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Management of blackgram root rot caused by *Macrophomina phaseolina* by antagonistic microorganisms

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Abstract: Biological control of *Macrophomina phaseolina* (Tassi.) Goid in blackgram was evaluated under glass house and field conditions. The antogonistic organisms used were *Trichoderma viride* and *T. harzianum* in biomass and in different carriers like talc and gypsum and along with *Rhizobium*. The incidence of root rot in blackgram was significantly reduced by 50% when treated with *Trichoderma* spp. alone or in combination with biofertilizer both under glass house and field conditions. The root length, shoot length, grain yield and nodulation significantly increased with the *T.harzianum* (biomass) + *Rhizobium* treated seeds (22.26 cm), *T.viride* (biomass) - Rhizobium treated seeds (36.93 cm), *T. harzianum* (gypsum formulation) - *Rhizobium* (661.66 kg/ha) and *T.viride* + *Rhizobium* (22.33 nodules/plant) respectively.

Key words : Biological control, Macrophomina phaseolina, Antagonists.

Introduction

Blackgram (*Vigna mungo* (L.) Hepper) is one of the important pulse crops gaining importance all over the world in recent years. It is rich in proteins and contains amino acids higher quantities than any other cereals and pulses. It is affected by number of diseases caused by fungi, bacteria and viruses. Among them the root rot caused by *Macrophomina phaseolina* (Tassi.) Goid is becoming more serious because of its seed and soil borne nature. Root rot can be controlled by using the fungicides. But the use of chemicals may pose danger to the environment by polluting the ecosystem. Further, the seed treatment with fungicides does not protect the crop for a longer period. Under rainfed conditions, soil drenching with fungicides is uneconomical. Hence biological control method has been considered as a promising approach for the management of soil borne diseases. If a biocontrol agent has consistently high antagonistic activity against *M.phaseolina*, it can be successfully exploited within the framework of integrated disease management system. Hence this study was undertaken to investigate the management of root rot in blackgram using antagonistic fungi.

Trichoderma isolates multiplied in organic substances, such as coir pith, groundnut shell and pressmud reduced the root rot caused by *Macrophomina phaseolina* in mungbean (Raghuchander *et al.* 1993). Haque and Abdul Ghaffer (1992) reported that *T.viride*, *T.hamatum* and *Rhizobium meliloti* used as seed dressing or soil drench reduced *Macrophomina phaseolina* infection by more than 50% on 30 days old fenugreek seedlings. Application of *T.viride* and *T.harzianum* together with carbendazim reduced dry root rot of soybeans caused by *Rhizoctonia bataticola* (Vyas, 1994). Shanker and Jeyarajan (1995) reported that seed treatment with *T.harzianum* and *T.viride* significantly reduced the root rot incidence of sesamum to 10.1% and 12.8% respectively.

Materials and Methods

The lab experiments were carried out in the Department of Plant Pathology, TNAU, Coimbatore during 1995-97. The biological agents used in this experiment were *Trichoderma viride* and *Trichoderma harzianum*. They were mass multiplied by inoculating in 250 ml conical flask containing 50 ml of yeast molasses medium (molasses 30g, yeast extract 5g in one litre of distilled water and sterilized). The flasks were incubated at room temperature for 15 days. After incubation, the mycelial mats along with the spores were blended with talc powder using blender @ one litre of each suspension with 2 kgs talc and 3 kgs gypsum separately. To one kg of this mixture 5g of carboxy

Table 1. Effect of bio-control agents on root rot incidence (pot culture study)

Treatments	Diseases incidence (%) Sampling intervals (days)					No. of nodules/ plant (60 days)
	15	30	45	60	75	
Trichoderma viride (biomass)	0 (9.09)	5.50	28.16	34.83	39.43	9.66
T. harzianum (biomass)	0 (9.09)	4.83	29.83	36.16	38.16	10.33
Rhizobium	3.16 (10.16)	14.16	37.50	48.50	53.83	18.66
Talc	3.66 (10.96)	14.50	35.50	50.83	67.83	7.66
Gypsym	3.66 (10.96)	12.16	34.33	45.83	63.50	7.33
T. viride (talc)	0 (9.09)	2.50	15.50	31.50	37.66	10.66
T. harzianum (talc)	0 (9.09)	3.66	17.50	34.16	39.16	11.33
T. viride (gypsum)	0 (9.09)	2.33	14.16	30.50	36.83	9.33
T. harzianum (gypsum)	0 (9.09)	3.33	15.16	31.50	37.66	9.66
T. viride (biomass + *Rhizobium*	0 (9.09)	1.66	20.83	32.83	39.50	19.33
T. harzianum (biomass) + *Rhizobium*	0 (9.09)	3.33	23.16	33.00	40.83	18.33
T. viride (talc) + *Rhizobium*	0 (9.09)	2.16	14.83	30.16	39.83	21.66
T. viride (gypsum) + *Rhizobium*	0 (9.09)	3.33	14.83	29.66	39.33	19.66
T. harzianum (talc) + *Rhizobium*	0 (9.09)	3.16	17.16	31.66	40.50	20.66
T. harzianum (gypsum) + *Rhizobium*	0 (9.09)	4.16	16.83	33.66	41.33	19.33
Control	4.50 (12.20)	18.50	32.83	50.66	84.50	10.33
Mean	0.82	6.20	23.01	36.59	46.24	14.00
CD (P=0.05)	0.50	0.62	0.90	1.38	1.95	0.94

