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Research Article

Seed treatment with bacterial antagonists – A simple technology to manage groundnut root rot under residual moisture conditions

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ABSTRACT: Seed treatment with talc based formulations of 11 bacterial antagonists was carried out to study their biocontrol activity against the root rot pathogen *Macrophomina phaseolina* and growth promotion in groundnut under residual moisture conditions during 2007 and 2008. Various growth parameters and incidence of root rot in different antagonist's treatment were recorded. Difference in the plant stand, increase in growth parameters at 60 days after treatment was insignificant. High rhizosphere colonization was reflected from the high population of RP6, IISR-6 and consortium of EB69+RP7, EB69+RBh42a, RP7+RBh42a obtained 60 days after treatment. Seed treatment with RP2, EB69 during 2007 and EB69+RBh42a, RSh5 during 2008 recorded the highest plant stand at the time of harvest. Least incidence of root rot was recorded in EB150 (9.26%) and in RSh5 (19.40%) during 2007 and 2008, respectively. EB150 reduced the incidence of root rot by 70% and increased the yield by 46% during 2007. RBh42a reduced the incidence of disease by 40% and increased the yield by 137% during 2008. Other bacterial antagonists also reduced root rot incidence and increased pod yield considerably. Based on this study it is concluded that a simple seed treatment with potential antagonistic bacteria during sowing would reduce the incidence of root rot and improve the yield in groundnut under rainfed conditions.

KEY WORDS: Seed treatment, bacterial antagonists, groundnut, root rot

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INTRODUCTION

Groundnut is a major oilseed crop in India accounting for 39 per cent of the total oilseed production and is grown in all seasons (Ghewande *et al*., 1997). Larger area under groundnut in Goa is during *Rabi* and mostly grown under residual soil moisture conditions after the harvest of paddy crop. A variety of diseases affect groundnut, majority of which are caused by fungi and lead to severe yield loss (Ganesan *et al.*, 2007). Root rot caused by *Macrophomina phaseolina* (Tassi) Goid is a major problem in Goa under dry conditions (Ramesh and Korikanthimath, 2006). This fungal pathogen is soil and seed borne; and causes root rot in more than 500 plant species (Ashraf and Javaid, 2007) posing serious problem in management. It has been reported that colonization of the roots and charcoal rot development occur only when the plants are drought stressed during reproductive growth (Diourte *et al.*, 1995). Biocontrol is an eco-friendly approach towards the management of *M. phaseolina* (Arora *et al.,* 2001). For the effective biocontrol, the survival of the antagonist in the carrier and its delivery to the rhizosphere are of prime importance. Kloepper and Schroth (1981) demonstrated the potentiality of the talc to be used as a carrier material for formulating antagonistic bacteria. Talc based formulations of endophytic and plant growth promoting rhizobacteria are applied in different forms for instance, soil amendment, and seed coating. In addition to these, bio-priming seed treatments which integrates biological and physiological aspects is a novel approach for controlling seed and soil borne pathogens (Callan *et al.,* 1990; El-Mohamedy, *et al*., 2006; El-Mohamedy and Abd El-Baky, 2008). In the present study, talc formulations of 11 antagonistic bacteria and three consortiums of bacteria were evaluated in the field for the management of root rot under residual moisture conditions.

MATERIALS AND METHODS

Pathogen and bacterial antagonists

M. phaseolina was isolated from the root rot infected groundnut plants on Potato Dextrose Agar (PDA) medium. Bacterial antagonists were isolated from rhizosphere and endophytic regions of various crops from different parts of Goa. Bacterial isolates namely IISR-6, PDBC-AB2 were obtained from IISR, Calicut and NBAII, Bangalore respectively. All the antagonists were maintained on Kings Medium B (King *et al*., 1954).

Screening of antagonists under in vitro

The bacterial antagonists were screened for their inhibitory activity against *M. phaseolina* under *in vitro* condition. Radial growth of *M. phaseolina* and inhibition zone caused by antagonists in the dual plate technique was recorded. The *in vitro* assay was repeated twice with three replications to select the potential antagonists for field study. Carbendazim was used as control along with other antagonists since it is a most commonly used fungicide in seed treatment and soil drenching.

Preparation of talc based formulation of antagonists

Talc based formulation of antagonists was prepared as described by Ramesh and Korikanthimath (2004). At the time of treatment, population in the talc formulation was 3 x 10^8 CFU g^{-1}.

