence of *Trichoderma* spp(figure 1). *T. viride* inhibit 75 % mycelial growth of *F. moniliforme* and 45 % of *F. sacchari*. *T. viride* was more effective for *F.moniliforme* than the *F.sacchari* as biocontrol agent. *T. harzianum* inhibit 70% growth of *F. moniliforme* and 55% of *F. sacchari*. (Table 2) *Yadav(2012) reported that in dual culture plates Trichoderma viride and T. harzianum completely colonized Fusarium oxysporum (over growth 71.6 and 66.66 cm).*

TABLE 1

S. No.	**Growth of *Trichoderma viride and Trichoderma harzianum* isolates on different agro-waste substrate medium after 20 days of inoculation .**						
		Growth rate		Colony colour	Growth rate		Colony colour
		Trichoderma harzianum			***Trichoderma viride***		
1	Sugarcane bagasse	Medium	+++	Green	Maximum	+++++	Yellowish green
2	Apple peel	Good	++++	White and green	Medium	+++	Light yellow and green
3	Mustard	No growth		-	Very poor	+	White
4	Wheat	Maximum	++++	Dark green	Good	++++	Yellowish green
5	Coriander	Poor	++	Green	Good	++++	Greenish white
6	Dry leaves	Poor	++	White	Poor	++	Green

TABLE 2

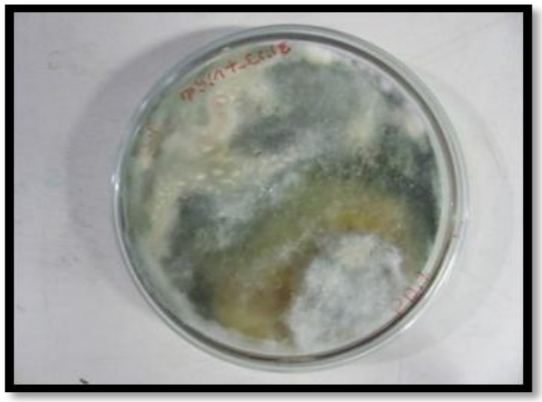
S.No.	**Antagonistic activities of *Trichoderma* sp (dual culture method)**		
	Pathogenic Fungi	**Percent inhibition in radial growth in:**	
		Trichoderma viride	***T. harzianum***

1	*Fusarium moniliforme*	77%	70%
2	*Fusarium sacchari*	45%	55%

FIGURE 1.Antagonistic activity of *Trichoderma* spp in dual culture method

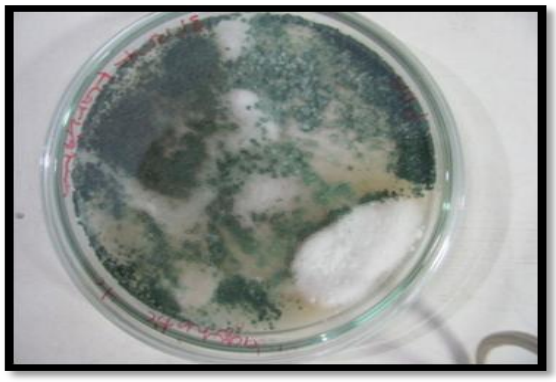
(a)

Inhibition in Radial Growth of (a) *F.moniliforme*



(b)

(b) *F. sacchari(*white mycelim*)* through activity of *T.viride*(yellowish green appearance)



(c)

Inhibition in Radial Growth of (c) *F. moniliforme*

(d)

(d) *F. sacchari*(white myceliam) by activity of *T.harzianum* (green and light green appearance)

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