Efficacy of Bioinoculants on Dry Root Rot Incidence of Blackgram Under *In Vitro* Conditions

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ABSTRACT

M. phaseolina is an important pathogen, distributed worldwide and blackgram at all stages are susceptible to infection. The present study was undertaken to investigate the bio control potential of the native antagonists *P. fluorescens, T. viride* and *G. mosseae* for the successful biological management of *M. phaseolina* causing root rot of blackgram. Combined delivery system (Seed treatment + soil application) with combination of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) proved much superior by recording the least incidence of root rot which was on par with the fungicide treatment and also recorded the maximum growth parameters of blackgram. Among the species of AM fungi tested, the treatment with *G. mosseae* recorded the minimum root rot incidence, maximum per cent root infection and maximum growth parameters of blackgram in pot culture conditions.The treatment (T_6) *viz.*, Seed treatment + soil application with combination of *T. viride* (Tv_3)and *P. fluorescens* (Pf_5) plus soil application of *G. mosseae* recorded the minimum incidence of blackgram root rot and maximum biometrics of blackgram in field trial.

Key words: ***Macrophomina phaseolina, Pseudomonas fluorescens, Trichoderma viride***

INTRODUCTION

Black gram is commonly called as Urd bean (*Vigna mungo* (L.) Hepper). The mention of blackgram has been made in vedic texts such as 'Kautilya's 'Arthashastra'. The word legume comes from 'legere' meaning 'to gather' and indicates that the seeds were collected by hand instead of being threshed from the plant as cereal grains. Archaeological studies have shown that urdbean was cultivated in the country as far back as 2200 B.C. Blackgram is mainly cultivated in Indian subcontinent. In India Black gram is popular as "Urad dal" and it is a highly prized pulse among all the pulses.

Pulses play a vital role in Indian agriculture. Pulses are important sources of food which is rich in protein. Pulse crops have unique mechanism to fix atmospheric nitrogen, thereby enriching the soil and tapping of stored soil moisture efficiently through the deep root system. Apart from India it is also cultivated in Pakistan, Afghanistan, Bangladesh and Myanmar. Most suitable climate to cultivate Blackgram is 27-30º C with heavy rainfall. This annual crop prefers loamy soil which has high water preservation capability. Blackgram grows normally in 90-120 days and it also enriches the soil with nitrogen. The 68th United nations General Assembly declared the year 2016 as the International Year of Pulses (IYP). India accounts for 33% of the world area and 25% of the world production of pulses. The crop is affected by number of pathogens and among them, *Macrophomina phaseolina* (Tassi) Goid. is one of the most serious seed and soil borne diseases causing significant yield loss of up to 25 to 36 per cent (Krishna Murthy *et al*., 2003).Charcoal rot caused by *M. phaseolina* is an economically important disease of a broad range of crops (Srivastava *et al.,* 2001), particularly in regions with warm and dry weather conditions during the growing season (Karthikeyan, 2015). *M. phaseolina* causes root rot, collar rot, stem rot, leaf blight, pod and seed infection in blackgram. 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). Biological control of soil borne plant pathogens by addition of antagonistic microorganisms is known to be a cheap and effective method for the management of soil and seed borne diseases (Cook and Baker, 1983). Further, the biocontrol agents also improve plant growth in addition to disease control resulting in higher yield (Sankar and Sharma,2001). Further, use of antagonistic organisms against *M. phaseolina* causing root rot has been well documented by earlier workers (Saransundar*et al*., 2013; Leelavathi *et al*., 2014) in several crops.

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. Memberskhb 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 these organisms, in recent years, mycorrhizal fungi as symbiotic organisms have been used against plant pathogens successfully. The arbuscular mycorrhizal fungi (AMF) symbiosis is a well-known terrestrial symbiotic association formed between fungi and roots of vascular plants (Douds and Siedel, 2012). Several studies indicated that, arbuscular mycorrhizal fungi (AMF) influenced fungal diseases caused by root pathogens (Trotta *et al*., 1996; Karagiannidis *et al*., 2002). AM symbiosis also increases resistance to biotic and abiotic stresses and reduces disease incidence, representing a key component of sustainable agriculture (St-Arnaud and Vujanovic, 2007). In particular, combinations of AMF and *Trichoderma* spp. may provide protection at different times or under different conditions, and occupy different or complementary niches. Thus, espousal of integrated management by AMF along with *Trichoderma* spp seems to be economic and promising way to improve to control *M. phaseolina* in low-input agricultural practice (Doley *et al*., 2014). Thus, the present study was undertaken with an aim to utilize the potential antifungal and plant growth promoting and plant defense inducing activities of biocontrol agents and AMF for the effective management of blackgram root rot disease.

