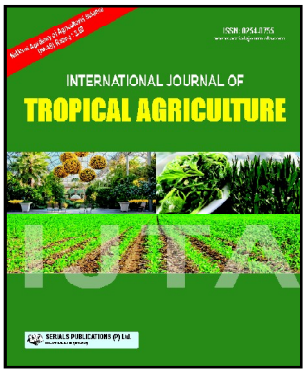

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Antagonistic Potential of *Trichoderma* Against Some Soil Borne Plant Pathogens

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Abstract: Seventeen *Trichoderma* cultures were isolated from soil samples of various location of Maharashtra, out of which two i.e. T1 and T7 isolates were selected for the development of mutants on the basis of highest antagonistic efficacy against *Sclerotium rolfsii* and *Macrophomina phaseolina* pathogen of groundnut and mungbean in an *in vitro* study in 2006-07. Conidial suspension with concentration of 10^7cfu/ml was treated with ethylmethylsulfonate with five concentrations @ 50, 75, 100, 125 and 150 µl/ml for 30 and 60 minutes. Selected single cell colonies were again treated with 0.1 and 0.2% colchicine. On the basis of highest antagonism total 15 cultures i.e. 13 mutants along with two mother cultures M_1 and M_7 (previously T1 and T7) were selected for the antagonistic potential against *Sclerotium rolfsii* and *Macrophomina phaseolina* using dual culture technique and production of volatile compounds. Mutant M_{10}, M_6 against *S. rolfsii* and M_3 against *M. phaseolina* were most efficient as recorded more than 80% inhibition in dual culture technique. The highest per cent inhibition of respective pathogen through the production of certain metabolites of *Trichoderma* mutants was recorded with M_6 and M_2 against *S. rolfsii* and *M. phaseolina* on third and fifth day after inoculation.

Key Words: Antagonistic potential, mutants, soil borne pathogens, *Trichoderma*, volatile compounds)

INTRODUCTION

The focus on the management of plant disease has been shifted from chemical pesticides to more eco-friendly bio pesticides to reduce environmental hazards. Several workers have reported the inhibitory effect of volatile compounds produce by *Trichoderma*

against some plant pathogen (Pan and Bhagat, 2008). *Trichoderma* spp. is known to produce many extracellular hydrolytic enzymes viz., β-1,3 glucanase, chitinase, cellulose etc. by which they cause lysis in many plant pathogenic fungi.

Trichoderma is also an effective biocontrol agent for protecting a number of crop plants from several soil borne plant pathogen (Mukhopadhyay *et al.*, 1994). Certain mechanism by which strains of *Trichoderma* functions are mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, induced resistance, solubilisation and sequestration of inorganic nutrients and inactivation on pathogens enzymes etc. (Harman, 2000). Sometime starvation condition could induce secretion of cell wall degrading enzymes (Ramot *et al.*, 2000) whereas in other cell wall or cell wall components were required to trigger the enzyme (Elad *et al.,* 1982). The present study was carried out to identify the antagonistic potential of *Trichoderma* mutants against *Sclerotium rolfsii* and *Macrophomina phaseolina* isolated from groundnut and mungbean crops. It was also found that there is a large variety of volatile secondary metabolites produced by *Trichoderma* such as ethylene, carbon dioxide, hydrogen cyanide, aldehydes and ketones which play an important role in controlling many plant pathogens (Vey *et al.*, 2001; Faheem *et al.*, 2010; Nagendra *et al.*, 2011, Pradeep Kumar *et al.*, 2012).

MATERIALS AND METHODS

In vitro antagonistic potential of total fifteen *Trichoderma* mutants were evaluated by dual culture technique (Morton and Stroube, 1955) against both test pathogens *Sclerotium rolfsii* and *Macrophomina phaseolina* isolated from groundnut and mungbean crops. The growth of pathogen and antagonist were recorded after 7th day of inoculation. For investigation of volatile antibiotic compound released by *Trichoderma* mutants, the antagonist and pathogen of the same age were grown separately in Petri plates containing PDA. The Petri plates containing pathogens were inverted over the Petri plates of antagonist. Edges of Petri plates were sealed with adhesive cellophane tape keeping the pathogen upside down. The colony diameter of the pathogen was measured daily up to seven days and per cent inhibition was calculated according to Vincent (1947).

