Seed and Soil Application with Antagonist on Plant Growth Promotion and Dry Root Rot Incidence of Blackgram Under *In Vitro* Condition

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Abstract

Blackgram is commonly known as Urd bean (*Vigna mungo* (L.) Hepper). The crop is affected by number of pathogens and among them, *Macrophomina phaseolina* is one of the most serious seed and soil borne diseases causing significant yield losses. The present study was taen up to investigate the bio control potential of the antagonists *P. fluorescens* and *T. viride*and assess the efficient delivery systems for the successful biological management of *M. phaseolina* causing root rot of blackgram. The results revealed that the combination of delivery systems Seed treatment + soil application) with consortium of antagonists viz., *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) recorded the maximum biometrics and minimum root rot incidence of blackgram.With regard to individual delivery system of the antagonist's seed treatment with the combination of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) @ 10 ml kg^{-1} of seed and soil application, at the dosage at 4 l ha^{-1} recorded the minimum root rot incidence and maximum growth parameters of blackgram.

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

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.

According to Directorate of Economics and Statistics, blackgram was cultivated in an area of 3.2 million hectare with production of 1.86 million tonnes during the year 2014-15. Blackgram production in the country is largely concentrated in five states viz, Madhya Pradesh, Uttar Pradesh, Andhra Pradesh, Tamil Nadu and Maharashtra. These five states together contribute for about 65 per cent of total blackgram production in the country.The major consuming cum importing countries of black gram are India, China, Pakistan, Japan and Thailand. In Tamil Nadu, blackgram is cultivated in an area of 4.0 lakhs hectare with production of 2.65 lakhs tonnes during the year 2014-15. Rice fallow pulses cultivation is a traditional practice followed in the delta region of the state. Tamil Nadu is one of the largest cultivators of black gram, an important pulse grown in both Kharif and Rabi seasons in the state that accounts for nearly

41% of the total area under pulses. It is cultivated in over two lakh hectares and the state produces over 70,000 tonnes annually. The crop is extensively grown in Nagapattinam, Thiruvarur, Cuddalore, Toothukudi, Tirunelveli, and Villupuram districts, which together account for 77.7% of the total area under the crop (Deccan Chronicle, 2016). Main and important use of Black gram is to make Dal, even split lentil is used for same purpose. Apart from this it is also used in making Uttappa, Dosa, Idli, Vada, Dal Makhhani etc.

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) *M. phaseolina* is an important pathogen, distributed worldwide and blackgram at all stages are susceptible to infection. This pathogen has a broad geographic distribution, and a large host range. 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. Paroda *et al.* (2000) believes that the "issue facing agriculture and particularly disease control is therefore, managing the plant disease severity below the economic threshold following ecologically safe, economically viable and easily operational procedure". 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). The biological control of *M. phaseolina*, using antagonistic bacteria and fungi have been reported (Pal *et al*., 2001). Combined application of two bacilli isolates reduced the *Macrophomina* induced charcoal rot of maize by 54% (Pal *et al*., 2001). Among the fungal antagonists, *Trichoderma* spp. are generally the most frequently reported. Application of *T. harzianum* as seed treatment, suspension for soil drenching or wheat husk bran culture reduced infection of *Rhizoctonia bataticola* to 18%, 28% and 14%, respectively, as compared to 70% in the control (Parakhia and Vaishnav, 1986). 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). With this background the present study was conducted to study the effect of antagonists in combination and standardize the delivery systems for the successful management of root rot of blackgram.

MATERIALS AND METHODS

Efficacy of biocontrol agents on blackgram plant growth promotion under *in vitro* condition

Preparation of inoculum of the antagonists

The isolates of *P. fluorescens* grown in conical flasks (250 ml) containing 100 ml of King's B for 48 h on a rotary shaker (100 rpm) at 28 ± 2ºC. Cells were removed by centrifugation at 8000 rpm for ten min at 4°C and washed in sterile water. The pellet was resuspended in small quantity of sterile dist. water until to obtain bacterial colonies of 10^7 cfu ml^{-1} measured by serial dilution plate technique. Fungal antagonist *viz., T.viride* isolate (Tv_3) were multiplied by inoculating a disc of actively growing mycelial disc in *Trichoderma* Selective broth and incubated for 15 days. Then the mycelial mats along with spores were harvested by filtration through Whatman No.1 filter paper. Then the contents were mixed with sterile distilled water and adequate number of colony forming units (10^7) was checked through dilution plate technique. For testing the combination effect of the most effective isolates of antagonists the culture suspensions of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) were mixed @ 1:1 ratio and used for the study.