Seed treatment with bacterial antagonists and field evaluation

The experiment was conducted during *Rabi* 2007 and *Rabi* 2008 at farmer's field in Sangolda village situated at 15º 31' N latitude and 75º 55' E longitude. Seeds of groundnut (variety TAG 24 during 2007 and Asha during 2008) were treated with the talc based formulation of antagonists (Table 2) @ 30 g kg^{-1} of seeds. 60ml water was added to 30 g talc product to make slurry. Seeds were mixed with the slurry thoroughly in such a way that uniform coating of the product on the seed surface. Then the seeds were allowed to remain overnight in a polythene bag under room temperature. The treated seeds were sown in the field at 10 cm depth in the spacing of 30 x 10 cm. All the treatments were replicated thrice in a randomized block design with 3mx 1m plot size. In case of consortium 15 g + 15 g from each was taken for treating the seeds. Carbendazim @ 2 g kg^{-1} of seeds was used as chemical control. Germination percentage was recorded after 2 weeks of sowing. The incidence of root rot was recorded at 60 and 120 days after sowing. After two months of sowing, samples were taken from the treatments to record the growth parameters like shoot length, root length, root weight, no of pods/plant, pod weight, plant weight. Soil sample was also taken from all the plots to assess the population of rhizosphere bacteria. During harvest time five plants from each plot was taken randomly to assess the growth parameters.

All the data were analysed statistically using ANOVA and the mean comparisons were carried out by using Least Significance Difference (LSD) test ($P \leq 0.05$).

RESUTS AND DISCUSSION

In vitro evaluation of antagonists

More than 100 bacterial isolates were screened for their antagonism against *M. phaseolina*. Based on the percentage inhibition and the previous data on the plant growth promoting ability of the isolates (data not shown) only 11 isolates *viz*., RP2, RP6, RP7, EB69, RCh62b, RBh42a, ERG1, RSh5, IISR 6, PDBC AB2 and EB150 were selected for the present study. RP6, RP7 and RCh62b identified as *Bacillus* spp. and the remaining isolates were species of *Pseudomonas*. All the isolates inhibited the growth of *M. phaseolina* and most of the isolates inhibited more than 80 per cent of the fungal growth (Table 1). Based on our earlier study, compatible combinations *viz.* EB69 + RP7, RBh42a + RP7, EB69 + Rbh42a were selected for field study. Fluorescent pseudomonads have been known to produce secondary metabolites, siderophores, HCN, chitinases (Gupta *et al.,* 2006), which may cause the inhibition of fungal growth. Lysis of the hyphae, shriveling and sclerotial deformities have been found to be caused by *Pseudomonas* spp as evident from the scanning electron micrographs of *M. phaseolina* (Gupta *et al*., 2001).

Table 1. Inhibition of *M. phaseolina* growth by selected antagonistic bacteria

Isolates	Growth of fungus*	% inhibition of fungal growth
RP2	1.28 ± 0.03	85.83
RP6	0.98 ± 0.23	89.17
RP7	0.93 ± 0.23	89.72
EB69	1.88 ± 0.17	79.17
RCh6	0.95 ± 0.05	89.44
RBh42	3.28 ± 1.43	63.61
ERG1	1.53 ± 0.28	83.06
RSH5	1.00 ± 0.35	88.89
IISR6	1.23 ± 0.22	86.39
PDBCAB2	1.38 ± 0.23	84.72
EB150	0.85 ± 0.10	90.56
Control	9.00 ± 0.00	0.00
LSD($P \leq 0.05$)	0.738	–

*Fungal diameter in cm; each value is mean of two experiments with two replications

Field evaluation of biocontrol agents

Growth promotion

Germination percentage was higher in the antagonists' treatment; however the difference is not statistically significant in both the years. Difference among treatments and control with regard to shoot length, root length, root weight, number of pods and weight of pods was not significant when observed after 60 days. RP6, IISR-6,

EB69 + RP7, EB69 + RBh42a, RP7 + RBh42a, recorded higher microbial population when analysed after 60 days (data not shown). Competent antagonistic bacteria should be able to establish themselves in the plant rhizosphere at population densities sufficient to produce beneficial effect after sowing. Efficient antagonistic bacteria survive in the rhizosphere by making use of the exudates secreted by the plant root, proliferate, colonize the entire root system and compete with the indigenous microorganisms (Bloemberg and Lugtenberg, 2001). Our results indicate better rhizosphere colonization by the applied antagonistic bacteria in groundnut, which is an essential criterion for the success of biological control.

