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EFFECT OF FUNGAL AND BACTERIAL ANTAGONISTS ON *MACROPHOMINA PHASEOLINA*(TASSI. GOID) CAUSING ROOT ROT IN GREEN GRAM

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Abstract: Fifteen fungal and seven bacterial antagonists were tested for their antagonism against *Macrophomina phaseolina* (Tassi. Goid) causing root rot in green gram by dual culture technique. The effect of fungal antagonists on sclerotia of *M. phaseolina* was also studied. Among the antagonistic fungi tested *Trichoderma viride* I_1 (native isolate) and 7". *harzianum* I_1 (native isolate) caused lower growth of the pathogen (42.33 and 44.25 mm) in dual culture. All the antagonists tried were found to reduce the number of sclerotia produced by the pathogen. However, *T. viride* I_3 caused appreciable reduction in the number of sclerotia produced by the pathogen (69.0 per 9 mm disc) and it also caused the maximum reduction in the sclerotial size (70.3µm) of the pathogen. Sclerotial germination was also the least (39.70%) with *T. viride* I_3treatment.

Key words: Antagonists, green gram, *Macrophominaphaseolina.*

INTRODUCTION

Green gram (*Vigna radiata* L.) is a leguminous crop grown for its protein rich seeds. Due to its rapid growth and early maturity, the green gram is adopted to multiple cropping systems in drier and warmer climates in lowland tropics and subtropics (Poehlman, 1991). Among the various diseases attacking green gram, root rot caused by *M. phaseolina* is of prime importance and it results in considerable reduction in yield and loss to the growers. Losses up to 60 per cent have been estimated due to the disease in green gram (Deshkar *e1 al.*, 1974). Chemical control of the pathogen was recommended (Kotasthane and Agrawal, 1976). But *M. phaseolina* was reported to have developed resistance to some of the fungicides (Anilkumar and Shastry, 1979). Biological control of plant pathogens was suggested as an alternative method to chemical control (Baker and Cook, 1974). Though the concept of biological control of plant diseases has been conceded much earlier, the realization of hazards caused by chemical on the ecosystem has generated a renewed interest in this field. Hence, *in vitro* studies were carried out with an aim to develop a commercial product of antagonists by selecting effective fungal and bacterial antagonists against *M. phaseolina* causing root rot in green gram.

MATERIALS AND METHODS

The fungal cultures listed in Table 1 obtained from the sources shown against them and maintained in the Department of Plant Pathology, AC & RI, Madurai were tested for their antagonism to *M. phaseolina.*

The fungal antagonists were tested for their antagonism against *M. phaseolina* by dual culture technique (Dennis and Webster, 1971). The fungal antagonists and *M. phaseolina* were grown on PDA medium for three days. A disc of 10 mm the test fungus was then cut out from the periphery of the colony and placed on one end of petridish containing 20 ml PDA medium. A similar disc of the pathogen was placed at the opposite end approximately 1.5 cm away from the edge of the plate. The plates were incubated for 96 h. The radial growth of the pathogen and the test fungi was measured.

Four mycelial discs of the pathogen (10 mm) were taken from each petridish at the place where the pathogen and antagonists interact 96 h after plating. The discs were placed in a beaker containing 10 ml of sterile distilled water and stirred for 30 min to separate the sclerotia from the medium. The entire contents were squeezed through cheesecloth and washed in several changes of distilled water and transferred to a glass vial containing 2.5 ml of 2.5 per cent ammonium sulphate. The sclerotia floated after 10 min. They were filtered through a filter paper and rinsed with distilled water. The sclerotia were counted with a stereo zoom microscope. Suitable control was maintained (Dhingra and Sinclair, 1975). From each dual culture plate, 25 sclerotia were harvested after seven days, dried for two hours in shade and the size was measured with a calibrated ocular micrometer.