Assay for blackgram 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 liquid formulation of biocontrol agents

For the preparation of liquid formulations the method suggested by Manikandan *et al*. (2010) was followed. The most effective isolate of *P.fluorescens* (Pf_5) and *T. viride* (Tv_3) identified in the present study was multiplied on King's B and PD broth respectively. The mother culture of *T. viride* (Tv_3) and log phase culture of *P. fluorescens* (Pf_5) was inoculated individually into respective broth and incubated at room temperature (28 ± 2°C). Further, the respective broths were added with glycerol at 2 per cent level. After the incubation period, the formulation was assessed for adequate CFU following serial dilution plating technique and the formulation thus prepared was sealed in plastic containers and used for further studies.

Liquid based formulation for combination studies

For assessing the efficacy of combination of antagonists the liquid based formulation of *T. viride* (Tv_3) and *P.fluorescens* (Pf_5) were prepared separately and equal amounts of individual preparations with the adequate number of colony forming units (CFU) were mixed well just before use.

Seed treatment with antagonist

Seeds of blackgram (VRI 2) were surface sterilized with two per cent sodium hypochlorite for 30 sec rinsed in sterile dist. water and dried overnight. Ten ml of antagonist inoculum was taken in a Petri dish. To this, 100 mg of Carboxy Methyl Cellulose (CMC) was added as an adhesive material. Seeds were soaked in antagonistic suspension for 2 h and air dried overnight in a sterile Petri dish.

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

Sterilized soil was mixed with the sand maize of *M. phaseolina* inoculum @ 5 per cent (w/w) level and filled in 30 cm diameter earthen pots. Seeds were separately treated with the liquid based formulations of the antagonists. Seed treatment was given as per the schedule given below were sown in pots and three plants were maintained in each pot. Seed treatment with carbendazim @ 2g kg^{-1} was used for comparison and pathogen alone inoculated pots served as control. The experiment was conducted with three replications in a randomized block design and five pots per replication were maintained. The per cent incidence of root rot, shoot and root length (cm) and biomass (g) of the plant were recorded at harvest. The biomass of the plant was recorded after drying the plants in hot air oven at 60°C until attaining a constant weight. In order to find out the rhizosphere competence of the antagonists and the reason for the reduced root rot incidence, the population of the antagonists and pathogen in the rhizosphere was estimated at harvest using suitable selective media and serial dilution technique.

Treatment schedule

T_1 : *T. viride* (Tv_3) seed treatment (2.5 ml kg^{-1} of seed)
T_2 : *T. viride* (Tv_3) seed treatment (5.0 ml kg^{-1} of seed)
T_3 : *T. viride* (Tv_3) seed treatment (10.0 ml kg^{-1} of seed)
T_4 : *P. fluorescens* (Pf_5) seed treatment (2.5 ml kg^{-1} of seed)
T_5 : *P. fluorescens* (Pf_5) seed treatment (5.0 ml kg^{-1} of seed)
T_6 : *P. fluorescens* (Pf_5) seed treatment (10.0 ml kg^{-1} of seed)
T_7 : *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) seed treatment (2.5 ml kg^{-1} of seed)
T_8 : *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) seed treatment (5.0 ml kg^{-1} of seed)
T_9 : *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) seed treatment (10.0 ml kg^{-1} of seed)
T_{10} : Carbendazim 50% WP as seed treatment @ 4.0 g kg^{-1} of seed
T_{11} : Control

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

Sterilized soil was mixed with the *M. Phaseolina* inoculum @ 5 per cent (w/w) level and filled in 30 cm earthen pots. The antagonists were applied as per below given the treatment schedule to the pots and incorporated well. Seeds sown in pathogen alone inoculated pots served as control and the pots drenched with carbendazim (0.1%) were used for comparison. The experiment was conducted with three replications in a randomized block design and five pots per replication were maintained with three plants maintained in each pot. All the observations as indicated under 3.11 were recorded.

