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Seed Bio-Priming for Management of Root Rot and Blight of Mungbean Incited by *Macrophomina phaseolina* (Tassi) Goid. and *Rhizoctonia solani* Kuhn

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Seed bio priming of mungbean with the biocontrol agents i.e. *Trichoderma viride, T. harzianum, T. virens and Pseudomonas fluorescens* individually and in combination for their efficacy against root rot and blight diseases of mungbean caused by *Macrophomina phaseolina* and *Rhizoctonia solani.* A combination of *T. viride + P. fluorescens* promoted the seed germination, shoot length, root length, fresh shoot weight, dry shoot weight, plant height and most effective in reducing root rot incidence of the mungbean under poly-house conditions as compared with other single or combined treatments or the untreated control. The activity of the defense-related enzymes peroxidase, polyphenol oxidase and phenyl alanine ammonia lyase was significantly greater in mungbean root tissue treated with a talc based formulation containing *T. viride + P. fluorescens* than other treatments or the untreated control. Moreover, a combination of *T. viride + P. fluorescens* significantly increased phenol content, total soluble sugar, protein content in mungbean leaves under poly house conditions.

Keywords: Antagonistic activity, *T. viride, T. harzianum, M. phaseolina, Rhizoctonia solani, bacterial bioagents,* defense-related enzymes peroxidase, polyphenol oxidase and phenyl alanine ammonia lyase.

Mungbean (*Vigna radiata* [L.] Wilczek) is one of the most important pulse crops and is grown in summer and *kharif* season in India. The crop is subject to diseases caused by fungi, bacteria and viruses. Of these diseases, root rot caused by *Macrophomina phaseolina* (Tassi.) Goid causes considerable losses (Raguchander *et al.*, 1993). Web blight caused by *Rhizoctonia solani* Kuhn is a destructive seed and soil borne disease of mungbean and it is considered as one of the important causes for stagnated productivity of these crops in the country. Management of *M. phaseolina and R. solani*, using chemical fungicides has been the prevailing control method for over fifty years. Though fungicides have shown good results in controlling *M. phaseolina* and *R. solani*, fungicide residue is a major problem and causes soil and environmental pollution, and human health hazards. In this context, the biocontrol of these diseases may represent an ecofriendly strategy for managing *M. phaseolina* and *R. solani* in crop plants. Mixtures of biocontrol agents will also have the advantage of exercising a broad spectrum activity, enhancing the efficacy and reliability of biological control generally and ensuring greater induction of defense enzymes over individual strains. Single antagonistic strains often result 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 tested different biocontrol strains in combination (Droby, 2001 and Thilagavathi, 2007).

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MATERIALS AND METHODS

Isolation of pathogen and maintenance of biocontrol agents

The root rot and blight pathogen *M. phaseolina* and *R. solani* were isolated from mungbean plants showing typical root rot symptoms and pure cultures of the pathogen were obtained by the single hyphal tip method (Rangaswami, 1972). The biocontrol agents *T. viride, T. harzianum, T. virens* and *P. fluorescens* were obtained from Department of Plant Pathology, B. A. College of Agriculture, Anand Agricultural University, Anand (Gujarat), India.

Effect of seed bio priming on mungbean growth *in vitro* by Rolled paper towel method

One hundred gram seeds of mungbean were taken separately in sterilized conical flasks. The liquid suspension of *T. viride, T. virens, T. harzianum* and *P. fluorescens* and the combined bioagents were prepared by mixing 5 gram of solid talc based formulation with spore load of 2×10^8 cfu / g in 25 ml of distilled water. The seeds were treated with the bioagents suspension for 10 hours at room temperature. After 10 hours of treatment, the seeds were shade dried. Bio-primed seeds were used for sowing after 10 hours of treatment. Untreated seeds were kept as control. The plant growth-promoting activity of the biocontrol agents were assessed based on the seedling vigour index by the standard Roll towel method (ISTA, 1993). The seedling vigour index was calculated using the formula as given by Abdul Baki and Anderson (1973).