From each dual culture plate, 25 matured sclerotia were taken and tested by slide germination method. They were incubated in moist chamber for 24 h. The number of germinated

Table 1. Fungal antagonists tested *in vitro* against *M. phaseolina*

Sl. No.	Antagonists	Source
1	*Trichoderma harzianum* Isolate I_1	Department of Plant Pathology, AC & RI, Madurai
2	*T. harzianum* I_2	Indian Cardamom Research Institute, Myladumpara
3	*T.koningii* Ij	No. 2170, ITCCF, I.A.R.I, New Delhi
4	*T. koningii* I_2	Kerala Agricultural University, Trichur
5	*T. viride* I_1	Tamil Nadu Agricultural. University, Coimbatore
6	*T. viride* I_2	Indian Cardamom Research Institute, Myladumpara
7	*T. viride* I_3	Department of Plant Pathology, AC & RI, Madurai
8	*T. viride* I_4	Agricultural Research Station, Virinchipuram
9	*Laetisaria arvalis*	Indian cardamom Research Institute, Myladumpara
10	*T. pileatus*	Department of Plant Pathology, AC&RI, Madurai
11	*T .longibrachiatum*	Centre for Advanced Studies in Botany, Univ. of Madras
12	*T, hamatum*	IARI, New Delhi
13	*T .piluliferum*	IARI, New Delhi
14	*T .reesei*	No. 2170, ITCCF, IARI, New Delhi
15	*T. pseudokoningii*	IARI, New Delhi

Table 2. *In vitro* effect of fungal antagonists on the growth of *M. phaseolina*

Sl. No.	Antagonist	Mycelial growth (mm)*		Inhibition over control (%)
		Antagonist	*M. phaseolina*	
1	*Trichoderma harzianum* Isolate I]	45.75	44.25	50.83
2	*T. harzianum* I_2	33.13	56.87	36.81
3	*T. koningii* \\	35.73	54.07	39.92
4	*T. koningii* I_2	36.43	53.57	40.48
5	*T. viride* I_1	33.27	56.73	36.91
6	*T. viride* I_2	34.13	55.87	37.92
7	*T. viride* I_3	47.67	42.33	52.97
8	*T. viride* I_4	33.63	56.37	37.37
9	*7".pileatus*	41.13	48.87	45.70
10	*T. longibrachiatum*	39.30	50.70	43.67
11	*T. hamatum*	41.77	48.23	46.41
12	*T. piliilifenun*	31.73	58.27	35.26
13	*T. reesei*	30.33	59.67	33.70
14	*T. pseudokoningii*	37.83	52.17	42.03
15	*Laetisaria arvalis*	32.37	57.63	35.97
16	Control	--	90.00	–

*Mean of three replications; CD (0.05) = 1.93

sclerotia and germ tubes put forth by each sclerotium was counted using a microscope (Montgomerg and Moore, 1938).

The cultures of bacterial antagonist were grown on nutrient agar medium (Rangaswami, 1972). Their antagonism against *M. phaseolina* were tested by the same method described for fungal antagonists except that in this case 1 cm long streak on antagonist was used instead of the culture disc. The plates were inverted and incubated at room tempera-

ture (28 ± 2°C) for 96 h. The growth of the pathogen, antagonistic bacteria and the width of the zone of inhibition, if any were recorded.

RESULTS AND DISCUSSION

The results obtained from the *in vitro* studies were presented in Table 2 to 4. Among the 15 antagonistic fungi screened for their effect on *M. phaseolina*, *T. viride* [3 and *T. harzianum* I_1 caused lower growth of the pathogen (42.33 and 44.25) in dual cultures. The next effective antagonist *T. hamatum* caused 48.23 mm growth of the pathogen. Full coverage of petriplates with the pathogen was observed in control. All the antagonists tried were found to reduce the number of sclerotia produced by the pathogen per unit area and they differed significantly. However, *T. viride* I_3 caused appreciable reduction in number of sclerotia produced by the pathogen (69.0 per 9 mm disc) followed by *T. harzianum* I_1, while in control the number of sclerotia produced by the pathogen was 162.33 per 9 mm disc. *T. viride* I_3 also caused maximum reduction (13.21%) in the sclerotial size (70.3μm) of the pathogen, while in control the sclerotial size of the pathogen was 81.0 μm. Sclerotial germination was also the least (39.70%) with *T. viride* I_3 treatment. Other antagonists also reduced the germination of sclerotia and in control, sclerotial germination was 97.3 per cent. *T. viride* I_3 significantly reduced the number of germ tubes (7 per sclerotium) produced by the sclerotia of the pathogen. The number of germ tubes per sclerotium of the pathogen was 18.0 in control.