Treatment schedule

T_1 : *T. viride* (Tv_3) soil application (1.5 lit ha^{-1})
T_2 : *T. viride* (Tv_3) soil application (3.0 lit ha^{-1})
T_3 : *T. viride* (Tv_3) soil application (4.0 lit ha^{-1})
T_4 : *P. fluorescens* (Pf_5) soil application (1.5 lit ha^{-1})
T_5 : *P. fluorescens* (Pf_5) soil application (3.0 lit ha^{-1})
T_6 : *P. fluorescens* (Pf_5) soil application (4.0 lit ha^{-1})
T_7 : *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) soil application (1.5 lit ha^{-1})
T_8 : *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) soil application (3.0 lit ha^{-1})
T_9 : *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) soil application (4.0 lit ha^{-1})
T_{10} : Carbendazim 50% WP soil drench @ 0.1%
T_{11} : Control

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 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 : Carbendazim 50% WP as seed treatment @ 4.0 g kg^{-1} + soil drench @ 0.1%
T_5 : Control

Table 1. Effect of seed treatment with antagonist on plant growth promotion and 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) @ 2.5 ml kg^{-1}	21.30^{g}	14.30^{e}	20.35^{f}	34.67^{h} (36.07)	50.06
T_2	*T. viride*(Tv_3) @ 5.0 ml kg^{-1}	23.10^{e}	14.90^{e}	20.44^{f}	31.56^{f} (34.17)	54.54
T_3	*T. viride*(Tv_3) @10.0 ml kg^{-1}	22.30^{f}	15.30^{d}	21.65^{c}	27.97^{d} (31.92)	59.71
T_4	*P. fluorescens* (Pf_5) @ 2.5 ml kg^{-1}	24.30^{d}	12.40^{h}	21.23^{e}	33.63^{g} (35.44)	51.56
T_5	*P. fluorescens*(Pf_5) @ 5.0 ml kg^{-1}	25.90^{c}	14.30^{f}	23.85^{c}	31.69^{f} (34.25)	54.35
T_6	*P. fluorescens*(Pf_5) @10.0 ml kg^{-1}	25.30^{c}	17.60^{b}	22.65^{d}	23.97^{c} (29.31)	65.47
T_7	*T. viride*(Tv_3) + *P. fluorescens*(Pf_5) @ 2.5 ml kg^{-1}	26.20^{b}	16.80^{c}	23.91^{c}	31.32^{f} (34.03)	54.88
T_8	*T. viride*(Tv_3) + *P. fluorescens*(Pf_5) @ 5.0 ml kg^{-1}	26.40^{b}	17.40^{b}	24.20^{b}	29.34^{e} (37.79)	57.74
T_9	*T. viride*(Tv_3) + *P. fluorescens*(Pf_5) @10.0 ml kg^{-1}	29.30^{a}	18.90^{a}	25.75^{a}	20.43^{a} (26.87)	69.29
T_{10}	Carbendazim 50% WP as ST @ 4.0 g kg^{-1}	22.30^{f}	14.40^{e}	20.10^{f}	21.32^{b} (21.49)	70.57
T_{11}	Control	21.49^{g}	13.30^{g}	18.78^{g}	69.43^{i} (56.43)	--