Vigour Index = (mean root length + mean shoot length) × per cent germination

Pot culture study

The soil in pots was sterilized in autoclave at temperature 121^0C for 6 hrs. Pathogens were multiplied on sand maize medium (9:1) for 15 day for mass production. The pots containing sterilized soil were inoculated with above sand maize inoculums @ 50 g/kg soil and left for 15 days for establishment of inoculum. The above treated seeds of mungbean was inoculated with pathogens i.e. *M. phaseolina* and *R. solani* separately and sown in pots containing pathogen inoculated soil in polyhouse condition.

Assay of phenol content, total soluble sugar, protein content and defense enzymes under poly house condition

Total phenol content from the leaves of healthy and inoculated mungbean was estimated by Folin cio-calteau method as described by Bhatnagar *et al.* (2005). Total soluble sugar from the leaves of healthy and inoculated mungbean was determined by phenol sulphuric acid method as described by Dubois (1956). Protein content was determined by the method developed by Lowry *et al.* (1951). Peroxidase activity was estimated at 7, 14, 21, 28 and 35 days after sowing following the Worthington's method as described by Guilbault (1976) was expressed as changes in absorbance at 460 nm min-1 g-1 of fresh tissue. PPO activity was determined at 7, 14, 21, 28 and 35 days after sowing following the procedure given by Malik and Singh (1980) and was expressed as changes in absorbance at 490 nm min-1 g-1 of fresh tissue. PAL activity was assayed at 7, 14, 21, 28 and 35 days after sowing following the method of Ross and Sederoff (1992) and was expressed as changes in absorbance at 290 nm min-1 g-1 of fresh tissue.

RESULTS

Effect of seed bio priming on mungbean growth in vitro by Rolled paper towel method

Seed bio priming of mungbean with combination of *T. viride* + *P. fluorescens* and *T. harzianum* + *P. fluorescens* produced mungbean seedlings with a significantly higher vigour index 2832.91 and 2357.07 than individual treatments *T. viride, P. fluorescens, T. harzianum* and *T. virens* whose vigour index was only 2119.15, 1787.8, 1637.58 and 1386.45, respectively. The untreated control seedlings had the lowest vigour index, 791.93 (Table 1).

Pot culture study

Seed bio priming of mungbean with combination of *T. viride* + *P. fluorescens* and *T. harzianum* + *P. fluorescens* in presence of *M. phaseolina* in pot condition produced mungbean seedlings with a higher vigour index 1417.2 and 1192.4 than individual treatments *T. viride, P. fluorescens, T. harzianum* and *T. virens* whose vigour index was only 1097.2, 990.9, 891.2 and 765.3, respectively. The untreated control seedlings had the lowest vigour index, 453.7. The combination of *T. viride* + *P. fluorescens* and *T.*

harzianum + *P. fluorescens* in presence of *R. solani* in pot condition produced mungbean seedlings with higher vigour index 1300.4 and 1197.4 followed by *T. virens* +*P. fluorescens, T. viride, P. fluorescens, T. harzianum* and *T. virens* with vigour index 1147.3, 1090.7, 1069.3, 955.1 and 839.7, respectively. The untreated control seedlings had the lowest vigour index, 446.5 (Table 2).

Plant height

Seed bio priming of mungbean with combination of *T. viride* + *P. fluorescens* and *T. harzianum* + *P. fluorescens* in presence of *M. phaseolina* in pot condition gave highest plant height at 60 DAS of 27.93 and 26.43 cm followed by *T. viride, T. virens* + *P. fluorescens, P. fluorescens, T. harzianum* and *T. virens* with plant

T. viride + *P. fluorescens*

T. harzianum + *P. fluorescens*

* T_5: *T. viride* + *P. fluorescens* + *M. Phaseolina*, T_{12}: *T. viride* + *P. fluorescens* + *R. solani*, T_{M-}: *M. Phaseolina*, T_R: *R. solani*

Table 1. Effect of seed biopriming of mungbean with bioagents individually and in combination on plant growth promotion by Rolled paper towel method

