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IN VIVO EVALUATION OF AM FUNGI ON DRY ROOT ROT DISEASE INCIDENCE AND BIOMETRICS OF GROUNDNUT

***Rajamohan, K. and Balabaskar, P.**

Department of Plant Pathology, Faculty of Agriculture, Annamalai Univertsity, Annamalainagar-608002

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ABSTRACT

Groundnut (Arachis hypogaea L.) is an important oilseed crop in Indian continent. Groundnut is being infected by several fungal, bacterial and viral diseases but dry root rot caused by *Macrophomina Phaseolina* (Tassi) Goid. is considered as the most devastating disease in all the groundnut growing areas of Cuddalore. Management of *M. phaseolina* using chemical control is arduous and uneconomical. Hence, the present study was conducted to assess the effect of native antagonists in combination with potential AMF (*G. mosseae*) for the successful non chemical management of root rot of groundnut under field conditions. The results revealed that among all the treatments tested, the treatment T_6 viz., *T. viride* (Tv_7) + *P. fluorescens* (Pf_5) as ST @ 10 ml kg^{-1} of seed + SA @ 2.5 lit ha^{-1}) plus *G. mosseae* (SA @ 10 kg ha^{-1}) recorded the least root rot incidence and also enhanced the biometrics of groundnut to the maximum under field conditions in both the field trials tested.

INTRODUCTION

Groundnut the 'king' of oilseeds is popularly called as wonder nut and poor men's cashew nut. While being a 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. The peanut, or groundnut (*Arachis hypogaea* L.), a species in the legume or "'bean" family (Fabaceae) was probably first cultivated in the valleys of Peru (World Geography of the Peanut, 2004). Groundnut is grown under rain fed and as well as irrigated conditions. 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. The fungus causes complex disease syndromes like charcoal rot, root rot, seedling blight, foliage blight, dry rot, pod rot, seed rot and causes considerable yield loss (Raguchander *et al.,* 1993).

Corresponding author: **Rajamohan, K.,**
Department of Plant Pathology, Faculty of Agriculture, Annamalai Univertsity, Annamalainagar-608002.

An early sign of stem infection is the presence of brown lesions at the base of the plant and where the branches join the main stem and ultimately the stem turn ashy-grey, often with small black minute (microsclerotia) within the affected area. Infected plants usually die prematurely. Yield can be significantly reduced. It generally occurs after flowering during a period of heat/ moisture stress, and results from infection of the roots by soil-borne microsclerotia. High temperature and moderately wet weather conditions favour disease. Disease severity increases with the increasing in temperature with optimum at 30-35˚C (Grover and Sakhuja, 1981). Management of *M. phaseolina* using chemical control is arduous, uneconomical and not advisable owing to the risk of ground water pollution, heavy metal toxicity, and death of non-target beneficial micro flora and evolution of fungicidal resistant pathogen variants (Rauf, 2000).

Meeting the future challenge for productive, but sustainable agriculture will require the use of all strategies that are effective, economical, safe and compatible. 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). Further, the biocontrol agents also improve plant growth in addition to disease control resulting in higher yield (Sankar and Sharma,2001). The biological control of *M. phaseolina*, using antagonistic bacteria and fungi have been reported (Pal *et al*., 2001; Saransundar *et al*., 2013; Leelavathi *et al*., 2014).

Among the antagonists, *Pseudomonas* spp. and *Trichoderma* spp. are generally the most frequently reported and have been known for their potential antifungal, plant growth promoting and plant defense inducing activities (Zaidi *et al.,* 2004). However, application of single antagonistic strain often results in inconsistent disease control. One of the strategies for overcoming such inconsistent performance is to combine two or more beneficial microbes in a biocontrol preparation (Raupach and Kloepper, 1998). Several researchers have also tested different biocontrol strains with different modes of actions in combination (Droby, 2001) to increase the efficacy and the consistency of disease control.

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). With this background the present study was conducted to assess the effect of antagonists in combination with potential AMF for the successful management of root rot of groundnut under field conditions.

MATERIALS AND METHODS

Isolation of the pathogen

The pathogen *Macrophomina phaseolina* (Tassi) Goid. was isolated from the diseased roots of groundnut plants showing the typical root rot symptoms by tissue segment method on potato dextrose agar (PDA) medium. The axenic cultures of the different isolates of the pathogen were obtained by single hyphal tip method (Rangaswami, 1972) and these were maintained on PDA slants for subsequent experiments.

