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Effect of Biocontrol Agents and PGPRs on Growth and Yield of Okra under *in vitro* Condition

A. Anantha Rama and L. Krishna Naik
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru -560 065
E-mail : ananthramboss4@gmail.com

Abstract

The aim of this study was to evaluate the efficiency of bioagents (*Trichoderma viride, Pseudomonas fluorescens*) and PGPRs (*Bacillus megaterium, Azotobacter chroococcum*) for the control of root rot pathogen (*Fusarium solani*) and its influence on growth and yield of okra plants under greenhouse conditions. Among all the treatments, T_{21} showed significant increase in plant height (135.67^{a} cm) compared to T_{25} (133.33^{b} cm), co-inoculation of *Trichoderma viride* 1 + *Pseudomonas fluorescens* 1 + *Bacillus megaterium* 1, significantly, increased number of fruits per plant T_{25} (28.33^{a}) compared to untreated control T_{1} (1.67^{p}), fruit length and fruit diameter also significantly increased in the treatment which received *T.v* 1 + *P.f* 1 + *B.m* 1 compared to all other treatments under green house conditions.

Keywords: Okra root rot, *fusarium solani, trichoderma viride*, *bacillus megaterium*

Okra (*Abelmoschus esculentus* L.) belongs to the family Malvaceae, genus *Abelmoschus* and species *esculentus*. It has many vernacular names *viz., Bhindi, Bhendi, Tori, Dhenrosh, Venda, Sapaid lori, Okra or Bende kayi* or Lady's finger. Okra plants are infected by a number of diseases caused by different fungi for example root (collar) rot and damping-off, root / stem rot; angular leaf spot and powdery mildew. According to reports, root rot is one of the most destructive diseases caused by *Fusarium solani* (Rahim *et al*., 2006). Its incidence ranges between 10-80 per cent with a maximum of 55-80 per cent in the crop grown in kitchen gardens and minimum of 10-45 per cent in the crop sown on large scale under field conditions. Abused use of chemical pesticides leads to ill effect on environment, with this concern there is a need to develop biological techniques to maintain fragile ecosystem. Many biological control agents such as *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp. and *Azotobacter* spp. could be effectively used in suppressing diseases caused by *Fusarium* spp. (Hashem and Hamada, 2002; Soleimani *et al*., 2005; Nourozian *et al.,* 2006; Abdel-Monaim, 2010).

With this concern the objective was setup to evaluate the effectiveness of isolated microorganisms under greenhouse conditions as bio-control and PGPR agents against the incidence of root rot disease caused by *Fusarium solani* and its impact on growth and yield of okra.

Material and Methods

Isolation of root rot pathogen of okra: Samples of okra plants exhibiting root rot and uninfected okra rhizosphere soils were collected from different fields in southern parts of Karnataka. Root rot infected samples were washed thoroughly with tap water. Small portions of the root rot diseased samples were surface sterilized with 1 per cent sodium hypochlorite solution for 5 min, rinsed in sterilized water and dried between folds of sterilized filter papers. The portions were placed on potato dextrose agar (PDA) and incubated at 25±1 °C. The fungal colonies were purified using single spore or hyphal tip technique. Identification of the fungi was made according to the procedure given by Booth (1985) and Gilman (1998). Stock cultures were maintained on PDA slants and kept in a refrigerator at 5 °C for further studies.

Isolation of potential antagonists and PGPRs: The antagonists against *Fusarium solani* were isolated and based on morphological, biochemical and molecular studies the organisms were identified as *Trichoderma viride*, *Pseudomonas fluorescens,* and PGPRs such as *Bacillus megaterium* and

Azotobacter chroococcum. Based on the different *in vitro* studies the potential antagonists and PGPRs were selected and used under greenhouse conditions.