Sr. no.	Treatments	Per cent Germination	Shoot length(cm)	Root length(cm)	Vigour index
1	*T. viride*	77.71*(95.47)**	16.37	10.90	2119.15
2	*T. harzianum*	66.84 (84.53)	14.83	9.67	1637.58
3	*T. virens*	65.00 (82.14)	12.23	9.10	1386.45
4	*P. fluorescens*	70.11(88.43)	15.57	9.93	1787.81
5	*T. viride* + *P. fluorescens*	85.69 (99.44)	18.93	14.13	2832.91
6	*T. harzianum* + *P. fluorescens*	79.55 (96.71)	17.04	12.60	2357.07
7	*T. virens* + *P. fluorescens*	70.11(88.43)	15.80	12.20	1963.08
8	Control (seeds soaked in sterilized distilled water)	55.77 (68.36)	8.43	5.77	791.93
	S. Em. ±	4.04	0.342	0.26	—
	CD at 5 %	12.10	1.03	0.79	—
	CV %	9.80	3.98	4.34	—

* Figures indicate Arcsine transformed values.
** Figures indicate retransformed values.

Table 2. Effect of seed biopriming of mungbean with bioagents individually and in combination on plant growth promotion under sick soil in pot condition

Sr. no.	Treatments	Per cent Germination	Shoot length(cm)	Root length(cm)	Vigour index	Fresh shoot weight at 15 DAS (g)	Dry shoot weight at 15 DAS (g)
T_1	*T. viride* + *M. Phaseolina*	71.57* (90.01)**	10.00	5.33	1097.2	1.91	0.30
T_2	*T. harzianum* + *M. Phaseolina*	63.93 (80.69)	9.47	4.47	891.2	1.54	0.27
T_3	*T. virens* + *M. Phaseolina*	61.22 (76.82)	8.43	4.07	765.3	1.51	0.25
T_4	*P. fluorescens* + *M. Phaseolina*	66.64 (84.28)	9.90	4.97	990.9	1.87	0.28
T_5	*T. viride* + *P. fluorescens* + *M. Phaseolina*	77.44 (95.27)	11.67	6.63	1417.2	2.28	0.37
T_6	*T. harzianum* + *P. fluorescens* + *M. phaseolina*	71.57 (90.01)	10.53	6.13	1192.4	2.01	0.33
T_7	*T. virens* + *P. fluorescens* + *M. Phaseolina*	63.93 (80.69)	10.30	5.50	1010.1	1.91	0.31
T_8	*T. viride* + *R. solani*	71.57 (90.01)	10.17	5.07	1090.7	1.97	0.32
T_9	*T. harzianum* + *R. solani*	68.86 (86.99)	9.50	4.37	955.1	1.57	0.27
T_{10}	*T. virens* + *R. solani*	66.64 (84.28)	8.47	4.13	839.7	1.54	0.26
T_{11}	*P. fluorescens* + *R. solani*	71.57 (90.01)	10.07	4.87	1069.3	1.90	0.30
T_{12}	*T. viride* + *P. fluorescens* + *R. solani*	71.57 (90.01)	11.70	6.47	1300.4	2.35	0.38
T_{13}	*T. harzianum* + *P. fluorescens* + *R. solani*	71.57 (90.01)	10.63	6.10	1197.4	2.05	0.33
T_{14}	*T. virens* + *P. fluorescens* + *R. solani*	71.57 (90.01)	10.33	5.70	1147.3	1.97	0.32
T_{15}	Control (*M. phaseolina*)	57.00 (70.34)	5.83	2.13	453.7	1.11	0.20
T_{16}	Control (*R. solani*)	63.43 (79.99)	4.87	2.17	446.5	1.14	0.19
	S. Em. ±	2.91	0.17	0.12	—	0.07	0.01
	CD at 5 %	8.37	0.50	0.35	—	0.20	0.04
	CV	7.39	3.17	4.29	—	6.84	7.62

* Figures indicate Arcsine transformed values.