*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 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)	Biomass (g plant^{-1})	Percent root rot	Per cent decrease over control
T_1	*T. viride*(Tv_3) @ 1.5 l ha^{-1}	22.90^{g}	16.30^{d}	20.44^{f}	36.67^{h} (37.26)	50.06
T_2	*T. viride*(Tv_3) @ 3.0 l ha^{-1}	24.60^{e}	16.90^{d}	21.65^{e}	33.56^{e} (35.40)	54.29
T_3	*T. viride*(Tv_3) @ 5.0 l ha^{-1}	23.50^{f}	17.30^{c}	20.35^{f}	29.97^{d} (31.29)	59.18
T_4	*P. fluorescens* (Pf_5)@ 1.5 l ha^{-1}	25.30^{d}	13.40^{f}	23.85^{c}	35.63^{g} (36.64)	51.47
T_5	*P. fluorescens*(Pf_5) @ 3.0 l ha^{-1}	26.70^{c}	15.30^{e}	22.65^{d}	34.69^{f} (36.08)	52.75
T_6	*P. fluorescens*(Pf_5) @ 5.0 l ha^{-1}	26.40^{c}	16.60^{d}	21.23^{e}	25.97^{c} (30.63)	64.63
T_7	*T. viride*(Tv_3) + *P. fluorescens*(Pf_5) @ 1.5 l ha^{-1}	28.90^{a}	19.80^{a}	25.20^{a}	33.32^{e} (35.25)	54.62
T_8	*T. viride*(Tv_3) + *P. fluorescens*(Pf_5) @ 3.0 lit ha^{-1}	27.10^{b}	17.40^{c}	23.91^{c}	25.54^{c} (30.35)	65.21
T_9	*T. viride*(Tv_3) + *P. fluorescens*(Pf_5) @ 5.0 l ha^{-1}	27.90^{b}	18.90^{b}	24.75^{b}	24.32^{b} (29.54)	66.88
T_{10}	Carbendazim 50% WP SD @ 0.1%	24.20^{e}	16.40^{d}	19.42^{g}	22.43^{a} (28.26)	69.45
T_{11}	Control	21.49^{h}	13.30^{f}	18.78^{h}	73.43^{i} (58.97)	--

*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 3. 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)

RESULTS AND DISCUSSION

Effect of seed treatment with antagonist on plant growth promotion and dry root rot incidence of blackgram under pot culture condition

The data depicted in table 1revealed that the seed treatment with antagonist either individually or as combination showed significant influence on the incidence of root rot of blackgram and all the antagonists significantly increased the plant growth parameters of blackgram when compared to control. Among the various treatments with the antagonists, the treatment combination with *T. viride* (Tv_3) and *P. fluorescens* (Pf_5)@ 10 ml kg^{-1} of seed recorded the significantly different minimum root rot incidence 20.43% which was followed by carbendazim treatment (21.32%). The same treatment also recorded the maximum growth parameters with 29.30 cm and 18.90 cm of shoot and root length and 25.75 g of plant biomass. The individual treatment with *T. viride* (Tv_3) 10 ml kg^{-1} of seed ranked next with 27.97 per cent root rot inhibition and 22.30 cm shoot length, 15.30 cm root length and 21.65 g plant biomass. Carbendazim @ 4g kg^{-1} treatment recorded the minimum root rot incidence of 20.43 per cent as compared to the maximum of 70.57 per cent in control. The dosage of bio control agents at 5 ml kg^{-1} of seed was proved to be insufficient as it recorded higher levels of disease incidence with all the antagonistic treatments.

Effect of soil application of antagonists on plant growth promotion and dry root rot incidence of blackgram (Pot culture)

The results presented in table 2showed that the treatments with different levels of antagonists as soil application differed in their efficacy in reducing the root rot incidence of blackgram. Among the treatments with antagonists, the combination treatment with *T. viride* (Tv_3) and *P. fluorescens* (Pf_5)treatment @ 5 l ha^{-1} recorded the least disease incidence of 24.32 per cent incidence of root rot and also recorded the maximum growth parameters of blackgram with 27.90 cm, 18.90 cm of shoot and root length and 24.75 g of plant biomass. Further, the level of 3.0 l ha^{-1} produced statistically at par results with that of 5.0 l ha^{-1} level in reducing the root rot incidence and improving growth parameters of blackgram. Hence, the dosage level of 3.0 l ha^{-1} was used for subsequent soil application studies with antagonists. Similarly, soil application of individual antagonists' *viz.*,*P. fluorescens* (Pf_5) @ 5.00 l ha^{-1} recorded 25.97 per cent root rot and *T. viride* (Tv_3) @ 5.00 lit ha^{1} recorded 29.97 per cent root rot incidence. Carbendazim as soil drenching (0.1%) recorded the incidence of 22.43 per cent root rot and 24.20 cm and 16.40 cm of shoot and root length and 19.42 g of plant biomass. The untreated control recorded the maximum disease incidence of 73.43 per cent and minimum growth parameters of 21.49 cm and 13.30 cm of shoot and root length and 18.78 g of plant biomass. In general the treatments with a dosage level of 1.5 lit ha^{-1} was proved to be the least effective as it recorded higher level of disease incidence in all the cases when compared with the other dosage levels tested.