Greenhouse experiment: Potential antagonist and PGPRs obtained from the *in vitro* evaluation studies were selected against *Fusarium solani* under green house condition to study the effect of these bioagents and plant growth promoting rhizobacteria in suppressing the root rot pathogen and influence on the growth and yield of okra.

Treatment Details

T_1 – Control (100 % RDF)
T_2 – *T.v* (ATV8)
T_3 – *T.v* (Std)
T_4 – *P.f* (APF19)
T_5 – *P.f* (Std)
T_6 – *B.m* (ABM6)
T_7 – *A.c* (AAC2)
T_8 – *T.v* (ATV8) + *P.f* (APF19)
T_9 – *T.v* (Std) + *P.f* (Std)
T_{10} – *T.v* (ATV8) + *P.f* (Std)
T_{11} – *T.v* (Std) + *P.f* (APF19)
T_{12} – *B.m* (ABM6) + *A.c* (AAC2)
T_{13} – *T.v* (ATV8) + *B.m* (ABM6)
T_{14} – *T.v* (Std) + *B.m* (ABM6)
T_{15} – *P.f* (APS19) + *B.m* (ABM6)
T_{16} – *P.f* (Std) + *B.m* (ABM6)
T_{17} – *T.v* (ATV8) + *A.c* (AAC2)
T_{18} – *T.v* (Std) + *A.c* (AAC2)
T_{19} – *P.f* (ATV8) + *A.c* (AAC2)
T_{20} – *P.f* (Std) + *A.c* (AAC2)
T_{21} – *T.v* (ATV8) + *P.f* (APF19) + *B.m* (ABM6)
T_{22} – *T.v* (Std) + *P.f* (Std) + *B.m* (ABM6)
T_{23} – *T.v* (ATV8) + *P.f* (Std) + *B.m* (ABM6)
T_{24} – *T.v* (Std) + *P.f* (APS19) + *B.m* (ABM6)
T_{25} – *T.v* (ATV8) + *P.f* (APF19) + *A.c* (AAC2)
T_{26} – *T.v* (Std) + *P.f* (Std) + *A.c* (AAC2)
T_{27} – *T.v* (ATV8) + *P.f* (Std) + *A.c* (AAC2)
T_{28} – *T.v* (Std) + *P.f* (APF19) + *A.c* (AAC2)

Plant growth and yield parameters: The plant growth parameters such as, Plant height (cm), Number of branches, plant and yield parameters like number of fruits, Fruit length (cm), Fruit diameters (cm) were measured and the data obtained from the green house experiments were subjected to CRD statistical analysis, the analysis of variance and interpretation of data were done as per procedures given by Fisher and Yates (2008).

Results and Discussion

Effect of Bioagents and PGPRs on plant height: In the greenhouse, the seedlings inoculated with the consortia of the antagonist and PGPR organisms grew better and showed higher plant height than uninoculated seedlings during the growth period. The treatment which received all the three inoculants *viz., T. viride, P. fluorescens* and *B. megaterium* showed significantly highest plant height (135.67^{a} cm) (Table I) at harvesting stage, whereas, the uninoculated control showed less plant height (96.33^{q} cm) compared to all other treatments.

Effect of Bioagents and PGPRs on Number of fruits / plants: Application of triple inoculants T_{21} (*T. viride, P. fluorescens* and *B. megaterium*) proved their efficacy in increasing the number of fruit set on plants, and it also resulted in significant increase in fruit yield per plant over the control (Table II).

Effect of Bioagents and PGPRs on Fruit Length: Among all the treatments, T_{21} (15.40^{a} cm) showed maximum fruit length followed by T_{25} (14.90^{ab} cm) and the least fruit length was recorded in the treatment T_1 (5.83^{o} cm). This shows that the treatment containing inoculants like *T. viride, P. fluorescens* and *B. megaterium* have positive influence on the fruit length compared to other treatments (Table II).