During harvest time five plants from each replication was taken randomly to assess the growth parameters and the results are presented in Table 2. Maximum shoot length was recorded in control (39.37 cm) during 2007 and in EB69 (34.88cm) during 2008. No significant difference was observed among the treatments in case of root length though maximum was in control (13.67cm) during 2007 and, in ERG1 (8.83) during 2008. Highest number of pods / plant (20.87) was observed in RP2 and RBh42a (19.60) treatments during 2007 and in PDBCAB2 (20.60) during 2008. Highest root weight was recorded in control (5.87g), RP2 (5.67g) in 2007 and in PDBCAB2 (10.87) during 2008. Similarly, highest plant weight was recorded in control (0.032kg), RP2 (0.024kg) in 2007 and in PDBCAB2 (0.021kg) during 2008. RBh42a (0.021kg), RSH5, IISR-6 and RP2 (0.019kg) recorded the maximum pod weight / plant in the 2007 and the difference is significant. During 2008, carbendazim recorded the maximum pod weight / plant (0.034kg) followed by PDBCAB2 (0.0293kg). These results signify that RBh42a and RP2 yielded high no. of pods per plant and pod weight / plant in the year 2007 whereas PDBCAB2 effectively increased the no. of pods per plant and pod weight / plant in the year 2008. These isolates being plant growth promoters are the best candidates for the biocontrol of plant diseases. Similar results have been reported by Arora *et al.* (2001) and Shanmugam *et al.* (2003) reported the ability of *Pseudomonas fluorescens* pf1 to simultaneously promote growth and reduce the root rot incidence in groundnut. Induced plant growth and yield could be due to effective bacterization of seeds and root colonization by the bacteria (Gupta *et al.,* 2006).

Disease control and yield

At the time of harvest, maximum plant stand was recorded in RP2, EB69 (61.48 %) and the least was recorded in control (34.07%) in 2007. In 2008, highest plant stand was recorded in EB69 + RBh42a (48.52%) and in RSh5 (48.15%). Incidence of root rot was recorded periodically and the per cent incidence at 60^{th} and 120^{th} day is presented in this paper. During 2007, the disease incidence in the treatments was below 4.1 per cent as compared to 10.4 per cent in control after 60 days. After 120 days of sowing, significant difference in the incidence among the treatments was observed. Less incidence was recorded in EB150 (9.26 %), EB69 + RBh42a (11.85 %) and EB69 + RP7 (13.33 %). In control the incidence was 32 per cent. However during 2008, incidence of root rot was higher in all the treatments compared to 2007. The least incidence was recorded in RBh42a (4.3%) and the highest in control (19.44%) when observed after 60 days. The least incidence was observed in RSh5 (19.40%) as compared to the 40.06% in the control after 120 days of sowing (Table 3).

Highest plant biomass during 2007 was recorded in EB69 (8.03t ha^{-1}), ERG1 (7.92t ha^{-1}) and RBh42a (6.71t ha^{-1}). In the year 2008, highest biomass was recorded in EB69+RBh42a (6.24t ha^{-1}) followed by 5.20t ha^{-1} in RSh5. Among the treatments RBh42a recorded highest pod yield (3.84t ha^{-1}), followed by EB69 (3.81t ha^{-1}), EB 150 (3.58t ha^{-1}) during 2007 with control recording only 2.4t ha^{-1}. Pod yield obtained was less in 2008 as compared to the year 2007 and the highest pod yield was recorded in RBh42a (1.83t ha^{-1}) treatment. (Table 3). The variety used during 2008 was Asha, which is bold nut and confectionery type. The variety is more susceptible to biotic and abiotic stress and the yield is comparatively less when compared to TAG 24. This could be the reason for higher disease incidence and lower yields irrespective of treatments during 2008. Amongst various strategies such as seed coating, bio-priming, soil amendment used for the delivery of antagonistic bacteria in the vicinity of the plants, seed coating was the most effective treatment for controlling root rot diseases (El-Mohamedy *et al.,* 2006).

