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BIO-EFFICACY OF NATIVE ANTAGONISTS AGAINST DRY ROOT ROT OF GROUNDNUT CAUSED BY *MACROPHOMINA PHASEOLINA*

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ABSTRACT

Groundnut grown under rainfed and irrigated conditions is subject to various diseases among which, *Macrophomina phaseolina* causing dry root rot is an important pathogen causing considerable yield loss. Management of *M. phaseolina* using chemical fungicides has been the prevailing control method for over fifty years. But the pathogen was reported to have developed resistance to fungicides and the pathogen is very difficult to be destroyed by drenching the soil with fungicides. Hence, the present investigation was conducted to test the efficacy of native antagonists along with *Glomus mosseae* (AM fungi) individually and as combination for the management of *M. phaseolina*. Among the native antagonist, *Trichoderma viride* isolate (Tv_7) and *Pseudomonas fluorescens* isolate (Pf_5) effectively inhibited the mycelial growth and sclerotia of *M. phaseolina* and showed compatibility between them. The combination of *T. viride* (Tv_7) and *P. fluorescens* (Pf_5) recorded the maximum germination, shoot and root length of groundnut. In the field studies the treatment combination involving seed treatment + soil application with combination of *T. viride* (Tv_3) and *P. fluorescens* (Pf_7) plus soil application of *G. mosseae* recorded the minimum incidence of root rot, maximum biometrics of groundnut.

INTRODUCATION

Groundnut (*Arachis hypogaea* L.) the 'king' of oilseeds is popularly called as wonder nut, poor men's cashew nut, earthnuts, goober peas, monkey nuts, pygmy nuts and pig nuts. It belongs to the family of Fabaceae and it contains the valuable source of all the nutrients It is a low priced commodity and is one of the most important food and cash crops of our country. Its plays a crucial role in the oilseed economy of India (Thamaraikannan *et al.,* 2009). The groundnut seed contain 45-50% oil, 27-33% protein as well as essential minerals and vitamins. The crop is affected by various diseases caused by fungi, bacteria and viruses. Of these pathogens, *Macrophomina phaseolina* (Tassi) Goid., is an important pathogen, distributed worldwide and groundnut at all stages are susceptible to infection. *M. phaseolina* attacks crop plants at different stages of plant growth and causes complex disease syndromes like root rot, seedling blight, charcoal rot, ashy stem blight, wilt, collar rot, dry rot, pod rot and seed rot in several crops (Ma *et al.,* 2010).

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In recent years, biocontrol has become a promising alternative to chemical control in the management of soil-borne diseases and has become one of the basic components in disease management practices (Karpagavalli and Ramabadran, 2001). Members of the genus *Pseudomonas* and *Trichoderma* have been known for their potential antifungal, plant growth promoting and plant defense inducing activities (Zaidi *et al*., 2004). Besides, in recent years, mycorrhizal fungi as symbiotic organisms have been used against plant pathogens successfully. Several studies indicated that, arbuscular mycorrhizal fungi (AMF) influenced fungal diseases caused by root pathogens (Trotta *et al.*, 1996; Karagiannidis *et al.*, 2002). Thus, biocontrol strains with different modes of actions in combination (Droby, 2001) may be used to increase the efficacy and the consistency of disease control. Hence, the present study was taken up with an objective of integrating AM fungi sp. *G. mosseae* along with *Trichoderma* species and *P.fluorescens* for the management of *M. phaseolina* causing dry root rot o groundnut which would be an economic and promising non chemical way to control *M. phaseolina* (Doley *et al.,* 2014).

MATERIALS AND METHODS

Isolation of pathogen and bio control agents: *M. phaseolina* was isolated from the infected Groundnut plants on potato dextrose

agar (PDA) medium. Biocontrol agents like *T. viride, P. fluorescens* were isolated from rhizosphere of various crops from different parts of Thiruvannamalai District. Isolation and maintenance of *Trichoderma* was done on Trichoderma specific medium (TSM) while King's Medium B was used for *P. fluorescens* following serial dilution technique.