MATERIALS AND METHODS

Efficacy of seed treatment plus soil application with the antagonists on plant growth and root rot incidence of blackgram (pot culture)

Sterilized soil was mixed with the pathogen inoculum @ 5 per cent (w/w) level and filled in 30 cm earthen pots. The most effective seed treatment and soil application dosages identified in the earlier experiments were used for testing the efficacy of combined delivery system of the antagonists. The antagonists meant for soil application were applied to the pots and incorporated well. Surface sterilized blackgram seeds were treated with the antagonists as per the schedule given below surface sterilized blackgram seeds sown in pot soil mixed with the inoculum of *M. Phaseolina* alone served as control. Seed treatment @ 2 g kg^{-1} of seed plus soil drench @ 0.1% with carbendazim was used for comparison. The experiment was conducted with three replications in a randomized block design with five pots per replication and three plants per pot. All the observations *viz.,* plant growth parameters, root rot incidence, population of the antagonist and population of pathogen were recorded as already indicated in earlier chapter.

Treatment schedule

T_1 : *T. viride*(Tv_3) seed treatment (10.0 ml $kg^{-1}$of seed) + soil application (3.0 $litha^{-1}$)
T_2 : *P. fluorescens*(Pf_5) seed treatment (10.0 ml $kg^{-1}$of seed) + soil application (3.0 lit ha^{-1})
T_3 : *T. viride* (Tv_3) + *P. fluorescens*(Pf_5) seed treatment (10.0 ml kg^{-1} of seed) + soil application (3.0 lit ha^{-1})
T_4 : Carbendazim 50% WP as seed treatment @ 4.0 g kg^{-1} + soil drench @ 0.1%
T_5 : Control

Isolation of AMF from blackgram rhizosphere

The rhizosphere soil collected from different blackgram 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 VA Mycorrhizal Fungi (Schenck and Perez, 1990) and recorded.

Mass production of AMF inoculum

For mass production of AMF inoculum the methodology suggested by Kumutha *et al.* (2010) was followed. A trench (3m × 1m × 0.3m lbh) lined with back 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.

Enumeration of AM Fungispore population

A quantity of 50 gm of black gram rhizosphere 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).

Determination of per cent AM Fungi infection

The roots of the blackgram plants were analysed for *G. mosseae* infection by clearing and staining method of Philips and Hayman (1970). The roots were thoroughly washed in tap water, without disturbing the external mycelium. The roots were cut into one cm pieces and immersed in 10 per cent KOH solution. The roots were cleared by autoclaving for 30 minutes at 15 lbs. pressure in 10 per cent KOH. Then the roots were rinsed in water for two to three times and acidified by soaking in two per cent HCl for five minutes. The acid was poured off and the root segments were stained by immersing in 0.05 per cent tryphan blue in lactophenol (lactic acid: glycerol, 1:1) and boiled for three minutes. The excess stain was poured off and added with lactophenol and kept overnight to destain the host tissues. Root bits were arranged on glass slides and examined under a microscope for mycorrhizal infection. 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.

Effect of AMF on plant growth promotion and root rot incidence of blackgram

Blackgram plants were grown in pots containing five kg of sterilized soil in greenhouse conditions. Prior to sowing, the pots were inoculated with *G. mosseae* @ 500 spores per pot Sundaresan *et al.* (1993) and with *M. phaseolina* inoculum @ five per cent (w/w) level. Three replications per treatment and suitable control were also maintained. Assessment on per cent root rot incidence and biometrics were recorded at the time of harvest. The plants uprooted on maturity were stained for assessing mycorrhizal infection by the method of Phillips and Hayman (1970). The 'P' content of the plant was also estimated using the method described by Allen (1940).