RESULTS AND DISCUSSION

The data presented in table 1 indicated that the inhibitory ability of *Trichoderma* mutants was within the range of 64.17 to 90.42% against *Sclerotium rolfsii* and 56.67-84.58% against *Macrophomina phaseolina.* Maximum inhibition of 90.42% and 90% was recorded by M_{10} and M_6 mutants whereas minimum 64.17% was recorded by M_9 and M_{14} followed by M_1 (66.67%) whereas the inhibiting ability of ten mutants was obtained in the range of 70 to 81.67% against *Sclerotium rolfsii*. Mutant M_3 recorded maximum 84.58% inhibition and M_5 recorded minimum 56.67% inhibition and remaining were in the range of 58.33 to 77.50% against *Macrophomina phaseolina.* (Plate 1 and 2).

Volatile compounds release by different *Trichoderma* mutants and mother cultures significantly restricted the growth of test pathogens (Table 2, Plate 3). The comparison of growth inhibition by volatile compound of *Trichoderma* mutants revealed that, maximum efficiency was shown by M_6 i.e. 70% on third day of inoculation followed by M_2 (67.78%) against *Sclerotium rolfsii*. Studies on the effect of volatile compound released by *Trichoderma* mutants against *Macrophomina phaseolina* revealed that the maximum efficiency was shown by M_2 i. e. 51.48% followed by 49.63% and 48.15% on third, fifth and sixth day of inoculation respectively. Maximum i.e. 41.85% inhibition was recorded by M_6 on fifth day of inoculation followed by 40.37% on third and fourth day.