** Figures indicate retransformed values.

height at 60 DAS of 25.50, 24.93, 24.40, 22.83 and 22.33 cm, respectively. The untreated control had the lowest plant height, 13.50 cm. The combination of *T. viride* + *P. fluorescens* and *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* in presence of *R. solani* in pot condition gave highest plant height of 27.20, 25.37 and 24.67 cm followed by *T. viride, P. fluorescens, T. harzianum* and *T. virens* with plant height 23.67, 23.20, 21.73 and 21.33 cm, respectively. The untreated control had the lowest plant height, 12.33 cm (Table 3).

Disease incidence

Seed bio priming of mungbean with combination of *T. viride* + *P. fluorescens, T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* in presence of *M. phaseolina* in pot condition gave highest per cent disease control of 89.41, 78.63 and 70.93 % followed by *T. viride, T. harzianum, T. virens* and *P. fluorescens* with per cent disease control of 68.71, 59.82, 58.04 and 52.48 %, respectively. The combination of *T. viride* + *P. fluorescens* in presence of *R. solani* in pot condition gave highest per cent disease control of 88.24 % followed by *T. viride, T. harzianum* + *P. fluorescens, T. virens* + *P. fluorescens, , T. virens, T. harzianum* and *P. fluorescens* with plant height 78.05, 71.36, 67.87, 67.87, 66.60 and 63.14 %, respectively (Table 3).

Induction of defense related enzymes by biocontrol agents in greengram

Activity of peroxidase, polyphenol oxidase and phenylamine ammonia lyase was increased from 7 DAS upto 21 DAS except the pathogen inoculated control and declined thereafter in all the inoculated treatments. Whereas in case of pathogen inoculated control, it gradually declined from 7 DAS itself. Combination of biocontrol agents induced a greater PO, PPO and PAL activity as compared to the untreated control or individual biocontrol agents. The greatest

Table 3. Effect of seed biopriming of mungbean with bioagents individually and in combination on number of branches/ plant, plant height and disease incidence under sick soil in pot condition

Sr. no.	Treatments	Number of branches /plant at 60 DAS	Plant height (cm) 30 DAS	60 DAS	Per cent disease incidence	Per cent disease control
T_1	*T. viride* + *M. Phaseolina*	4.67	20.50	25.50	25.76* (18.89)**	68.71
T_2	*T. harzianum* + *M. Phaseolina*	4.00	17.50	22.83	33.08 (29.79)	59.82
T_3	*T. virens* + *M. Phaseolina*	3.67	17.83	22.33	34.54 (32.15)	58.04
T_4	*P. fluorescens* + *M. Phaseolina*	4.33	20.17	24.40	39.12 (39.81)	52.48
T_5	*T. viride* + *P. fluorescens* + *M. Phaseolina*	5.67	21.83	27.93	8.72 (2.30)	89.41
T_6	*T. harzianum* + *P. fluorescens* + *M. phaseolina*	5.00	20.50	26.43	17.60 (9.14)	78.63
T_7	*T. virens* + *P. fluorescens* + *M. Phaseolina*	4.67	19.50	24.93	23.94 (16.47)	70.93
T_8	*T. viride* + *R. solani*	4.33	20.17	23.67	17.60 (9.14)	78.05
T_9	*T. harzianum* + *R. solani*	3.33	18.33	21.73	26.77 (20.29)	66.60
T_{10}	*T. virens* + *R. solani*	3.33	16.00	21.33	25.76 (18.89)	67.87
T_{11}	*P. fluorescens* + *R. solani*	4.00	19.17	23.20	29.55 (24.32)	63.14
T_{12}	*T. viride* + *P. fluorescens* + *R. solani*	5.67	21.50	27.20	9.43 (2.68)	88.24
T_{13}	*T. harzianum* + *P. fluorescens* + *R. solani*	4.67	20.50	25.37	22.96 (15.22)	71.36
T_{14}	*T. virens* + *P. fluorescens* + *R. solani*	4.67	19.50	24.67	25.76 (18.89)	67.87
T_{15}	Control (*M. phaseolina*)	1.67	10.00	13.50	82.32 (98.21)	0.00
T_{16}	Control (*R. solani*)	2.00	10.50	12.33	80.16 (97.08)	0.00
	S. Em. ±	0.29	0.47	0.53	3.00	—
	CD at 5 %	0.83	1.36	1.54	8.68	—
	CV	12.18	4.47	4.02	23.71	—

* Figures indicate Arcsine transformed values.