Efficacy of antagonists and *G. mosseae* on plant growth and the incidence of root rot of blackgram (Pot culture)

A separate pot culture experiment was conducted as per the treatment schedule to the *M. phaseolina* inoculated (5% level) sick soil to assess the efficacy of antagonists and *G. mosseae* on the management of blackgram root rot disease. The following are the treatments.

Treatment schedule

T_1 : *T. viride*(Tv_3) seed treatment (10.0 ml $kg^{-1}$of seed) + soil application (3.0 lit ha^{-1})
T_2 : *P. fluorescens*(Pf_5) seed treatment (10.0 ml kg^{-1} of seed) + soil application (3.0 lit ha^{-1})
T_3 : *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) seed treatment (10.0 ml kg^{-1} of seed) + soil application (3.0 lit ha^{-1})
T_4 : *T. viride* (Tv_3) seed treatment (10.0 ml $kg^{-1}$of seed) + soil application (3.0 lit ha^{-1}) + *G. mosseae* soil application @ 10 kg ha^{-1}
T_5 : *P. fluorescens*(Pf_5) seed treatment (10.0 ml kg^{-1} of seed) + soil application (3.0 lit ha^{-1}) + *G. mosseae* soil application @10 kg ha^{-1}
T_6 : *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) seed treatment (10.0 ml kg^{-1} of seed) + soil application (3.0 lit ha^{-1}) + *G. mosseae* soil application @10kg ha^{-1}
T_7 : Carbendazim 50% WP as seed treatment@ 4.0 g kg^{-1} + soil drench @ 0.1%
T_8 : Control

The experiment was conducted in a randomized block design with three replications wherein three plants per pot were maintained. The incidence of root rot (per cent), shoot and root length (cm), biomass of the plant (g) and number of pods per plant were recorded at harvest. The blackgram root samples were stained for assessing per cent mycorrhizal colonization (Phillips and Hayman, 1970) and rhizophere soil samples were analysed for *G. mosseae* chlamydospore count by employing wet sieving and decanting method (Gerdemann and Nicolson, 1963)

Table 1. Effect of seed treatment plus soil application with antagonist on plant growth promotion and dry root rot incidence of blackgram under Pot culture

Tr. No	Treatment	Shoot length (cm)	Root length (cm)	Bio mass (g plant^{-1})	Percent root rot	Percent decrease over control
T_1	*T. viride*(Tv_3) ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1}	24.10^{d}	15.40^{d}	25.30^{c}	30.50^{c} (33.52)	56.11
T_2	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1}	27.12^{b}	17.4^{b}	26.20^{b}	27.70^{b} (31.75)	60.14
T_3	*T. viride*(Tv_3)+ *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 3.0 l ha^{-1}	29.20^{a}	19.70^{a}	28.23^{a}	23.30^{a} (28.86)	66.47
T_4	Carbendazim 50% WP as ST @ 4.0g/kg and SD @ 0.1%	26.80^{c}	16.90^{c}	20.60^{d}	22.20^{a} (28.11)	68.05
T_5	Control	22.90^{e}	12.30^{e}	16.75^{e}	69.50^{d} (56.47)	--

*Mean of three replications
*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

Table 2. Effect of AM Fungi on plant growth promotion and dry root -rot incidence of blackgram under pot culture

S. No	Treatment	Shoot length (cm)	Root length (cm)	Biomass (g plant-1)	Per cent root rot (PDI)	Per cent decrease over control	Per cent root infection by AMF
T1	*Glomus mosseae*	46.76a	17.76b	20.73c	37.98b	42.43	65.54a
T2	*Glomus fasciculatum*	45.75b	16.79c	22.94a	38.45a	41.72	59.43b
T3	*Gigaspora margarita*	43.87c	18.78a	21.43b	43.87c	33.51	34.64c
T4	Inoculated Control	32.58d	15.98d	16.78d	65.98d	–	11.68d

Table 3. Testing compatibility between biocontrol isolates and *G. mosseae*under Pot culture