During 2007, EB150 and EB69 performed better in the field evaluation. More than 70 per cent disease reduction and 46 per cent yield increase was observed in EB150 treatment. But during 2008, 46 per cent disease reduction and 44 per cent increase in yield was obtained with the EB150 treatment. EB69 recorded over 50 per cent increase in yield and disease reduction in the year 2007. But in 2008 treatment, less than 40 per cent yield and disease reduction were observed with the same isolate. In 2007 trial, RBh42a showed more than 50 per cent disease reduction and up to 57 per cent increase in yield. RBh42a treatment recorded the maximum increase in yield (137%) in 2008 as compared to the other treatments with more than 40 per cent disease reduction. The increased yield might be due to increased number of pods per plant and pod weight coupled with reasonably good plant population. Further, RBh42a possess growth promoting

Table 2. Effect of seed treatment with talc based formulation of antagonists on groundnut growth parameters

Treatments	Shoot length (cm)		Root length (cm)		No. of pods / plant		Root wt (g)		Plant wt (kg)/plant		Pod wt (kg)/plant	
	2007-08*	2008-09**	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09
RP2	30.80	30.60	11.24	8.11	20.87	12.67	5.67	6.93	0.024	0.019	0.019	0.020
RP6	23.19	29.60	10.49	7.75	13.60	11.40	3.80	6.00	0.017	0.011	0.014	0.016
RP7	22.87	28.09	11.51	8.10	16.27	15.67	3.40	8.33	0.014	0.016	0.016	0.023
EB69	29.01	34.88	13.30	8.07	15.93	13.80	3.80	8.33	0.023	0.016	0.016	0.019
RCh6-2b	21.76	29.23	10.87	8.67	17.20	14.67	4.07	5.80	0.016	0.015	0.015	0.018
RBh42a	29.29	25.83	12.83	7.67	19.60	17.20	4.60	6.27	0.016	0.015	0.021	0.025
ERG1	23.98	34.83	11.96	8.83	13.60	19.60	3.87	7.20	0.014	0.021	0.012	0.029
RSh5	29.31	32.33	12.49	6.27	18.27	14.33	4.13	7.47	0.018	0.015	0.019	0.017
IISR-6	28.65	34.33	12.19	6.77	19.07	16.27	3.87	7.93	0.016	0.019	0.019	0.024
PDBCAB2	25.37	34.15	11.51	7.87	15.20	20.60	3.47	10.87	0.015	0.022	0.015	0.030
EB150	28.19	29.46	11.87	6.96	14.33	19.27	4.80	5.13	0.021	0.011	0.012	0.024
EB69 + RP7	24.29	33.08	10.59	7.63	12.33	17.33	3.13	6.07	0.012	0.018	0.011	0.025
EB69 + RBh42a	28.71	31.57	11.95	8.70	17.60	19.27	5.93	4.80	0.023	0.012	0.018	0.028
RP7 + RBh42a	24.76	27.10	11.09	8.00	15.87	17.33	3.40	7.10	0.011	0.014	0.016	0.025
Carbendazim	31.93	30.59	11.45	7.17	16.07	16.87	4.73	5.80	0.018	0.011	0.016	0.030
Control	39.37	29.90	13.67	6.63	15.67	13.93	5.87	4.53	0.032	0.009	0.017	0.020
CD ($P \leq 0.05$)	8.11	ns	ns	ns	3.48	ns	ns	ns	ns	ns	0.005	Ns

Mean of three replications and each replication contains five plants; groundnut variety TAG 24 was used; **groundnut variety Asha was used

Table 3. Effect of seed treatment with talc based formulation of antagonists on groundnut root rot and yield