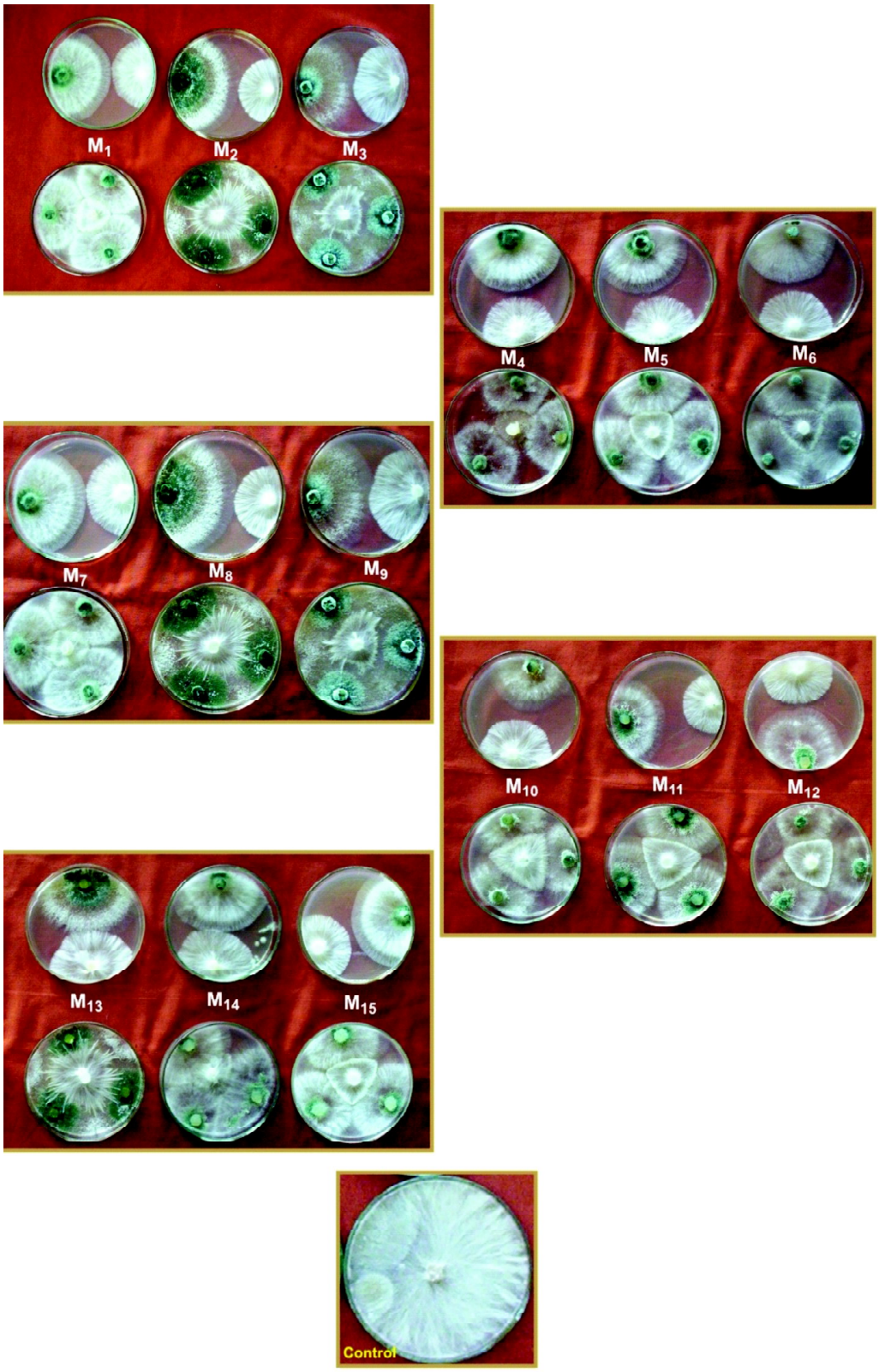

Plate 1: Antagonistic effect of *Trichoderma* mother culture and mutants against *Sclerotium rolfsii*