Sl. No	Treatment	Population antagonistic and pathogenic organisms (g^{-1} oven dry soil)			Per cent root infection *G. mosseae*	Compatibility
		T. viride(10^3 cfu)	*P. fluorescens* (10^6 cfu)	*M. phaseolina* (10^3 cfu)		
T_I	*T. viride* (Tv_3) ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1}	17.60	--	18.23	--	---
T_2	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1}	--	22.45	16.44	--	---
T_3	*T. viride*(Tv_3) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 3.0 l ha^{-1}	16.42	21.43	14.43	--	---
T_4	*G. mosseae* SA @ 10 kg ha^{-1}	--	--	23.12	63.98	Compatible
T_5	*T. viride* (Tv_3) + ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	17.43	--	16.90	61.84	Compatible
T_6	*P. fluorescens* (Pf_5) + ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1} + *G. mosseae* SA @ 10 kg ha^{1}	--	20.24	13.31	63.65	Compatible
T_7	*T. viride*(Tv_3) + *P. fluorescens* (Pf_5) + ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1} + *G. mosseae* SA @ 10 kg ha^{1}	14.43	18.53	11.60	61.77	Compatible

*Mean of three replications
*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

Table 4. Effect of combined application of antagonist and *G. mosseae* on dry root rot incidence of blackgram under Pot culture

Tr. No	Treatments	Shoot length (cm)	Root length (cm)	Biomass (g plant^{-1})	Per cent root rot	Per cent decrease over control
T_1	*T. viride*(Tv_3) ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1}	26.33^{g}	14.55^{e}	20.45^{g}	30.87^{g} (33.75)	51.75
T_2	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1}	28.43^{e}	15.92^{d}	24.32^{d}	29.87^{f} (33.12)	53.31
T_3	*T. viride*(Tv_3) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 3.0 l ha^{-1}	27.36^{f}	16.01^{c}	23.75^{e}	24.76^{d} (29.84)	61.30
T_4	*T. viride*(Tv_3) ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	29.77^{d}	15.64^{d}	26.42^{c}	27.87^{e} (31.86)	56.43
T_5	*P. fluorescens* (Pf_5) ST (10 ml kg^{-1}) + SA 3.0 l ha^{-1} + *G. mosseae* SA @ 10 kg ha^{1}	32.83^{c}	17.57^{b}	27.16^{b}	23.76^{c} (29.17)	62.86
T_6	*T. viride*(Tv_3) + *P. fluorescens* (Pf_5) ST(10 ml kg^{-1}) + SA 3.0 l ha^{-1} + *G. mosseae* SA @ 10 kg ha^{-1}	35.97^{a}	18.76^{a}	28.58^{a}	21.89^{a} (27.89)	65.78
T_7	Carbendazim 50% WP as ST @ 4.0 g kg^{-1} and SD @ 0.1%	34.69^{b}	16.64^{c}	21.64^{f}	22.76^{b} (28.49)	64.42
T_8	Control	24.53^{h}	13.43^{f}	12.45^{h}	63.98^{h} (53.11)	--

*Mean of three replications
*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

RESULTS AND DISCUSSION

Efficacy of seed treatment plus soil application of the antagonists against root rot incidence and plant growth of blackgram Under Pot culture

The results obtained on the efficacy of combined delivery system of the antagonists *viz.*, se soil treatment are furnished in table 1. Among the antagonists tested by seed treatment + soil appl combination treatment (T_3) of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) recorded the minimum incidence of root rot with (23.30%) which was on par with the fungicide treatment (22.20%)and also recorded the maximum shoot length (29.20 cm), root length (19.70 cm) and plant biomass (28.23 g). Carbendazim as seed treatment @ 4g kg^{-1} of seed plus soil drenching @ 0.1% recorded 26.80 cm of shoot length, 16.90 cm of root length and 20.60 g of plant biomass. Individual application of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) as seed and soil treatment recorded a disease incidence of 30.50 and 27.70 per cent respectively. The untreated control recorded the maximum disease (69.50%) incidence and minimum growth parameters of blackgram.

Occurrence of AMF in blackgram rhizosphere

The results revealed the occurrence of AMF in all the blackgram growing localities. The AMF were isolated from the soil and root samples collected during the survey and observed for the morphological characters. Generally the AMF *viz.,Glomus mosseae, G. fasciculatum* and *G.margarita* were found occurring in blackgram fields. In most locations (Six locations) the AMF *viz.,G. mosseae* was found to occur followed by *G. fasciculatum* (Three locations) and G.*margarita*(One location)Hence*G. mosseae* was used in the subsequent studies.