Table 3. *In vitro* effect of fungal antagonists on sclerotia of M. *phaseolina*

Sl. No.	Antagonists	SN	DSN (%)	SS* (μm)	DSS (%)	SG (%)*	DSG (%)	GT	DGS
1	*T. viride* I_3	69.0	57.49	70.30	13.21	39.7 (39.06)	59.20	7.0	61.11
2	7. *harzianum* I_1	76.0	53.18	71.00	12.35	42.3 (40.57)	56.53	10.0	44.44
3	*T .koningii*	92.00	43.33	77.60	4.19	51.0 (44.57)	47.58	12.5	30.55
4	*T. pseudokoningii*	117.33	27.72	79.00	2.47	47.7 (43.68)	50.98	13.6	24.44
5	*T.pileatus*	106.33	34.50	76.00	6.17	49.7 (44.83)	48.92	14.7	18.33
6	7".*longibrachiatum*	120.67	25.66	72.00	11.11	44.7 (41.96)	54.06	11.3	37.22
7	*T. hamatum*	119.33	26.49	76.50	5.56	40.3 (39.41)	58.58	12.3	31.67
8	Control	162.33	--	81.00	--	97.3 (80.54)	--	18.	--
	CD (0.05)	4.03		1.02		1.03		2 71	

SN = Sclerotial number per 9 mm disc; DSN = Decrease of sclerotial number over control; SS = Sclerotial size; DSS = Decrease of sclerotial size over control; SG = Sclerotial germination; DSG = Decrease in sclerotial germination over control; GT = Germ tube per sclerotia; DGS'= Decrease in germ tube per sclerotia; *Mean of three replications; Data in parentheses are arc sine transformed values

Table 4. *Invitro* effect of bacterial antagonists on growth of M. *phaseolina*

Sl.No.	Antagonistic bacteria	Growth (mm)*		Width of antagonistic zone (mm)
		Antagonistic bacteria	*M. phaseolina*	
1	*Pseudomonas fluorescens* - I_1	29.3	59.40	1.30
2	*P. fluorescens* - I_{10}	–	90.00	0:00
3	*P. fluorescens* - I_7	–	90.00	0.00
4	*Bacillus subtilis*	43.0	43.00	3.30
5	*P. fluorescens* - I_{27}	30.7	57.60	1.70
6	*P. fluorescens* - I_{13}	15.0	75.00	0.00
7	*P. fluorescens* - I_2	6.3	82.70	0.00
8	Control	–	90.00	–
	CD (0.05)		1.73	

*Mean of three replications

Observations of Elad *et al.* (1987) revealed that *T. harzianum* I_1 coils around the hyphae of *R. solani* in the interaction zone in dual culture. Claydon *et al.* (1987) demonstrated that the potent inhibitory effect of *T. harzianum* against wide range of soil borne fungi might be due to the production of volatile metabolite *viz.*, 6-n-pentlyl-2 hyran-2-one. Ramasingh *et al.* (1993) reported that *T. viride* and *T. harzianum* exhibited strong antagonism against *M. phaseolina* of green gram. They also reported that *T. viride* showed antibiosis mechanism against the pathogen while *T. harzianum* exhibited hyperparasitism as the mechanism of inhibition of pathogen. Elad *et al.* (1983) stated that the sclerotial production and viable propagules of *M. phaseolina* were inhibited by *T. harzianum.*

Among the bacterial antagonists tested for their inhibitory effect on *M. phaseolina, Bacillus subtilis* caused significantly lower growth (43.00 mm) of the pathogen when compared to other antagonistic bacteria. *Pseudomonas fluorescens* I_{27} stands next where the mean mycelial growth of the pathogen was 57.60 mm as against 90 mm in control. The failure of the native strains of *P. fluorescens* in effectively inhibiting the growth of *M. phaseolina* was reported by Jacob (1989). But Ganesan and Gnanamanickam (1987) observed that native strains of *P. fluorescens* restricted the mycelial growth of *S. rolfsii* and provided protection against the pathogen. Saxena and Saxena (1995) reported that good zone of inhibition of *Rhizoctonia* by *Pseudomonas* sp. However, Weller (1988) pointed out that the strains of *P. fluorescens* differed in their rhizosphere competence and antagonistic activity. The present study indicates the existence of difference in antagonistic activity among the antagonistic fungi and bacteria. It also emphasizes the need to select specific antagonistic fungi and bacteria against a specific pathogen for the effective disease management.

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