Effect of Bioagents and PGPRs on Fruit diameter: Fruit diameter of the okra also increased with the application of *T. viride* + *P. fluorescens* + *B. megaterium* (1.67^{a} cm) in combination, followed by the treatment which received *T. viride* + *P. fluorescens* + *A. chroococcum* (1.43^{ab} cm) and the treatment in which only pathogen (*Fusarium solani*) was inoculated showed significantly less fruit diameter compared to all other treatments (Table II).

TABLE I

Influence of biocontrol agents on plant height in okra

Treatments	Plant height (cm)			
	30 DAS	60 DAS	90 DAS	At harvest
T_1 – Control (100 % RDF)	5.33 ^{r}	36.33 ^{q}	65.00 ^{s}	96.33 ^{q}
T_2 – *T.v* (ATV8)	15.33 ^{m}	48.33 ^{l}	81.67 ^{m}	109.33 ^{l}
T_3 – *T.v* (Std)	13.33 ^{n}	46.33 ^{m}	77.67 ^{n}	106.67 ^{m}
T_4 – *P.f* (APF19)	15.00 ^{m}	48.00 ^{l}	80.67 ^{m}	109.00 ^{l}
T_5 – *P.f* (Std)	13.00 ^{n}	46.00 ^{m}	75.67 ^{o}	106.33 ^{m}
T_6 – *B.m* (ABM6)	9.33 ^{p}	42.00 ^{o}	70.00 ^{q}	101.67 ^{o}
T_7 – *A.c* (AAC2)	7.33 ^{q}	39.67 ^{p}	67.33 ^{r}	98.33 ^{p}
T_8 – *T.v* (ATV8) + *P.f* (APF19)	28.33 ^{g}	60.33 ^{g}	97.67 ^{g}	122.67 ^{g}
T_9 – *T.v* (Std) + *P.f* (Std)	27.33 ^{h}	59.33 ^{g}	96.33 ^{h}	121.67 ^{g}
T_{10} – *T.v* (ATV8) + *P.f* (Std)	28.00 ^{gh}	60.00 ^{g}	97.33 ^{gh}	122.33 ^{g}
T_{11} – *T.v* (Std) + *P.f* (APF19)	27.67 ^{gh}	59.67 ^{g}	96.67 ^{gh}	122.00 ^{g}
T_{12} – *B.m* (ABM6) + *A.c* (AAC2)	11.33 ^{o}	44.33 ^{n}	72.67 ^{p}	104.33 ^{n}
T_{13} – *T.v* (ATV8) + *B.m* (ABM6)	25.33 ^{i}	57.33 ^{h}	94.00 ^{i}	118.67 ^{h}
T_{14} – *T.v* (Std) + *B.m* (ABM6)	20.33 ^{k}	53.33 ^{j}	88.00 ^{k}	114.33 ^{j}
T_{15} – *P.f* (APS19) + *B.m* (ABM6)	24.67 ^{i}	57.00 ^{h}	93.67 ^{i}	118.33 ^{h}
T_{16} – *P.f* (Std) + *B.m* (ABM6)	20.00 ^{k}	53.00 ^{j}	87.67 ^{k}	114.00 ^{j}
T_{17} – *T.v* (ATV8) + *A.c* (AAC2)	22.33 ^{j}	55.33 ^{i}	90.67 ^{j}	116.33 ^{i}
T_{18} – *T.v* (Std) + *A.c* (AAC2)	18.00 ^{l}	51.00 ^{k}	84.67 ^{l}	111.33 ^{k}
T_{19} – *P.f* (ATV8) + *A.c* (AAC2)	22.00 ^{j}	55.00 ^{i}	90.33 ^{j}	116.00 ^{i}
T_{20} – *P.f* (Std) + *A.c* (AAC2)	17.67 ^{l}	50.67 ^{k}	84.33 ^{l}	111.00 ^{k}
T_{21} – *T.v* (ATV8) + *P.f* (APF19) + *B.m* (ABM6)	39.33 ^{a}	71.33 ^{a}	109.67 ^{a}	135.67 ^{a}
T_{22} – *T.v* (Std) + *P.f* (Std) + *B.m* (ABM6)	36.00 ^{c}	67.33 ^{c}	105.33 ^{c}	131.33 ^{c}
T_{23} – *T.v* (ATV8) + *P.f* (Std) + *B.m* (ABM6)	31.33 ^{e}	63.33 ^{e}	101.33 ^{e}	126.33 ^{e}
T_{24} – *T.v* (Std) + *P.f* (APS19) + *B.m* (ABM6)	31.00 ^{ef}	63.00 ^{ef}	101.00 ^{ef}	126.00 ^{ef}
T_{25} – *T.v* (ATV8) + *P.f* (APF19) + *A.c* (AAC2)	37.67 ^{b}	69.33 ^{b}	107.33 ^{b}	133.33 ^{b}
T_{26} – *T.v* (Std) + *P.f* (Std) + *A.c* (AAC2)	33.67 ^{d}	65.67 ^{d}	103.33 ^{d}	129.33 ^{d}
T_{27} – *T.v* (ATV8) + *P.f* (Std) + *A.c* (AAC2)	30.67 ^{ef}	62.33 ^{ef}	100.67 ^{ef}	125.67 ^{ef}
T_{28} – *T.v* (Std) + *P.f* (APF19) + *A.c* (AAC2)	30.33 ^{f}	62.00 ^{f}	100.00 ^{f}	125.00 ^{f}
S.Em±	0.223	0.280	0.286	0.275
CD at 1%	0.842	1.055	1.080	1.035