Treatments	Final plant population (%)		% Root rot (60 DAS)		% Root rot (120 DAS)		Biomass (t ha^{-1})		Pod Yield (t ha^{-1})	
	2007-08*	2008-09**	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09
RP2	61.48^{a}	21.48^{f}	2.96^{bc}	8.15	20.74^{bcd}	31.55^{abc}	4.98^{cdef}	3.28	2.84^{cdef}	0.91^{bcd}
RP6	56.30^{abcd}	29.63^{cdef}	2.96^{bc}	15.70	15.93^{cde}	26.05^{bcde}	4.52^{def}	3.94	2.88^{cdef}	0.84^{bcd}
RP7	58.15^{abc}	33.33^{bcdef}	2.59^{bc}	9.71	16.67^{cde}	26.42^{bcde}	5.08^{cedf}	4.26	3.10^{abcdef}	0.90^{bcd}
EB69	61.48^{a}	33.70^{bcde}	2.96^{bc}	18.46	14.81^{def}	26.25^{bcde}	8.03^{a}	4.28	3.81^{ab}	1.01^{bcd}
RCh6-2b	57.41^{abc}	41.48^{abc}	1.85^{bc}	8.97	23.70^{abc}	23.09^{cde}	5.91^{bcde}	4.59	2.92^{cdef}	1.25^{b}
RBh42a	57.78^{abc}	40.37^{abcd}	1.48^{bc}	4.30	15.19^{def}	23.76^{cde}	6.71^{abc}	5.13	3.84^{a}	1.83^{a}
ERG1	44.81^{bcde}	42.22^{abc}	2.96^{bc}	9.63	21.48^{bcd}	23.22^{cde}	7.92^{ab}	5.03	3.29^{abcd}	1.10^{bcd}
RSh5	51.85^{abcd}	48.15^{a}	4.07^{b}	6.31	23.33^{bc}	19.40^{e}	5.19^{cdef}	5.20	3.07^{abcdef}	1.09^{bcd}
IISR-6	50.37^{abcd}	34.44^{bcde}	1.11^{bc}	6.07	23.70^{abc}	22.59^{de}	5.78^{cde}	4.81	3.26^{abcde}	1.19^{bcd}
PDBCAB2	48.52^{abcde}	28.15^{def}	2.96^{bc}	7.34	21.11^{bcd}	28.48^{bcd}	6.43^{abcde}	4.59	2.78^{cdef}	0.92^{bcd}
EB150	52.59^{abcd}	45.56^{db}	1.48^{c}	8.53	9.26^{f}	21.32^{de}	6.39^{abcde}	4.73	3.58^{abc}	1.11^{bcd}
EB69+RP7	58.52^{ab}	29.26^{cdef}	1.11^{c}	14.84	13.33^{ef}	29.12^{bcd}	6.50^{abcd}	4.73	3.37^{abcd}	1.05^{bcd}
EB69+RBh42a	60.37^{a}	48.52^{a}	1.11^{c}	6.15	11.85^{ef}	20.73^{de}	4.51^{def}	6.24	2.64^{def}	1.25^{bc}
RP7+RBh42a	41.48^{de}	23.33^{ef}	1.85^{bc}	15.69	18.52^{cde}	35.06^{ab}	4.41^{def}	3.68	3.01^{bcdef}	0.78^{cd}
Carbendazim	43.33^{cde}	43.33^{ab}	2.22^{bc}	9.75	27.41^{ab}	25.09^{cde}	3.66^{f}	3.35	2.31^{f}	0.85^{bcd}
Control	34.07^{e}	27.78^{def}	10.37^{a}	19.44	32.22^{a}	40.06^{a}	4.34^{ef}	2.82	2.44^{ef}	0.77^{d}
CD (P < 0.05)	8.72	7.89	5.79	ns	5.71	5.73	2.1	ns	0.83	0.47

*Groundnut variety TAG 24 was used; **groundnut variety Asha was used; mean of three replications and each replication data is from 3 m^2 area; all the percentage data were analysed after arc sine transformation; in a column, means followed by a common letter are not significantly different at LSD (P d" .05)