Isolation of native antagonists from rhizosphere soil

Trichoderma spp

Groundnut rhizosphere soil samples collected from four different locations were used for the isolation of *Trichoderma* isolates by soil dilution plating technique using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983). These *Trichoderma* cultures were purified by single hyphal tip method and used for the studies. Micrometric measurements of conidia and phialides were done by mounting four days old culture stained with lactophenol cotton blue and observed under high power of research microscope. *Trichoderma* spp., thus isolated was subjected for identification based on the key to species suggested by Domsch *et al*. (1980). On the whole thirteen isolates of *Trichoderma* spp. were isolated and among them ten isolates were identified as *T.viride* and three isolates were identified as *T. harzianum*. The identified *T. viride* isolates were designated as Tv_1-Tv_{10}. Similarly, the *T. harzianum* isolates were designated as Th_{11}-Th_{13}.

Isolation of native antagonistic bacteria

Antagonistic bacteria were isolated from the rhizosphere soil collected from different groundnut growing areas of Cuddalore district by serial dilution method on Nutrient agar medium, King's B medium, for *Bacillus* and *Pseudomonas,* respectively by incubating at room temperature for 24 h. Colonies with characteristics of *Bacillus* sp. and *Pseudomonas* sp., were isolated individually and purified by streaking them on Nutrient agar medium and King's B medium, respectively. For comparison standard cultures of *P. fluorescens*, *B. subtilis* were obtained from the culture collections of Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, Tamil Nadu. *In vitro* assays were conducted following dual culture and poisoned food technique to identify the potential native antagonists. The studies revealed the supremacy of native antagonists viz., *T. viride* (Tv_7), *P. fluorescens* (Pf_5) in suppressing *M.phaseolina.* These isolates were also found compatible with each other and also with *G.mossae.* Hence, only *T. viride* (Tv_7), *P. fluorescens* (Pf_5) alone were tested as consortium along with *G. mosseae* under field conditions for the management of root rot of groundnut.

Isolation of AMF from groundnut rhizosphere

The rhizosphere soil collected from different groundnut growing fields were examined for the presence of AM fungal spore by wet sieving and decanting method described by Gerdemann and Nicolson (1963) followed by sucrose centrifugation (Smith and Skipper, 1979). These spores were cleaned of soil particles by sucrose density gradient centrifugation method and washed with distilled water. The spore suspension was observed under stereo zoom microscope and morphologically similar spores were separated into groups, mounted and identified. The AM fungi were identified based on the Manual for Identification of Mycorhizal Fungi (Schenck and Perez, 1990) and recorded. The AM fungi *G. mosseae* was found commonly associated in all the locations and hence the same was used for the field studies.

Mass production of *G. mosseae* inoculums

For mass production of *G. mosseae* inoculum the methodology suggested by Kumutha *et al.* (2010) was followed. A trench (3m × 1m × 0.3m lbh) lined with black polythene sheet was used as plant growth tub. 500 kg of vermiculite and 50 kg of sterilized soil was mixed and packed in the trench up to a height of 20 cm. To this ten kg of mother inoculum of *G. mosseae* containing 250-300 spores per 100 g soil was spread 2-5 cm below the surface of vermiculite. Surface sterilized maize seeds were sown and applied with 20 gm of urea, super phosphate and 10 gm of muriate of potash per trench. Further 10 gm of urea was applied twice on 30 and 45 DAS. Thus the stock plants

were grown for eight weeks and de-topped. The inoculum was prepared by collecting the vermiculite in the pit along with root bits infected with *G. mosseae*. Thus, approximately 55 kg of inoculum could be produced from one square meter area and used for the field studies. The propagules in soil-based culture consisted of both spores and (250-300 spores per 100 g soil) chopped, colonized root fragments (Kumutha *et al.,* 2010).

Enumeration of *G. mosseae* spore population

A quantity of 50 gm of peanut rhizophere soil sample was suspended in 200 ml water and mixed well. Heavier particles were allowed to settle for a new seconds and the suspension was decanted through a 710 µm sieve to remove the larger particles of organic matter. The residue was resuspended in more water and sieving was repeated. The suspension that passed through this sieve was stirred to resuspend all particles. The heavier particles were allowed to settle for few seconds and the liquid decanted through 250 µm sieve.