Note : *T.v – Trichoderma viridae, P.f – Pseudomonas fluorescens, B.m – Bacillus megaterium, A.c – Azotobacter chroococcum,* Std – Standard culture

TABLE II

Influence of biocontrol agents on growth and yield parameters in okra

Treatments	Number of fruits / plan	Fruit Length (cm)	Fruit Diameter (cm)
T_1 – Control (100 % RDF)	5.33 r	36.33 q	65.00 s
T_1 – Control (100 % RDF)	1.67 p	5.83 o	0.93 f
T_2 – *T.v* (ATV8)	9.67 k	8.27 jk	1.13 bcdef
T_3 – *T.v* (Std)	8.33 l	7.83 kl	1.10 cdef
T_4 – *P.f* (APF19)	9.67 k	8.23 k	1.30 bcd
T_5 – *P.f* (Std)	8.00 l	7.80 kl	1.10 cdef
T_6 – *B.m* (ABM6)	4.67 n	6.73 mn	1.00 def
T_7 – *A.c* (AAC2)	3.33 o	6.27 no	0.97 ef
T_8 – *T.v* (ATV8) + *P.f* (APF19)	19.67 f	12.33 f	1.27 bcde
T_9 – *T.v* (Std) + *P.f* (Std)	19.00 f	12.13 f	1.23 bcdef
T_{10} – *T.v* (ATV8) + *P.f* (Std)	19.33 f	12.23 f	1.27 bcde
T_{11} – *T.v* (Std) + *P.f* (APF19)	19.33 f	12.20 f	1.27 bcde
T_{12} – *B.m* (ABM6) + *A.c* (AAC2)	6.33 m	7.17 lm	1.00 def
T_{13} – *T.v* (ATV8) + *B.m* (ABM6)	17.33 g	11.67 f	1.23 bcdef
T_{14} – *T.v* (Std) + *B.m* (ABM6)	13.67 i	9.83 h	1.13 bcdef
T_{15} – *P.f* (APS19) + *B.m* (ABM6)	17.00 g	11.63 f	1.20 bcdef
T_{16} – *P.f* (Std) + *B.m* (ABM6)	13.33 i	9.77 h	1.13 bcdef
T_{17} – *T.v* (ATV8) + *A.c* (AAC2)	15.67 h	10.63 g	1.17 bcdef
T_{18} – *T.v* (Std) + *A.c* (AAC2)	11.67 j	9.00 i	1.13 bcdef
T_{19} – *P.f* (ATV8) + *A.c* (AAC2)	15.33 h	10.60 g	1.13 bcdef
T_{20} – *P.f* (Std) + *A.c* (AAC2)	11.33 j	8.97 ij	1.13 bcdef
T_{21} – *T.v* (ATV8) + *P.f* (APF19) + *B.m* (ABM6)	28.33 a	15.40 a	1.67 a
T_{22} – *T.v* (Std) + *P.f* (Std) + *B.m* (ABM6)	25.33 c	14.43 bc	1.40 abc
T_{23} – *T.v* (ATV8) + *P.f* (Std) + *B.m* (ABM6)	22.00 e	13.33 de	1.33 bc
T_{24} – *T.v* (Std) + *P.f* (APS19) + *B.m* (ABM6)	21.67 e	13.20 e	1.37 abc
T_{25} – *T.v* (ATV8) + *P.f* (APF19) + *A.c* (AAC2)	26.67 b	14.90 ab	1.43 ab
T_{26} – *T.v* (Std) + *P.f* (Std) + *A.c* (AAC2)	23.67 d	14.00 cd	1.37 abc
T_{27} – *T.v* (ATV8) + *P.f* (Std) + *A.c* (AAC2)	21.33 e	13.10 e	1.33 bc
T_{28} – *T.v* (Std) + *P.f* (APF19) + *A.c* (AAC2)	21.00 e	13.07 e	1.30 bcd
S.Em±	0.284	0.189	0.081
CD at 1%	1.072	0.712	0.304