Figures in parentheses indicate mean transformed values

methyl cellulose was added and shade dried for 1-2 hours and packed in polybags. For the biomass, the mycelial mats along with the spores were harvested, shade dried, ground with pestle and mortar and stored in room temperature. These talc and gypsum-based formulations and the biomass of the antagonists were further used for the seed treatment.

Pot experiment

The pot culture experiment was conducted in the Department of Plant Pathology, TNAU, Coimbatore under glass house conditions during 1995-97. The experiment was conducted in completely randomized block design and all treatments were replicated thrice. The treatments were as follows:

Seed treatment with

T_1 *T.viride* (biomass) (4g kg of seed)
T_2 *T.harzianum* (biomass)
T_3 *Rhizobium* (30g/kg seed)
T_4 Talc
T_5 Gypsum
T_6 *T.viride* (talc formulation)
T_7 *T.harzianum* (talc formulation)
T_8 *T.viride* (gypsum)
T_9 *T.harzianum* (gypsum)
T_{10} *T.viride* (biomass) + Rhizobium
T_{11} *T.harzianum* (biomass) + Rhizobium
T_{12} *T.viride* (talc) + Rhizobium
T_{13} *T.viride* (gypsum) + Rhizobium
T_{14} *T.harzianum* (talc) + Rhizobium
T_{15} *T.harzianum* (gypsum) + Rhizobium
T_{16} control

Blackgram seeds (variety TMV-1) were treated with the fungal antagonist at the rate of 4g/kg seed as dry treatment. For the treatments

Table 2. Effect of bio-control agents on root rot incidence and biometric observations of blackgram

Treatments	Disease incidence (%) Sampling intervals (days) 15	30	45	60	75	Root length (cm)	Shoot length (cm)	Single plant dry wt. (g)	Tricho-derma popula-tion x 10^3 cfu/g	Grain yield (kg ha^{-1})	No.of nodules/ plant (60 days)
T. viride (biomass)	0 (2.02)	16.93	19.73	35.23	40.60	21.00	35.83	4.00	52.00	638.33	10.66
T. harzianum (biomass)	0 (2.02)	19.50	23.33	36.26	41.16	15.70	26.23	6.00	74.00	637.33	11.66
Rhizobium	0 (2.02)	26.93	28.10	37.10	69.50	21.50	34.00	6.00	21.00	640.33	20.00
Talc	13.66 (21.62)	25.76	28.76	36.50	64.83	17.83	30.50	6.36	53.00	531.33	9.33
Gypsym	13.16 (21.26)	24.50	30.33	39.50	67.83	15.83	34.10	5.10	17.66	539.33	9.66
T. viride (talc)	0 (2.02)	20.00	22.10	24.66	62.33	19.06	32.53	7.80	26.33	638.00	11.33
T. harzianum (talc)	0 (2.02)	21.50	23.20	23.83	45.00	20.96	24.53	6.26	26.66	645.66	11.66
T. viride (gypsum)	0 (2.02)	19.30	19.90	24.73	45.33	15.83	25.00	6.30	122.00	647.66	12.33
T. harzianum (gypsum)	0 (2.02)	20.86	26.33	27.50	44.16	18.66	36.16	4.80	152.00	652.00	12.33
T. viride (biomass + *Rhizobium*	0 (2.02)	18.00	24.83	27.33	40.93	16.73	36.93	7.30	31.00	642.00	21.00
T. harzianum (biomass) + *Rhizobium*	0 (2.02)	16.93	20.16	25.16	42.16	22.26	31.53	4.30	35.00	645.66	19.33
T. viride (talc) + *Rhizobium*	0 (2.02)	17.50	24.50	27.83	45.16	18.00	30.50	5.20	255.00	651.66	22.33
T. viride (gypsum) + *Rhizobium*	0 (2.02)	17.50	23.83	26.50	42.50	18.03	34.33	5.06	183.33	652.00	20.66
T. harzianum (talc) + *Rhizobium*	0 (2.02)	19.40	27.26	30.00	43.83	21.60	34.56	6.13	169.66	656.66	20.00
T. harzianum (gypsum) + *Rhizobium*	0 (2.02)	18.00	25.26	28.16	43.50	17.00	35.03	4.16	104.00	661.66	19.66
Control	0 (23.72)	18.60	35.33	53.00	91.83	18.93	32.23	4.26	18.66	538.33	10.66
Mean	16.26	20.07	24.70	31.45	50.68	18.68	32.43	5.58	81.95	626.14	15.16
CD (P=0.05)	1.05	0.97	1.95	2.28	3.33	0.64	1.46	0.31	6.70	5.84	1.15