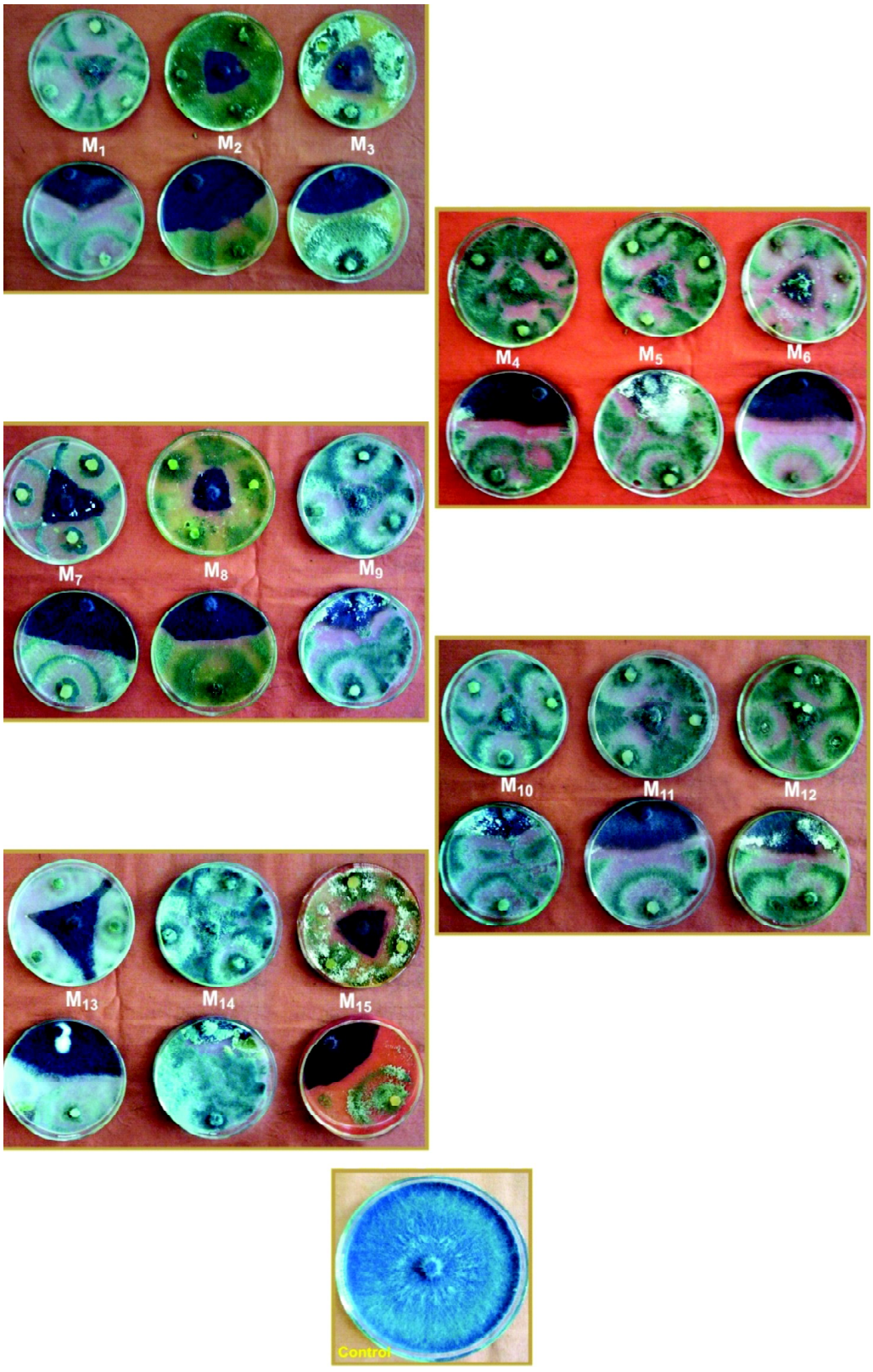

Plate 2: Antagonistic effect of *Trichoderma* mother culture and mutants against *Macrophomina phaseolina*

Table 1
Effect of *Trichoderma* mutants on radial mycelial growth of *Sclerotium rolfsii* and *Macrophomina phaseolina*

Trichoderma mutants	*Sclerotium rolfsii*		*Macrophominaphaseolina*	
	Mycelial growth (mm)	*% inhibition*	*Mycelial growth (mm)*	*% inhibition*
M_1	26.67	66.67	23.67	70.42
M_2	14.67	81.67	32.00	60.00
M_3	18.00	77.50	12.33	84.58
M_4	18.67	76.67	18.67	76.67
M_5	20.00	75.00	34.67	56.67
M_6	8.00	90.00	22.67	71.67
M_7	18.67	76.67	27.00	66.25
M_8	21.00	73.75	22.33	72.08
M_9	28.67	64.17	18.00	77.50
M_{10}	7.67	90.42	19.00	76.25
M_{11}	16.00	80.00	21.00	73.75
M_{12}	16.00	80.00	19.67	75.42
M_{13}	24.00	70.00	30.00	62.50
M_{14}	28.67	64.17	33.33	58.33
M_{15}	17.00	78.75	21.00	73.75
Control	80.00		80.00	
SE (m)±	0.76		0.88	
CD(P=0.01)	2.91		3.35	

Table 2
Growth inhibition (%) of *Sclerotium rolfsii* and *Macrophomina phaseolina* by volatile compounds released by *Trichoderma* mutants

Mutants	*Days*	*Inhibition (%) over control*	
		Sclerotium rolfsii	*Macrophominaphaseolina*
M_1	1	48.57	27.56
	2	14.55	12.37
	3	30.00	8.52
	4	27.78	7.04
	5	24.44	8.15
	6	24.44	5.19
M_2	1	34.29	29.49
	2	52.73	29.57
	3	67.78	51.48
	4	64.44	47.41
	5	61.11	49.63
	6	55.56	48.15
M_3	1	40.00	33.97
	2	14.55	25.81
	3	24.44	27.41
	4	13.33	15.56
	5	5.56	15.56
	6	0.00	14.07
M_4	1	34.29	30.77
	2	25.45	19.35
	3	52.22	28.89
	4	51.10	25.19
	5	50.00	26.30
	6	50.00	25.56
M_5	1	45.71	30.13
	2	25.45	13.44
	3	30.00	22.96
	4	22.22	17.78
	5	16.67	19.26
	6	12.22	8.15
M_6	1	57.14	33.33
	2	65.45	26.88
	3	70.00	40.37
	4	66.67	40.37
	5	61.10	41.85
	6	61.11	39.26
M_7	1	51.43	28.85
	2	47.27	9.68
	3	62.22	30.37
	4	37.78	19.26
	5	23.33	19.26
	6	18.89	15.56