Compatibility test between selected antagonists

Dual culture technique (solid medium): Compatibility among *T. viride, P. fluorescens* and *B. subtilis* was tested by following the dual culture technique (Dennis and Webster, 1971) and observed for the mycelial over growth of *T. viride* onto the PGPR isolates without forming any inhibition zone.

Dual culture in broth: The broth containing both the composition of PDA and KB (mixed at equal volume) was inoculated with the mycelial disc (9 mm diameter) of seven days old culture of *T. viride*. After one day a loopful of *P. fluorescens* isolates were inoculated separately into the broth and incubated for two more days. Similarly, the broth containing both the composition of PDA and NA (mixed at equal volume) was used to assess the compatibility of *T. viride* and *B. subtilis*. Third day after bacterial inoculation the observations on mycelial dry weight and bacterial population was assessed and recorded

Assay for groundnut plant growth promotion under *in vitro* condition by roll towel method: Plant growth-promoting activity of the antagonists was assessed based on the seedling vigour index by the standard roll towel method (ISTA, 1993). Twenty five seeds treated with culture filtrate of Tv_3, Pf_5, Tv_3+Pf_5 were kept over the presoaked germination paper. The seeds were held in position by placing another presoaked germination paper strip over it and gently pressed. The sheets along with seeds were then rolled and incubated in growth chamber for 10 days. Three replications were maintained for each treatment. The root length and shoot length of individual seedlings were measured and the per cent germination of seeds was also calculated. The seedling vigour index was calculated by using the formula as described by Abdul Baki and Anderson (1973). Vigour Index = (Mean root length + Mean shoot length) × Germination (%) Based on the results of the above studies, the most effective isolate of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) were tested for compatibility for to be used in combination against *M. phaseolina*.

Preparation of solid formulation of biocontrol agents: A loopful of *P. fluorescens* (Pf_7) isolates was inoculated into the sterilized KB broth and incubated in a rotary shaker at 150 rpm for 72 h. at room temperature (28 ± 2°C). After 72 h, 400 ml of bacterial suspension containing 9×10^8 cfu ml^{-1}, one kg of the carrier material (talc powder), 15 g calcium carbonate and 10 g CMC were thoroughly mixed, shade dried to reduce the moisture content below twenty per cent and packed in polythene bags (Vidhyasekaran *et al*., 1996).

The effective isolate of *T. viride* (Tv_3) was inoculated into molasses yeast broth (Papavizas *et al*., 1984) and incubated for 15 days. The biomass (containing 3×10^8 cfu ml^{-1}) along with the medium was homogenized and incorporated into the sterile talc powder carrier material @ 50 ml suspension per 100 g and thoroughly mixed with addition of 500 mg CMC (Jeyarajan *et al.,* 1994). The contents were shade dried for 12 h. and stored in polythene bags.

Determination of per cent *G. mosseae* infection: The roots of the peanut plants were analysed for *G. mosseae* infection by clearing and staining method of Philips and Hayman (1970). The mycorrhizal colonization was expressed using the following formula:

$$\text{Per cent root colonization} = \frac{\text{Number of root segments positive for colonizati on}}{\text{Number of root segments examined}} \times 100$$

The root segment was considered mycorrhizal even if one of the three structures, *i.e.,* hyphae, arbuscules or vesicles was present.

Integrated management of root rot of ground nut: Based on the best results obtained from the pot culture experiments, field trial were conducted in root rot prone farmer's field at Kuppam in Thiruvannamalai district of Tamil Nadu during Nov to Jan (Field trial) of 2015-2016, representing irrigated conditions by integrating the best treatments identified in the pot culture experiments. The blanket fertilizer schedule of 35:23:23 NPK ha^{-1} recommended by the State Agricultural Department was followed. A plot size of 5 × 4 m was used for each treatment. Each treatment was replicated thrice and a suitable control was also maintained. The variety VRI 2 was used in this study. Carbendazim 50 WP @ 2 g kg^{-1} as seed treatment was used for comparison. All the agronomic practices were followed as per the standard procedure as recommended by the State Agricultural Department.