Effect of AMF on plant growth and root rot incidence of blackgram (pot culture)

The data depicted in table 2 revealed that the soil application of AMF showed significant influence on the incidence of root rot of blackgram and all the AMF species significantly increased the plant growth parameters of blackgram when compared to control. Among the three different species of AMF, the treatment with *G. mosseae* recorded the minimum root rot incidence with 37.98 per cent and also recorded the maximum shoot and root length (46.76 cm, 17.76 cm respectively) and 20.73 g of plant biomass. Also the treatment with *G. mosseae* recorded the maximum per cent root infection (65.54%) in blackgram. This was followe *fasciculatum* which showed 59.43 per cent root infection and recorded 38.45 per cent of root rot in 45.75 cm, 16.79 cm of shoot length, root length respectively and 22.94 g plant biomass and. *G. margarita* treatment showed the minimum root infection (34.64%).

Testing compatibility between biocontrol agents and *G. mosseae* (Pot culture)

The results on the compatibility of antagonists both individually and in combination with *G. mosseae* were recorded in table 3. The results revealed that co-inoculation of individual antagonists and also combination of antagonists *T. viride* (Tv_3) + *P. fluorescens* (Pf_5)along with *G. mosseae* showed no inhibitory effect and all the antagonists showed normal rhizosphere population. The treatment with application of *G. mosseae* plus combination of antagonists showed a rhizosphere population of 14.43 cfu g^{-1} of *T. viride* (Tv_3), 18.53 cfu g^{-1} of *P. fluorescens* (Pf_5), 11.60 cfu g^{-1} of *M. phaseolina* and 61.77 per cent root infection by *G. mosseae* which were at par with the results obtained in the treatments with individual antagonists.

Effect of combined application of antagonists and *G. mosseae* on dry root rot incidence and plant growth parameters of blackgram (Pot culture)

The results obtained on the efficacy of combined application of antagonists and *G. mosseae* is furnished in table 4. Among the treatments, seed treatment + soil application with combination of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) plus soil application of *G. mosseae* treatment (T_6) recorded the minimum incidence of root rot (21.89%). This was Significantly followed by the treatment (T_5) with *P. fluorescens* (Pf_5) as seed and soil treatment plus soil application of *G. mosseae* and combination of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) as seed and soil treatment (T_3) in reducing the root rot

incidence and enhancing the growth parameters of blackgram. The untreated control recorded the maximum disease (63.98%) incidence and minimum growth parameters.

Similar to the present findings Yadav and Aggarwal (2015) opined that possibility of AM fungal applicationwith different bioinoculants to improve the cultivation of valuable oil yielding plants**.** El-Sayed Hussein Ziedan *et al.* (2011) also opined that the use of mixed inocula of mycorrhizal symbionts and biocontrol agents can be more effective than the use of a single species.AM fungi are the important basis of sustainable agricultural systems and over the past few years the enormous advances in research on mycorrhizal physiology and ecology have led to a greater understanding of the many roles of AM fungi in the ecosystem. *P. fluorescens* commonly known as mycorrhiza helperbacteria are found to be associated with mycorrhiza topromote the symbiosis between fungus and plant bystimulating fungal growth or protecting the fungusagainst other fungal competitors. It acts as a systemicBio-control agent against various fungal and bacterial diseases by producing a number of secondary hydrogen cyanide. *T. viridae* has also been recognized as potent biological agent to control plant diseases by producing antibiotics and cell walls degrading enzymes that can kill the pathogen (Alpa Yadav *et al*., 2015).

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). Similar 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_3) + *P. fluorescens* (Pf_5) + *G. mosseae* would have exerted a synergism in promoting the plant growth parameters and enhancing yield of blackgram. Likewise, the inoculation of *P.fluorescens* and *G. fasciculatum* in *Ocimum sanctum* enhanced seed germination and enhanced all the growth parameters of plant under pot trials (Pravita Pandey *et al.*, 2014).Thus, 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*.

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