Note : *T.v – Trichoderma viridae, P.f – Pseudomonas fluorescens, B.m – Bacillus megaterium, A.c – Azotobacter chroococcum,* Std – Standard culture

TABLE III

Influence of biocontrol agents on number of branches of okra

Treatments	No. of branches / plant	Treatments	No. of branches / plant
T_1 – Control (100 % RDF)	0.00 ^{i}	T_{15} – *P.f* (APS19) + *B.m* (ABM6)	1.33 ^{efg}
T_2 – *T.v* (ATV8)	0.67 ^{ghi}	T_{16} – *P.f* (Std) + *B.m* (ABM6)	1.00 ^{fgh}
T_3 – *T.v* (Std)	0.67 ^{ghi}	T_{17} – *T.v* (ATV8) + *A.c* (AAC2)	1.00 ^{fgh}
T_4 – *P.f* (APF19)	0.67 ^{ghi}	T_{18} – *T.v* (Std) + *A.c* (AAC2)	1.00 ^{fgh}
T_5 – *P.f* (Std)	0.67 ^{ghi}	T_{19} – *P.f* (ATV8) + *A.c* (AAC2)	1.00 ^{fgh}
T_6 – *B.m* (ABM6)	0.33 ^{hi}	T_{20} – *P.f* (Std) + *A.c* (AAC2)	1.00 ^{fgh}
T_7 – *A.c* (AAC2)	0.33 ^{hi}	T_{21} – *T.v* (ATV8) + *P.f* (APF19) + *B.m* (ABM6)	4.00 ^{a}
T_8 – *T.v* (ATV8) + *P.f* (APF19)	1.67 ^{def}	T_{22} – *T.v* (Std) + *P.f* (Std) + *B.m* (ABM6)	2.67 ^{bc}
T_9 – *T.v* (Std) + *P.f* (Std)	1.67 ^{def}	T_{23} – *T.v* (ATV8) + *P.f* (Std) + *B.m* (ABM6)	2.00 ^{cde}
T_{10} – *T.v* (ATV8) + *P.f* (Std)	1.67 ^{def}	T_{24} – *T.v* (Std) + *P.f* (APS19) + *B.m* (ABM6)	2.00 ^{cde}
T_{11} – *T.v* (Std) + *P.f* (APF19)	1.67 ^{def}	T_{25} – *T.v* (ATV8) + *P.f* (APF19) + *A.c* (AAC2)	3.00 ^{b}
T_{12} – *B.m* (ABM6) + *A.c* (AAC2)	0.67 ^{ghi}	T_{26} – *T.v* (Std) + *P.f* (Std) + *A.c* (AAC2)	2.33 ^{bcd}
T_{13} – *T.v* (ATV8) + *B.m* (ABM6)	1.33 ^{efg}	T_{27} – *T.v* (ATV8) + *P.f* (Std) + *A.c* (AAC2)	2.00 ^{cde}
T_{14} – *T.v* (Std) + *B.m* (ABM6)	1.00 ^{fgh}	T_{28} – *T.v* (Std) + *P.f* (APF19) + *A.c* (AAC2)	2.00 ^{cde}