In the present study, seed treatment with combination of *T. viride* (Tv_3) + *P. fluorescens* (Pf_5) @ 10 ml kg^{-1} recorded significant reduction in the root rot incidence and increased the growth parameters of blackgram to the maximum. Also, seed treatment of the antagonist was found superior over soil application of the antagonists. Seed treatment is the cheapest method of delivery of the antagonists to the rhizosphere and this aims at providing protection to the germinating seeds by creating a biological shield. Patil *et al*. (2003) also opined that the biological control agents were more effective and economical when applied as seed treatment than as soil treatment as observed in the present study. Bacterization of sunflower seeds with fluorescent *Pseudomonas* resulted in increased seed germination, biometrics, and yield reduced the collar rot incidence of sunflower (Shivani Bhatiya *et al*., 2005). These earlier reports corroborates with the present findings. However, Rajbir Singh and Sinha (2005) and Ashraf Alikhan and Sinha (2005) have reported soil application of *P. fluorescens* and *T. harzianum* respectivelywas found better than the seed treatment in reducing sheath blight disease in rice.

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

The results obtained on the efficacy of combined delivery system of the antagonists *viz.*, seed plus soil treatment are furnished in table 3. Among the antagonists tested by seed treatment + soil application,

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.

Seed treatment plus soil application of *T. viride* and *P. fluorescens* in combination resulted in significantly the lowest root rot incidence of blackgram (Sethuraman *et al*., 2003). Seed and soil application with the mixture of *T. viride* and *P. fluorescens* was more effective in reducing the root rot incidence and increasing seed germination of blackgram (Sajeena *et al*., 2004). These reports are in line and lend support to the present findings. The combination of delivery systems might have supported better rhizosphere competence of the antagonists and the various mechanisms elicited by the biocontrol agents might have suppressed the pathogen resulting in the reduced incidence of the disease. Also the growth promoting substances produced by the combination of antagonists might have increased the growth parameters of blackgram.

Efficacy of antagonists on seed germination and plant growth promotion

In the present study, the combination of the culture filtrate of *T. viride* (Tv_3) + *P.fluorescens* (Pf_5) treatment recorded the maximum germination per cent, shoot length; root length and vigour index . The growth promotion might be probably due to the production of growth promoting substances by the antagonists as observed by Chang *et al.* (1986) and also the growth promoting substances produced by *T. viride* and *P. fluorescens* might have exerted a synergistic action and enhanced the growth of blackgram. *Pseudomonas* spp. were also reported to produce amino acids, salicylic acid and IAA (O' Dowling and O'Gara, 1994) which might have improved the plant growth and seedling vigour. In the pot culture studies also, all the antagonistic treatments showed phytotonic effect and significantly increased the plant growth parameters of blackgram. However, the maximum root length and shoot length was recorded by the treatment consisting of combination of antagonists.

Similar to the present study, the combinations *T. harzianum* and *P. fluorescens* increased greengram plant growth in pot culture and in the field more than did individual biocontrol strains (Thilagavathi *et al.*, 2007). Also, the combinatorial efficacy of *P. fluorescens* and *T. harzianum* in enhancing the biometrics of biometrics of sweet pepper (Sunilkumar *et al*., 2012) was reported. Enhanced plant growth by the siderophore producing strains of fluorescent *Pseudomonas* was reported by several workers (Dutta *et al*., 2005). *P. fluorescens* might have stimulated plant growth by improving uptake of minerals into the host plants particularly phosphate (Kloepper *et al.*, 1980), siderophore mediated iron uptake (Jurkevitch *et al.,* 1988), association with N_2 fixation (Hong *et al*., 1991), production of IAA (Dubeikovsky *et al.,* 1993), promotion of mycorrhizal function (Garbaye, 1994) and solubilizing nutrients such as phosphorus (Whitelaw, 2000). Thus, the results of the present study and the earlier reports have confirmed that the growth promoting substances produced by *P. fluorescens* and *T. viride* (Tv_3+Pf_5) would have exerted a synergism in promoting the growth parameters of blackgram.

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