Treatment schedule

T_1: *T. viride* (Tv_3) seed treatment (10.0 ml kg^{-1} of seed) + soil application (2.5 lit ha^{-1})
T_2: *P. fluorescens* (Pf_7) seed treatment (10.0 ml kg^{-1} of seed) + soil application (2.5 lit ha^{-1})
T_3: *T. viride* (Tv_3) + *P. fluorescens* (Pf_7) seed treatment (10.0 ml kg^{-1} of seed) + soil application (2.5 lit ha^{-1})
T_4: *T. viride* (Tv_3) seed treatment (10.0 ml kg^{-1} of seed) + soil application (2.5 lit ha^{-1})+ *G. mosseae* soil application @10 kg ha^{-1}
T_5: *P. fluorescens* (Pf_7) seed treatment (10.0 ml kg^{-1} of seed) + soil application (2.5 lit ha^{-1}) + *G. mosseae* soil application @10 kg ha^{-1}
T_6: *T. viride* (Tv_3) + *P. fluorescens* (Pf_7) seed treatment (10.0 ml $kg^{-1}$of seed) + soil application (2.5 lit ha^{-1}) + *G. mosseae* soil application @10 kg ha^{-1}
T_7: Carbendazim 50% WP as seed treatment @ 4.0 g kg^{-1}
T_8: Control

The incidence of root rot (per cent) was assessed periodically, plant height (cm), biomass of the plant (g), number of pods per plant and yield (kg) were recorded at harvest. The biomass of the plant was recorded after drying the plants in the hot air oven at 60°C until attaining a constant weight.

RESULTS AND DISCUSSION

Compatibility among native antagonists: The results depicted in table 1and 2 revealed that the native antagonists viz., *T.viride* over grew the *P.fluorescens* and *B.subtilis* without any inhibition zone thus indicating compatibility among them. The other combinations showed inhibition zone and were not compatible.

Table 1. Testing the compatibility among native antagonists (Dual culture-solid medium)

Fungal and Bacterial antagonist	Remarks
***T. viride* (Tv_7) *Vs P. fluorescens* (Pf_5)**	Compatible
***T. viride* (Tv_7) *Vs B. subtilis* (Bs_3)**	Compatible
***P. fluorescens* (Pf_5) *Vs B. subtilis* (Bs_3)**	Non compatible
***T. viride* (Tv_7) *Vs P. fluorescens* (Pf_5) *Vs B. subtilis* (Bs_3)**	Non compatible

Table: 2. Testing compatibility among the native antagonists (Dual culture-liquid medium)

Antagonist	Number of bacterial cells $\times 10^6$	Mycelial dry weight (mg)
***T. viride* (Tv_7)**	-	480.60
***P. fluorescens* (Pf_5)**	51.00	-
***B. subtilis* (Bs_3)**	43.06	-
***T. viride* (Tv_7)+ *P. fluorescens* (Pf_5)**	49.75	476.40
***T. viride* (Tv_7)+ *B. subtilis* (Bs_3)**	37.45	402.60

Table 3. Efficacy of antagonists on groundnut seed germination and plant growth promotion under *in vitro* conditions (Roll towel method)

Treatments	Seed germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
T. viride (Tv_7)	92.30^b	6.4^c	9.2^c	1439.88^c
P. fluorescens (Pf_5)	93.70^b	7.1^b	9.5^b	1555.42^b
B. subtilis (Bs_3)	90.40^c	6.2^d	9.0^d	1374.08^d
T. viride (Tv_7)+ *P. fluorescens* (Pf_5)	95.80^a	7.4^a	9.8^a	1647.76^a
Carbendazim 50% WP as ST @ 2.0 g kg^{-1}	95.60^a	7.1^b	9.5^b	1586.96^b
Control	89.90^c	4.1^e	5.1^e	827.08^e