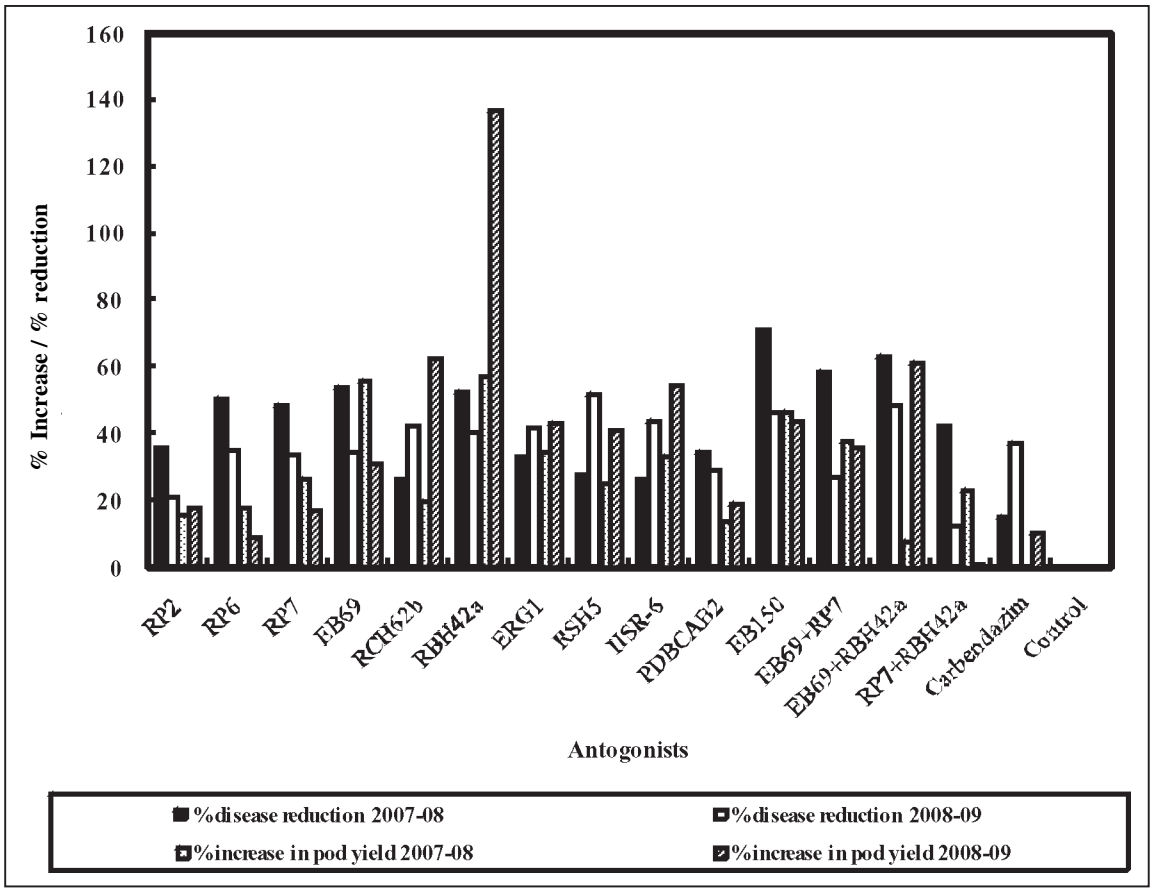

Fig. 1. Effect of antagonistic bacteria on groundnut root rot and pod yield

attributes with high rhizosphere competence (Henriqueta, 2009) which is very important for any bioagent. All the other biocontrol treatments also decreased the incidence of root rot and increased the yield considerably (Fig. 1).

The mechanisms responsible for the biocontrol activity against fungal pathogens include competition for nutrients, niche exclusion, induced systemic resistance (ISR) and the production of antifungal metabolites (AFMs) (Bloemberg and Lugtenberg, 2001) such as chitinases and antimycotics. Moreover, volatile compounds and HCN are the other mechanisms contributing to the biocontrol activity as observed in our other studies. *Pseudomonas fluorescens* isolated from the groundnut rhizosphere which produced HCN, salicylic acid, siderophores and 1, 3-endoglucanase was used as a biocontrol agent against groundnut root rot (Meena *et al.*, 2001). Our further studies indicated that the promising antagonistic isolates (RBh42a, EB150 and EB69) are species of *Pseudomonas* and produced siderophores, HCN and DAPG, an antifungal antibiotic. Electron microscopic examination showed hyphal coiling, vacuolation, coagulation and granulation of the cytoplasm resulting in the lysis of the hyphae of *M. phaseolina* by pseudomonads (Bhatia *et al.*, 2003).

Based on our results it is found that RBh42a is a potent plant growth promoter which increased the pod yield and reduced the incidence of root rot in both the years. Other two isolates, *viz*., EB150 and EB69 also recorded less disease incidence and higher pod yield during the field experiments.

A consortium of the above promising isolates would be effective in reducing root rot caused by *M. phaseolina* in conjunction with growth promotion in groundnut. Consortium of *P. fluorescens* and *Bacillus subtilis* had been used for the biocontrol of root rot caused by *M. phaseolina* in green gram (Thilagavathi *et al.*, 2007). From this study, it is concluded that a simple seed treatment with the antagonistic bacteria during sowing would reduce the loss caused by the root rot in the rainfed groundnut.

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