contd. table 2

Mutants	Days	Inhibition (%) over control	
		Sclerotium rolfsii	*Macrophominaphaseolina*
M_8	1	42.86	24.36
	2	34.55	25.27
	3	51.11	35.56
	4	42.22	30.00
	5	38.89	28.52
	6	35.56	30.74
M_9	1	48.57	15.38
	2	36.36	4.07
	3	43.33	18.89
	4	33.33	17.41
	5	26.67	16.30
	6	23.33	17.78
M_{10}	1	60.00	26.92
	2	60.00	14.52
	3	65.56	21.48
	4	63.33	17.78
	5	60.00	19.63
	6	53.33	18.89
M_{11}	1	54.29	19.23
	2	38.18	4.83
	3	46.67	12.96
	4	25.56	11.85
	5	21.11	10.74
	6	13.33	9.63
M_{12}	1	57.14	15.38
	2	34.55	5.50
	3	54.44	7.04
	4	51.11	0.37
	5	42.22	1.11
	6	38.89	0.74
M_{13}	1	45.71	15.38
	2	30.91	6.65
	3	38.89	3.70
	4	32.22	1.48
	5	23.33	0.74
	6	20.00	1.11
M_{14}	1	40.00	28.85
	2	40.00	27.96
	3	32.22	35.93
	4	25.56	24.81
	5	13.33	24.44
	6	8.89	18.15
M_{15}	1	60.00	26.92
	2	23.64	13.44
	3	46.67	23.70
	4	44.44	19.26
	5	38.89	17.10
	6	36.67	18.52
SE (m)±		4.07	1.56
CD(P=0.01)		12.37	4.75

It was observed that maximum growth inhibition of both the pathogens i.e. *Sclerotium rolfsii* and *Macrophomina phaseolina* was exhibited on third day of inoculation and the maximum inhibition of *Sclerotium rolfsii* was recorded by mutant M_6 (70%) followed by M_{10} (65.56%) and M_7 (62.22%). Maximum inhibition of *Macrophomina phaseolina* was recorded by M_2 i.e. 51.48% followed by M_6 40.37% respectively. Regarding first day of inoculation maximum inhibition of *Sclerotium rolfsii* was recorded by M_{10} and M_{15} i.e. 60% followed by M_6 and M_{12} (57.14%). The volatile compounds of all the *Trichoderma* mutants and mother culture inhibited the growth of *S. rolfsii* and *M. phaseolina* but degree of inhibition varied with type of antagonist. *In vitro* conformation test like dual plate can be coupled to extracellular enzyme and protein analysis because they play a important role in inactivation of fungal plant pathogens. *Trichoderma* species excrete a large number of chitinolytic and glucanoltic enzymes, degrading cell walls and hence playing a key role in mycoparasitism. Similar observations have been recorded by Tapwal *et al.* (2004) with *Trichoderma* and