The suspension that passed through this sieve was again collected and the sieving was repeated using 105 µm sieve and 45 µm sieve. The larger particles of organic matter were caught on the top sieves of higher pore size. The soil particles and spores collected in 105 µm and 45 µm sieves were taken in 10 ml conical flasks separately. The suspension in each flask was shaken thoroughly and allowed to settle for 30 seconds. The spores present in these suspensions were trapped on Whatman No. 1 filter paper. The spores on the filter paper were then spread on a marked Petri dish and the number of spores was counted by observing under a stereoscopic microscope (Gerdemann and Nicolson, 1963).

Field studies

Integrated management of root rot of ground nut

Based on the best results obtained from the *in vitro* and pot culture experiments, two field trials were conducted in root rot prone farmer's field at Vengadankuppam and Aduragaram in Cuddalore district of Tamil Nadu during July to October (Field trial 1) and December to March (Field trial 2) of 2010 - 2011, representing rainfed and irrigated conditions. 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 @ 4 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. The per cent disease index was worked out according to "Phytopathometry" by Mayee and Datar (1986) as mentioned below.

$$\text{Per cent Disease Index (PDI)} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

The experiment was conducted in a randomized block design with three replications per treatment. The chemical carbendazim @ 4 g/g of seed as seed treatment was used as comparison. The PDI was assessed periodically and the biometrics were assessed at the time of harvest following standard procedures.

RESULTS AND DISCUSSION

Effect of combined application of antagonists and *G. mosseae* on the dry root rot incidence of groundnut (Field trial 1 - Rainfed): The results obtained in the field studies are furnished in table 1. In general the root rot incidence showed an increasing pattern with an increase in the age of the crop in all the treatments and also control plots.

Observations taken at harvest revealed that the treatment T_6 *T. viride* (Tv_7) + *P. fluorescens* (Pf_5) as ST @ 10 ml kg^{-1} of seed +SA @ 2.5 lit ha^{-1}) plus *G. mosseae* (SA @ 10 kg ha^{-1}) recorded 13.00% of root rot incidence which was followed by the treatment T_5 *P. fluorescens* (Pf_5) + *G. mosseae* which recorded an incidence of 15.66 per cent. The treatments T_4 and T_3 came next in the decreasing order of merit. The treatments with integration of *G. mosseae* showed enhanced disease suppression when compared to individual antagonistic treatments. The chemical treatment with carbendazim (ST @ 4 g kg^{-1}) recorded 16.00 % root rot incidence and the untreated control recorded 32.50 per cent root rot incidence at harvest.

Effect of combined application of antagonists and *G. mosseae* on the biometrics of groundnut (Field trial 1 - Rainfed)

Generally, the antagonistic treatments with integration of *G. mosseae*, showed enhanced growth and yield attributes when compared to other treatments and control. However, among the treatments the treatment T_6 *T. viride* (Tv_7) + *P. fluorescens* (Pf_5) as ST @ 10 ml kg^{-1} of seed + SA @ 2.5 lit ha^{-1}) plus *G. mosseae* (SA @ 10 kg ha^{-1}) recorded 45.30 cm of plant height, 31.10 g of plant biomass, 27.60 numbers of pods $plant^{-1}$, 44.60 g of hundred seed weight, 1980 kg ha^{-1} seed yield.. This was followed by the treatment T_5 *P. fluorescens* (Pf_5) + *G. mosseae* which recorded 43.70 cm of plant height, 30.67 g of plant biomass, 26.10 numbers of pods $plant^{-1}$, 43.30 g of hundred seed weight, 1905 kg ha^{-1} seed yield (Table - 2)

Effect of combined application of antagonists and G. mosseae on dry root rot disease incidence of groundnut (Field trial 2 - Irrigated)

The results revealed that the treatment T_6 *T. viride* (Tv_7) + *P. fluorescens* (Pf_5) 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 4.60, 8.22, 10.20, 12.42 per cent dry root rot incidence at 45, 60, 75 and at harvest respectively. The chemical treatment recorded 15.35% while the control ecorded 28.70% root rot incidence at harvest.