Table 4. Bio-efficacy of native antagonists against dry root rot of Groundnut caused by *Macrophomina phaseolina*

Tr.No	Treatments	Plant height (cm)	Biomass (g)	Number of pod $plant^{-1}$	100 seed weight	Yield kg ha^{-1}	Root rot incidence (%)				Mean
							45 DAS	60 DAS	75 DAS	At harvest	
T_1	*T. viride* (Tv_3) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	50.10^e	29.67^e	32.41^e	38.49^e	2010^e	10.84^f	15.98^f	18.61^f	20.36^f	16.44
T_2	*P. fluorescens* (Pf_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	51.30^d	31.70^d	35.63^c	39.20^d	2125^d	09.40^e	14.33^e	16.44^e	18.44^g	14.65
T_3	*T. viride* (Tv_3) + *P. fluorescens* (Pf_7) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	51.52^d	32.00^c	34.12^d	40.10^d	2150^d	08.10^d	13.88^d	15.77^d	17.10^e	13.71
T_4	*T. viride* (Tv_3) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	52.42^c	32.55^c	34.70^d	41.33^c	2200^c	07.30^c	11.40^c	13.30^c	15.22^d	11.80
T_5	*P. fluorescens* (Pf_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	52.60^b	33.67^b	35.53^b	44.74^b	2250^c	05.40^b	09.44^b	11.11^b	13.25^c	09.92
T_6	*T. viride* (Tv_3) + *P. fluorescens* (Pf_7) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @10 kg ha^{-1}	54.70^a	34.10^a	36.83^a	45.40^a	2400^a	03.60^a	07.22^a	09.20^a	11.42^a	07.86
T_7	Carbendazim 50% WP as ST @ 4.0 g kg^{-1}	53.90^a	33.10^b	35.93^b	43.60^b	2350^b	06.66^b	10.80^b	12.30^b	14.35^b	11.02
T_8	Control	39.66^f	24.33^f	31.65^f	34.53^f	1990^f	11.21^g	18.75^g	26.50^g	27.70^h	21.04

Even in the liquid medium assay the population of both *T.viride* and *P.fluorescens* was not affected when co-inoculated in the same medium. Hence, the compatible isolates were used as consortium in the field studies.

Efficacy of antagonists on groundnut seed germination and plant growth promotion under *in vitro* conditions (Roll towel method): The Table 3 results revealed that among the various treatments the combination treatment involving *T. viride* (Tv_7)+ *P. fluorescens* (Pf_5) recorded the maximum seed germination (95.80%), shoot length (7.4 cm), root length (9.8 cm) and vigour index (1647.76) which was better than the chemical treatment. The untreated contro recorded the minimum values with regard to the germination and growth parameters.

Effect of combined application of antagonists and *G. mosseae* on dry root rot disease incidence of groundnut (Field trial): The results revealed that the treatment T_6 *T. viride* (Tv_3) + *P. fluorescens* (Pf_7) as ST @ 10 ml kg^{-1} of seed + SA @ 2.5 lit ha^{-1}) plus *G. mosseae* (SA @ 10 kg ha^{-1}) maintained its superiority over other treatments in reducing the root rot incidence to the minimum by recording an incidence of 3.60, 7.22, 9.20, 11.42 per cent dry root rot incidence at 45, 60, 75 and at harvest respectively. The chemical treatment recorded 14.35% while the control recorded 27.70% root rot incidence at harvest. The root rot incidence showed an increasing trend with an increase in the age of the crop in all the treatments and also control plots. Under irrigated conditions, *P. fluorescens* maintained its superiority over *T. viride* (Table 4). The results indicated that different plant colonization pattern and different mechanism of disease suppression elicited by the combination of *T. viride* (Tv_3), *P. fluorescens* (Pf_7) and *G. mosseae* might have offered greater protection to the groundnut plants against the attack of *M. phaseolina*. Besides, the reduction in the disease incidence might have occurred because of the reduction in the inoculum density of *M. phaseolina* through changes in the general