Note : *T.v – Trichoderma viridae, P.f – Pseudomonas fluorescens, B.m – Bacillus megaterium, A.c – Azotobacter chroococcum,* Std – Standard culture

Effect of Bioagents and PGPRs on Number of branches per plant: Number of branches per plant of the okra also increased with the application of *T. viride* + *P. fluorescens* + *B. megaterium* (4.00^{a}) in combination, followed by the treatment which received *T. viride* + *P. fluorescens* + *A. chroococcum* (3.00^{b} cm) and the treatment in which only pathogen (*Fusarium solani*) was inoculated showed significantly less number of branches per plant compared to all other treatments (Table III)

Generally, treatments involving, *T.v* + *P.f* + *B.m* recorded the highest reduction of root rot incidence and increased the growth and yield components of crop plants especially in soil application method. The results obtained are in good accordance with previous studies which have been concluded that *Trichoderma viride, Pseudomonas fluorescens* and *Bacillus megaterium* can effectively protect many plant species against root rot diseases (Hashem and Hamada, 2002; Soleimani *et al*., 2005; Nourozian, *et al.,* 2006; Atef, 2008; Abdel-Monaim, 2010). According to Harman (2001) natural factors limiting the number of soil borne pathogens occur through a combination of antagonism by other soil fungi and bacteria, natural release of antibiotics from other bacteria and fungi and by competitive exclusion of habitat in the root zone or rhizosphere. The mechanism of *Trichoderma* and *Bacillus* acts on pathogens may be by attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Soleimani *et al*., 2005; Zaghloul *et al.,* 2007), produce siderophores and hydrogen cyanide (Soleimani *et al*., 2005), production of secondary metabolites such as Phenazine-1-Carboxilic acid (PCA), 2, 4-Pyrrolnitrin, Oomycin.

Such enhancement may be due to induced plant resistance, production extracellular enzymes and antifungal or antibiotics, which decrease biotic stress on plant, and produce growth promoting substances

(Szczech and Shoda, 2004). In addition, Egamberdiyeva (2007) hypothesized that there are several mechanisms by which rhizosphere bacteria and fungi may stimulate plant growth, such as production of plant growth substances, nitrogen fixation, phytohormones, vitamins, solublizing minerals besides, their role in direct inhibition of pathogen growth and suppression of diseases and increased plant growth and yield.

The obtained results are in harmony with that obtained by Zahoor *et al*. (2012); Siddiqui *et al.,* 2000 on okra**.** The inoculation of bioagents and PGPRs showed effective control on root rot disease of okra and influenced the growth and yield of okra plants.

Specific antagonist can influence disease suppression and could be considered as part of disease control strategy under an integrated pest management which offers a successful approach for the deployment of both agro-chemicals and biocontrol agents. This study suggest that effective screening of bioagents for growth and yield promotion under greenhouse experiment is a good tool to select efficient antagonist for biocontrol agent development.

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