Table 1. Effect of combined application of antagonist and *G. mosseae* on dry root rot disease incidence of groundnut (Field trial 1 - Rainfed)

Tr. No	Treatments	Root rot incidence (%)				Mean
		45 DAS	60 DAS	75 DAS	At harvest	
T_1	*T. viride* (Tv_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	11.40^{d}	16.70^{e}	18.15^{d}	20.65^{e}	16.72
T_2	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	12.80^{e}	17.66^{e}	19.49^{e}	21.43^{e}	17.84
T_3	*T. viride* (Tv_7) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	10.99^{d}	15.80^{d}	17.99^{d}	19.75^{d}	16.13
T_4	*T. viride* (Tv_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	07.70^{b}	11.25^{b}	15.80^{c}	17.25^{c}	13.00
T_5	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	09.30^{c}	13.66^{c}	13.00^{a}	15.66^{b}	12.90
T_6	*T. viride* (Tv_7) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA@10 kg ha^{-1}	05.40^{a}	09.33^{a}	11.80^{a}	13.00^{a}	09.88
T_7	Carbendazim 50% WP as ST @ 4.0 g kg^{-1}	07.40^{b}	11.45^{b}	14.60^{b}	16.00^{b}	12.36
T_8	Control	14.22^{f}	26.75^{g}	30.49^{f}	32.50^{f}	25.99

Table 2. Effect of combined application of antagonist and *G. mosseae* on biometrics of groundnut (Field trial 1 - Rainfed)

Tr. No	Treatments	Plant height (cm)	Biomass (g)	Number of pod $plant^{-1}$	100 seed weight	Yield kg ha^{-1}
T_1	*T. viride* (Tv_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	42.90^{c}	29.00^{c}	26.00^{b}	40.95^{c}	1795^{d}
T_2	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	40.20d	26.45^{e}	23.31^{d}	38.40^{d}	1690^{e}
T_3	*T. viride* (Tv_7) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	43.10^{c}	29.55^{c}	25.70^{c}	42.80^{b}	1850^{c}
T_4	*T. viride* (Tv_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	43.10^{c}	28.70^{d}	25.90^{c}	41.60^{b}	1780^{d}
T_5	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	43.70^{b}	30.67^{b}	26.10^{b}	43.30^{a}	1905^{b}
T_6	*T. viride* (Tv_7) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @10 kg ha^{-1}	45.30^{a}	31.10^{a}	27.60^{a}	44.60^{a}	1980^{a}
T_7	Carbendazim 50% WP as ST @ 4.0 g kg^{-1}	44.80^{b}	30.10^{b}	26.00^{b}	43.70^{a}	1900^{b}
T_8	Control	28.55^{e}	20.66^{f}	21.65^{e}	35.65^{e}	1530^{f}

Table 3. Effect of combined application of antagonist and *G. mosseae* on dry root rot disease incidence of ground nut (Field trial 2 - Irrigated)

Tr. No	Treatments	Root rot incidence (%)				Mean
		45 DAS	**60 DAS**	**75 DAS**	**At harvest**	
T_1	*T. viride* (Tv_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	11.84^{f}	16.98^{f}	19.61^{f}	21.36^{f}	17.44
T_2	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	10.40^{e}	15.33^{e}	17.44^{e}	19.44^{g}	15.65
T_3	*T. viride* (Tv_7) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	09.10^{d}	14.88^{d}	16.77^{d}	18.10^{e}	15.46
T_4	*T. viride* (Tv_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	08.30^{c}	12.40^{c}	14.30^{c}	16.22^{d}	12.80
T_5	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	06.40^{b}	10.44^{b}	12.11^{b}	14.75^{c}	10.92
T_6	*T. viride* (Tv_7) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @10 kg ha^{-1}	04.60^{a}	08.22^{a}	10.20^{a}	12.42^{a}	08.86
T_7	Carbendazim 50% WP as ST @ 4.0 g kg^{-1}	07.66^{b}	11.80^{b}	13.30^{b}	15.35^{b}	12.02
T_8	Control	12.22^{g}	19.75^{g}	27.50^{g}	28.70^{h}	22.04

Table 4. Effect of combined application of antagonist and *G. mosseae* on biometrics of groundnut (Field trial 2 - Irrigated)