** Figures indicate retransformed values.

increase in PO activity was produced by combinations of *T. viride* + *P. fluorescens*, *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* in presence of *M. phaseolina* at 21 DAS of 22.11, 20.37 and 19.38 ” OD/min/ g tissue, respectively followed by *P. fluorescens*, *T. viride*,

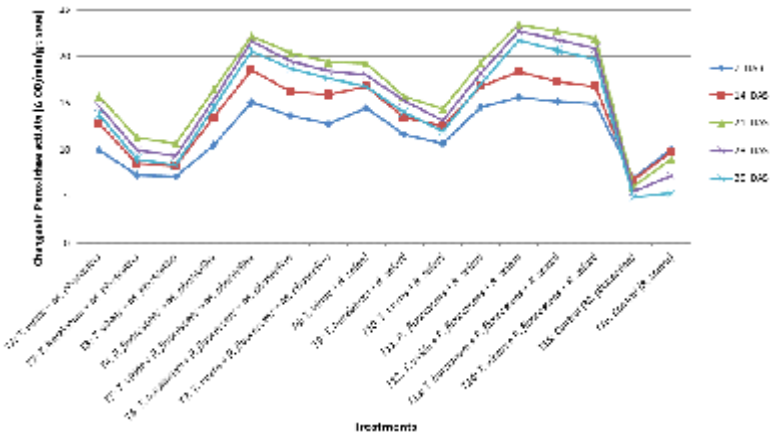

Fig. 1. Changes in Peroxidase activity (” OD/min/g tissue) in mungbean root tissue due to seed bio-priming with different bioagents under sick soil in pot condition

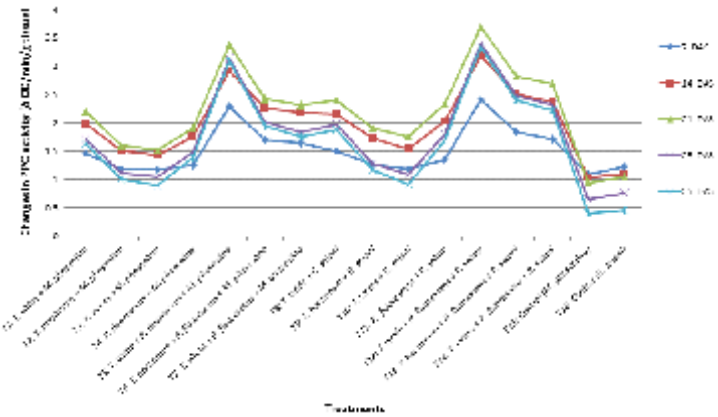

Fig. 2. Changes in Polyphenol oxidase (PPO) activity (”À OD/min/g tissue) in mungbean root tissue due to seed bio-priming with different bioagents under sick soil in pot condition

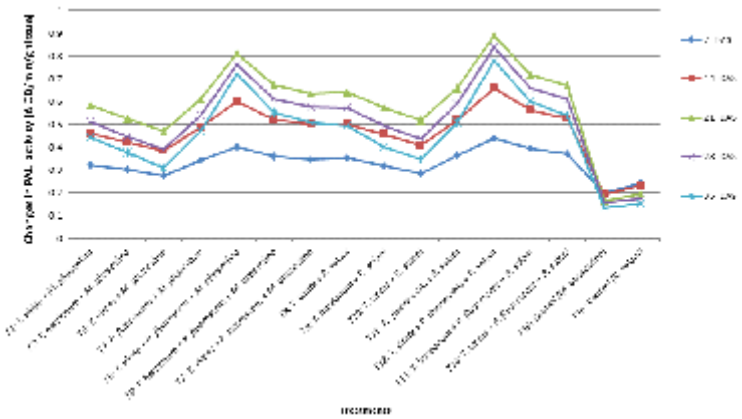

Fig. 3. Changes in Phenylamine ammonia lyase (PAL) activity (” OD/min/g tissue) in mungbean root tissue due to seed bio-priming with different bioagents under sick soil in pot condition