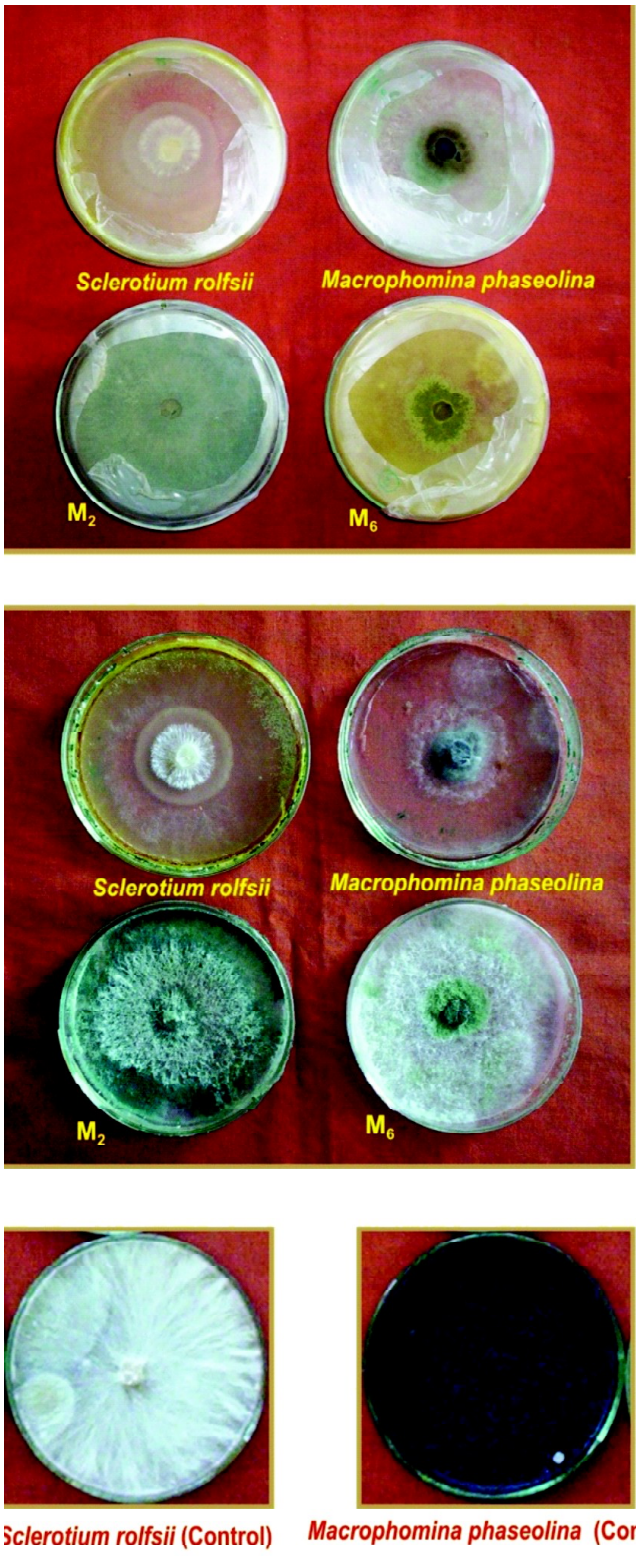

Plate 3: Effect of volatile compound released by *Trichoderma* on growth of *Macrophomina phaseolina* and *Sclerotium rolfsii*

Gliocladium virens against *Dematophora necatrix* and recorded that the volatile metabolites of *T. viride* were most effective against *F. oxysporium* and least against *R. solani* and almost reverse situation was observed when non volatile metabolites were examined. Baiswar *et al.*(2006) tested four biocontrol agents *viz*., *Trichoderma viride*, *T. harzianam*, *T. virens* and *Aspergillus terms* against *Penicillium gladioli* and *Aspergillus niger* causing corn rot of gladiolus during storage and observed that mycoparasitism and production of volatile compounds were the prominent mechanisms of biocontrol by *Trichoderma* species. *A. terms* was least effective against both the pathogens. Rini and Sulochana, (2007) observed that out of twenty-six local isolates of *Trichoderma* spp. and 56 isolates of fluorescent pseudomonads from Kerala were evaluated for their antagonistic activity against *R. solani* and *F. oxysporum in vitro* conditions, different isolates showed varying degrees of antagonism. Pan and Bhagat (2008) stated that *Trichoderma* isolates have strong selectivity in their antagonistic potential towards a particular pathogen. Similar results were also recorded by Patil *et al.* (2009).When studied for their potential to be used together as biological control agents of plant pathogens, the volatiles emitted by *Trichoderma viride* increased the expression of a primary biocontrol gene of *Pseudomonas fluorescens* (Lutz *et al.*, 2004). Despite some methodological and technological constraints, researchers have detected and characterized approximately 250 fungal VOCs, many of which have characteristic odors and are produced during primary and secondary metabolism. Fungal VOCs may contribute to a controversial medical diagnosis called "sick building syndrome" and may also be important in the success of some biocontrol species of *Trichoderma*. (Morath *et al.*, 2012).

Barakat *et al.* (2013) observed that antagonistic fungi naturally occurring on faba bean leaf surface were isolated and evaluated for their activity as bioagents for *Botrytis fabae* the causative agent of chocolate spot disease. Thirty isolates were purified

and identified as 26 isolates of *Trichoderma* species (*Trichoderma album, T. aureoviride , T. hamatum*, *T. harzianum* and *T. viride*) and 4 isolates belonging to the genera of *Cladosporium, Gliocladium, Epicoccum* and *Paecilomyces*. The inhibitory effect of *Trichoderma* spp. ranged between 51.11 - 77.78%. Bandopadhyay *et al.* (2008) recorded that production of volatile components from *T. viride* species of *Gliocladium* and *Aspergillus niger* could inhibit the sclerotial development of *M. phaseolina.*

Production of antibiotics and degree of reduction of both mycelial and sclerotial growth of pathogen varied with type and age of antagonist. Strains of *Trichoderma* species against a wide range of challenging pathogens, their mechanism of action, survivability and rhizospheric competence with the changing climate, compatibility with the various components of integrated pest management, quality formulations and field success are the major issues which need to be further studied intensively.

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