Tr.No	Treatments	Plant height (cm)	Biomass (g)	Number of pod $plant^{-1}$	100 seed weight	Yield kg ha^{-1}
T_1	*T. viride* (Tv_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	51.10^{e}	30.67^{e}	33.41^{e}	39.49^{e}	2010^{e}
T_2	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	52.30^{d}	32.70^{d}	36.63^{c}	40.20^{d}	2125^{d}
T_3	*T. viride* (Tv_7) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1}	52.52^{d}	33.00^{c}	35.12^{d}	41.10^{d}	2150^{d}
T_4	*T. viride* (Tv_7) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	53.42^{c}	33.55^{c}	35.70^{d}	42.33^{c}	2200^{c}
T_5	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	53.60^{b}	34.67^{b}	36.53^{b}	45.74^{b}	2250^{c}
T_6	*T. viride* (Tv_7) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 2.5 lit ha^{-1} + *G. mosseae* SA @10 kg ha^{-1}	55.70^{a}	35.10^{a}	37.83^{a}	46.40^{a}	2400^{a}
T_7	Carbendazim 50% WP as ST @ 4.0 g kg^{-1}	54.90^{a}	34.10^{b}	36.93^{b}	44.60^{b}	2350^{b}
T_8	Control	40.66^{f}	25.33^{f}	32.65^{f}	35.53^{f}	1990^{f}

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 - 3). The results indicated that different plant colonization pattern and different mechanism of disease suppression elicited by the combination of *T. viride* (Tv_7), *P. fluorescens* (Pf_5) 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. Application of *Glomus* spp. together with other *T. viride* and *B. subtilis* significantly effected than individual treatments for controlling root rot and increasing growth and yield of sesame (Ziedan *et al*., 2011).

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. 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.

It is noteworthy to observe that *T. viride* performed well under rainfed condition and *P. fluorescens* performed better in reducing the root rot incidence under irrigated condition confirming the statement by Fukui *et al*. (1994) who reported that a single strain may not grow equally well in a variety of environmental conditions. The field soil in the present study had a favourable pH of 6.8 for the multiplication of *T. viride* as observed by Harman (1991). Probably these factors might be the reason for the better antagonistic activity by *T. viride* under rainfed conditions. Fluorescent *Pseudomonas* preferred heavy textured soil than light soil (Van Elsas *et al.,* 1989) and moist soil (Harman, 1991) for its multiplication and activity. The sandy loam soil with slightly low soil pH observed in the present experimental field and the dry conditions therefore, might be the reasons for the reduced biocontrol activity of *P. fluorescens* in rainfed conditions.

Effect of combined application of antagonists and *G. mosseae* on the biometrics of groundnut (Field trial 2 - Irrigated)

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_5) + *T. viride* (Tv_7) combination" recorded the maximum plant height (55.70 cm), biomass content (35.10 g), number of pods per plant (37.83) .This was followed by the treatment consisting of seed and soil application of *P. fluorescens* (Pf_5) + combined with *G. mosseae*. The untreated control recorded the minimum plant height (40.66 cm), biomass (25.33) and number of pods per plant (32.65). With regard to individual antagonist, *P.fluorescens* (Pf_5) combined with *G. mosseae* recorded better values when compared with the treatment of *T. viride* (Tv_7) combined along with *G. mosseae* (Table - 4).

The present observations have clearly demonstrated that the biocontrol efficiency and plant growth promotion efficiency could be improved by combining biocontrol agents along with *G. mosseae*. 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). Similarly, 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 growth hormones and metabolites produced by the combination of antagonists *T. viride* (Tv_7) + *P. fluorescens* (Pf_5) + *G. mosseae* would have exerted a synergism in promoting the plant growth parameters and enhancing yield of groundnut. The ability of *P. fluorescens* and AMF to promote plant growth by improved nutrient acquisition and suppression of soil borne pathogens is well documented (Gamalero *et al.*, 2003; Yusran *et al.*, 2009). Thus, the results of the present study clearly demonstrated that co inoculation of *G. mosseae* with *T. viride* (Tv_7) and *P. fluorescens* (Pf_5) would have exerted different mechanisms

of disease control and enhanced greater disease suppression and improved the consistency of biological control under varied climatic conditions and has provided an ecofriendly and sustainable strategy for the management of the root rot disease of groundnut caused by *M. phaseolina*.

Further the combination treatment maintained its superiority under both rainfed and irrigated conditions as observed by Janisiewicz (1996) who stated that compatible multiple strains might be advantageous when dealing with soilborne diseases and combining antagonists that occupy different nutritional niches and coexist in the infection court are more effective biological control treatments than individual antagonists. Consequently, application of a mixture of introduced biocontrol agents would more closely mimic the natural situation (Mishra *et al.,* 2011) and might broaden the spectrum of biocontrol activity, enhance the efficacy and reliability of biological control.

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