T. harzianum and *T. virens* of 16.42, 15.67, 11.29 and 10.62 OD/min/ g tissue, respectively. The greatest increase in PO activity was produced by combinations of *T. viride* + *P. fluorescens*, *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* in presence of *R. solani* at 21 DAS of 23.35, 22.68 and 21.96 " OD/min/ g tissue, respectively followed by *P. fluorescens*, *T. viride*, *T. harzianum* and *T. virens* of 19.30, 19.19, 15.73 and 14.40 OD/min/ g tissue, respectively (Fig. 1). The greatest increase in PPO activity was produced by combinations of *T. viride* + *P. fluorescens*, *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* in presence of *M. phaseolina* at 21 DAS of 3.37, 2.42 and 2.31 " OD/min/ g tissue, respectively followed by *T. viride*, *P. fluorescens*, *T. harzianum* and *T. virens* of 2.20, 1.91, 1.61 and 1.52 OD/min/ g tissue, respectively. The greatest increase in PPO activity was produced by combinations of *T. viride* + *P. fluorescens*, *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* in presence of *R. solani* at 21 DAS of 3.69, 2.82 and 2.68 " OD/min/ g tissue, respectively followed by *T. viride*, *P. fluorescens*, *T. harzianum* and *T. virens* of 2.40, 2.32, 1.90 and 1.76 OD/min/ g tissue, respectively (Fig. 2). The greatest increase in PAL activity was produced by combinations of *T. viride* + *P. fluorescens*, *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* in presence of *M. phaseolina* at 21 DAS of 0.813, 0.673 and 0.637 " OD/min/ g tissue, respectively followed by *P. fluorescens*, *T. viride*, *T. harzianum* and *T. virens* of 0.610, 0.583, 0.527 and 0.470 OD/ min/ g tissue, respectively. The greatest increase in PAL activity was produced by combinations of *T. viride* + *P. fluorescens*, *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* in presence of *R. solani* at 21 DAS of 0.890, 0.720 and 0.670 " OD/min/ g tissue, respectively followed by *P. fluorescens*, *T. viride*, *T. harzianum* and *T. virens* of 0.660, 0.643, 0.573 and 0.517 OD/min/ g tissue, respectively (Fig. 3).

Phenol content

The greatest increase in phenol content was produced by combinations of *T. viride* + *P. fluorescens*, *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* in presence of *M. phaseolina* of 0.385, 0.349 and 0.317 mg 100mg^{-1} FW, respectively followed by *P. fluorescens*, *T. viride*, *T. harzianum* and *T. virens* of 0.302, 0.295, 0.260 and 0.241 mg 100mg^{-1} FW, respectively. The greatest increase in phenol content was produced

Table 4. Changes in phenol content, total soluble sugar and protein content (mg 100mg^{-1} FW) in mungbean leaves due to seed bio-priming with different bioagents under sick soil in pot condition