microbial balance as observed by Lukade (1992). The earlier results on the use of mixed inocula for the management of soil borne diseases also have proved that the use of mixed inocula of mycorrhizal symbionts and biocontrol agents can be more effective than the use of a single species. Kavitha *et al.* (2003) also opined that dual inoculations (*i.e.,* use of two biological control entities other than the pathogen) have been found more effective in disease suppression than single inoculations. Kanchan Singh (2010) reported that inoculations of AM fungi in combination with *P. fluorescens* and mustard oilcake showed best results against root rot disease besides increasing growth parameters of cluster bean. In general, disease severity could be reduced by root colonization of AMF via several mechanisms including increasing the mineral absorption and increased plant growth (Smith and Read, 1997), phenolic compounds (Devi and Reddy, 2002) and pathogenesis-related proteins (Pozo *et al.,* 1999). Also AMF fungi increases lignin content in root system (Ziedan *et al.* 2010) which could have been attributed as the reason for the reduced incidence of root rot in groundnut observed in the present study. O'Dowling and O'Gara (1994) listed as many as 38 secondary metabolites produced by *Pseudomonas* spp. involved in the suppression of various diseases. Similarly, *Trichoderma* spp. also produces a variety of antifungal metabolites including antibiotics (Worasatit *et al*., 1994) and cell wall degrading enzymes that differ from those produced by *Pseudomonas* spp. The combined activity of these strains with different antifungal compounds might have expanded the spectrum of biocontrol activity and brought about the reduction in the disease incidence.

Effect of combined application of antagonists and *G. mosseae* on the biometrics of groundnut (Field trial): Generally all the treatments with antagonists and *G. mosseae* showed increased growth and yield parameters when compared to control. Among the various treatments, the treatment consisting of "basal application of *G. mosseae* @ 10 kg ha^{-1} + soil application (2.5 lit ha^{-1}) + seed treatment (10 ml $kg^{-1}$of seed) of *P. fluorescens* (Pf_7) + *T. viride* (Tv_3) combination" recorded the maximum plant height (54.70 cm), biomass content (34.10 g), number of pods per plant (36.83). This was followed by the treatment consisting of seed and soil application of *P. fluorescens* (Pf_7) + combined with *G. mosseae*. The untreated control recorded the minimum plant height (39.66 cm), biomass (24.33) and number of pods per plant (31.65) (Table 4). The growth hormones and metabolites produced by the combination of antagonists *T. viride* (Tv_3) + *P. fluorescens* (Pf_7) + *G. mosseae* would have exerted a synergism in promoting the plant growth parameters and enhancing yield of groundnut. Synergistic effects on plant growth under several conditions when coinoculated with biocontrol agents and AMF are reported (Vivas *et al.*, 2003; Artursson *et al.*, 2006). Similarl, to the results obtained in the present study Marulanda *et al.* (2008), also reported that *Bacillus megaterium* inoculated with *G. intraradices* showed the highest percentage of root length of *Lactuca sativa* plants compared to the single inoculation of *G. intraradices*. Combined inoculation of AM fungi and phosphate-solubilizing bacteria *Bacillus polymyxa* and *Azospirillum brasilense* resulted in maximum growth response (Muthukumar and Udaiyan, 2006). Sukhada *et al.* (2010) found that application of *G. mosseae* + *T. harazianum* to banana field soil infested by *F. oxysporum* f. sp. *cubense* improved plant height and reduced the population of *Fusarium*. The ability of *P. fluorescens* and AMF to promote plant growth by improved nutrient acquisition and suppression of soil borne pathogens is also well documented (Gamalero *et al.*, 2003; Yusran *et al.*, 2009). AMF could have also facilitated mineral and water uptake, and increased the defense against *M. phaselina* (Smith *et al.*, 2001; Marulanda *et al.*, 2008).

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