Figures in parentheses indicate mean transformed values

in combination with *Rhizobium* (30g/kg seed) blackgram seeds with first treated with Rhizobium slurry prepared with rice kanji. It is then shade dried. After 24h of *Rhizobium* treatment, antagonist treatment @ 4g/kg of seed was given 24h before sowing. Root rot incidence was recorded at 15 days interval and number of nodules was recorded at maturity.

Field experiments

The field trial was conducted in randomized block design, each treatment with three-replication using plot size of 3x2m². The treatments used were as in pot culture experiment.

The seeds were sown in plots with 30x10 cm spacing. The root rot incidence was recorded at 15 days interval till harvest. After maturity (60 DAS) 10 plants were pulled out with entire root system intact from each plot carefully. The roots were washed with water and root length, shoot length, number of nodules, single plant dry weight and grain yield (kg/ha) were recorded.

Results and Discussion

The results revealed that, seed treatment with *Trichoderma* spp. alone or in combination with biofertilizer significantly reduced the root rot incidence by 50% both under glass house and field conditions when compared to control. At 15 DAS root rot incidence was minimum in *Trichoderma* treated plants (Table 1). At 60 DAS, minimum incidence of root rot was recorded in the treatment T_{13} (*T.viride* in gypsum formulation with *Rhizobium* treated seeds (29.6%)) followed by T_{12} (*T.viride* in talc formulation with *Rhizobium* treated seeds (30.16%)). However, the treatments *T.viride* in gypsum formulation (T_8) (36.83%) and *T.harzianum* in gypsum (T_9) (37.66%) was on par in controlling the root rot incidence at 75 DAS. Also there was no reduction in nodule formation in combined seed treatment of antagonist with *Rhizobium*. Similar results were obtained by Jeyarajan *et al.* (1991) that under pot culture condition root rot incidence in blackgram was reduced by *T.viride* and *G.virens* to 35% and 45% respectively against 70% in control. Robert *et al.* (1993) reported that seed treatment with *T.harzianum* and *T.viride* was more effective in controling *R.solani* in *Phaseolus vulgaris* both under greenhouse and field condition.

In field experiments, all the treatments in combination with the *Rhizobium* significantly reduced the root rot incidence when compared to control. At 15 DAS, disease incidence was minimum in all the *Trichoderma* treated plots. At 75 DAS, least incidence of root rot was recorded in treatment T_{10} *T.viride* (biomass) + *Rhizobium* (40.93%), *T.harzianum* (biomass) + *Rhizobium* (42.16%), (T_{11}) *T.viride* (gypsum) + *Rhizobium* (42.5%), (T_{13}) *T.harzianum* (gypsum) + *Rhizobium* (43.5%) and *T.harzianum* (talc) + *Rhizobium* (43.83). Maximum incidence of 93.13% was recorded in untreated control. The shoot length, root length, single plant dry weight, antagonist population and grain yield was maximum in the treatments T_{10}, *T.viride* (biomass) + *Rhizobium* (36.93 cm), followed by T_{11} - *T.harzianum* (biomass) + *Rhizobium* (22.26 cm), *T.viride* (talc) (7.8g), *T.viride* (talc) + *Rhizobium* (255 x 103 colonies/g of soil), *T.harzianum* (gypsum) + *Rhizobium* (661.66 kg/ha). The highest nodulation was registered in treatment T_{12}, *T.viride* + *Rhizobium* (22.33 nodule/plant) which was significantly higher than other treatments.

Similar results were reported by Ramakrishnan *et al.* (1994) that the talc based *T.viride* formulation was found to be effective in reducing the root rot incidence in urd bean. Muthamilan and Jeyarajan (1996) reported that the integration of *T.harzianum*, *Rhizobium* and carbendazim remarkably reduced the root rot of groundnut caused by *Sclerotium rolfsii*. Windham *et al.* (1986) concluded that *Trichoderma* species produced a growth regulation factor that increases the rate of seed germination and dry weight of the seeds. *Trichoderma viride* treatment increased the yield of blackgram by 97% was reported by Raguchander *et al.* 1993.

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