Sr. no.	Treatments	Phenol content	Total soluble sugar	Protein content
T_1	*T. viride* + *M. Phaseolina*	0.295	0.813	3.35
T_2	*T. harzianum* + *M. phaseolina*	0.260	0.761	3.05
T_3	*T. virens* + *M. Phaseolina*	0.241	0.730	2.97
T_4	*P. fluorescens* + *M. phaseolina*	0.302	0.824	3.46
T_5	*T. viride* + *P. fluorescens* + *M. phaseolina*	0.385	0.993	3.93
T_6	*T. harzianum* + *P. fluorescens* + *M. phaseolina*	0.349	0.875	3.67
T_7	*T. virens* + *P. fluorescens* + *M. phaseolina*	0.317	0.840	3.76
T_8	*T. viride* + *R. solani*	0.329	0.938	3.21
T_9	*T. harzianum* + *R. solani*	0.294	0.843	3.04
T_{10}	*T. virens* + *R. solani*	0.267	0.789	2.96
T_{11}	*P. fluorescens* + *R. solani*	0.337	0.952	3.24
T_{12}	*T. viride* + *P. fluorescens* + *R. solani*	0.445	1.104	3.87
T_{13}	*T. harzianum* + *P. fluorescens* + *R. solani*	0.386	1.000	3.31
T_{14}	*T. virens* + *P. fluorescens* + *R. solani*	0.358	0.967	3.55
T_{15}	Control (*M. phaseolina*)	0.146	0.328	2.32
T_{16}	Control (*R. solani*)	0.186	0.344	2.24
	S. Em. ±	0.01	0.01	0.03
	CD at 5 %	0.02	0.02	0.09
	CV %	3.91	1.25	1.59

by combinations of *T. viride + P. fluorescens, T. harzianum + P. fluorescens* and *T. virens + P. fluorescens* in presence of *R. solani* of 0.445, 0.386 and 0.358 mg 100mg^{-1} FW, respectively followed by *P. fluorescens, T. viride, T. harzianum* and *T. virens* of 0.337, 0.329, 0.294 and 0.267 mg 100mg^{-1} FW, respectively (Table 4).

Total soluble sugar

The greatest increase in total soluble sugar was produced by combinations of *T. viride + P. fluorescens, T. harzianum + P. fluorescens* and *T. virens + P. fluorescens* in presence of *M. phaseolina* of 0.993, 0.875 and 0.840 mg 100mg^{-1} FW, respectively followed by *P. fluorescens, T. viride, T. harzianum* and *T. virens* of 0.824, 0.813, 0.761 and 0.730 mg 100mg^{-1} FW, respectively. The greatest increase in total soluble sugar was produced by combinations of *T. viride + P. fluorescens, T. harzianum + P. fluorescens* and *T. virens + P. fluorescens* in presence of *R. solani* of 1.104, 1.000 and 0.967 mg 100mg^{-1} FW, respectively followed by *P. fluorescens, T. viride, T. harzianum* and *T. virens* of 0.952, 0.938, 0.843 and 0.789 mg 100mg^{-1} FW, respectively (Table 4).

Protein content

The greatest increase in total soluble sugar was produced by combinations of *T. viride + P. fluorescens, T. virens + P. fluorescens* and *T. harzianum + P. fluorescens* in presence of *M. phaseolina* of 3.93, 3.76 and 3.67 mg 100mg^{-1} FW, respectively followed by *P. fluorescens, T. viride, T. harzianum* and *T. virens* of 3.46, 3.35, 3.05 and 2.97 mg 100mg^{-1} FW, respectively. The greatest increase in phenol content was produced by combinations of *T. viride + P. fluorescens, T. virens + P. fluorescens* and *T. harzianum + P. fluorescens* in presence of *R. solani* of 3.87, 3.55 and 3.31 mg 100mg^{-1} FW, respectively followed by *P. fluorescens, T. viride, T. harzianum* and *T. virens* of 3.24, 3.21, 3.04 and 2.96 mg 100mg^{-1} FW, respectively (Table 4).

DISCUSSION

Multiple strain mixtures of microbial agents have been employed successfully against several plant pathogens in earlier studies. Mixtures include those of bacteria and fungi (Leibinger *et al.*, 1997), and those of bacteria and yeast (Janisiewicz and Bors, 1995). In the present study, seed biopriming of mungbean with talcbased formulations containing a mixture of bacterial and fungal biocontrol agents and individual biocontrol agents were tested against the mungbean root rot and blight disease causing agent *M. phaseolina* and *R. solani in vitro* and in the polyhouse condition. Several authors have suggested that combinations of introduced biocontrol agents have to be compatible with each other for better and more consistent disease suppression (Raaijmakers *et al.*, 1995). In the present study, the *P. fluorescens* were compatible with *T. viride, T. harzianum* and *T. virens*. Georgakopoulos *et al*. (2002) identified potentially useful and compatible antagonist combinations by cultivating antagonists in a liquid medium containing a substrate previously used by other antagonists of the prospective combination. Also, a single biocontrol strain may not grow equally well in a variety of environmental conditions (Fukui *et al*., 1994). In the present study, the biocontrol agents *T. viride* produced the greatest reduction in mycelial growth of *M. phaseolina* and *R. solani*, followed by *T. harzianum, T. virens* and *P. fluorescens* as compared with the control. The biocontrol agents *T. viride* showed maximum growth inhibition of *M. phaseolina* (Chirame and Padule, 2005; Khan *et al.*, 2012). Thilagavathi *et al.* (2007) tested the antagonistic effect of *Trichoderma viride* (strains Tv1 and Tv13), *Pseudomonas fluorescens* (Pf1 and Pf15) and *Bacillus subtilis* (Bs16) individually and in combination against *Macrophomina phaseolina* causing root rot in greengram. Among all individual biocontrol agents, *Trichoderma viride* (strains Tv1 and Tv13) individually showed maximum growth inhibition of the pathogen. Biocontrol agent, *T. viride* gave the highest mycelial growth inhibition of *R. solani* over control (Seema and Devaki, 2012; Kamalakannan et *al.*, 2004). The lower mycelial pathogen growth may be due to antibiotics produced by the biocontrol agents, as has been reported by many workers (Ramamoorthy and Samiyappan, 2001, Viswanathan and Samiyappan, 2001). In the present study, a combinations *T. viride + P. fluorescens* increased mungbean seed germination, shoot length, root length, vigour index by rolled paper towel method as compared to other combinations, individual biocontrol agents and control. Results similar to the present investigation were reported by Thilagavathi *et al.* (2007). The

combinations *T. viride* + *P. fluorescens* increased mungbean seed germination, shoot length, root length, vigour index, plant height in pot culture as compared to other combinations, individual biocontrol agents and control. Similarly, a combined application of *T. viride* + *P. fluorescens* increased root and shoot length in chilli (Manoranjitham *et al*., 2000). Mixtures of PGPR strains achieved better disease suppression of sheath blight in rice than when they were applied singly (Nandakumar *et al*., 2001). In the present study, the combinations of *T. viride* + *P. fluorescens* gave better disease suppression of root rot and blight in mungbean plants as compared to other combinations, individual biocontrol agents and control. In the present study, mungbean plants infected with *M. phaseolina* and treated with the bioformulation combinations *T. viride* + *P. fluorescens*; *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* had higher levels of PO, PPO and PAL activity than infected plants treated with single biocontrol agents. Mungbean plants infected with *R. solani* and treated with the bioformulation combinations *T. viride* + *P. fluorescens*; *T. harzianum* + *P. fluorescens* and *T. virens* + *P. fluorescens* had higher levels of PO, PPO and PAL activity than infected plants treated with single biocontrol agents. Similarly, a combined application of biocontrol agents increased levels of PO, PPO and PAL activity in greengram against root rot caused by *M. phaseolina* as compared to individual biocontrol agents (Thilagavathi *et al.,* 2007). Increased levels of PO and PPO have been shown to result from a number of resistant interactions involving plant pathogenic fungi, bacteria and viruses (Kandan *et al*., 2002). PAL was reported to be induced in cucumber by fluorescent pseudomonads against *P. aphanidermatum* (Chen *et al.,* 2000), in tomato against *Fusarium oxysporum* f. sp. *lycopersici* (Ramamoorthy *et al.,* 2002) and in bean against *Botrytis cinerea* (Zdor and Anderson, 1992). The results suggest that disease suppression by these combinations of biocontrol agents in the pot culture was related to the *in vitro* interaction that occurred between the biocontrol agents. The biocontrol agents not only controlled dry root rot but also promoted plant growth, and this gives them an advantage over the use of chemical fungicides against root rot in disease management. The work has to be intensified to study the mechanisms involved in disease control by mixtures of biocontrol agents.

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