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EFFICACY OF SEED PLUS SOIL APPLICATION OF *BACILLUS CEREUS* ON THE ROOT ROT (*MACROPHOMINA PHASEOLINA* (TASSI.) GOID.) INCIDENCE AND PLANT GROWTH OF GROUNDNUT

K. Sanjeev Kumar, T. Sivakumar, K.R. Saravanan, P. Balabaskar and S. Sudhasha

Department of Plant Pathology, Faculty of Agriculture, Annamalai University,
Annamalai Nagar - 608 002 (Tamil Nadu) India.

Abstract

Groundnut dry root rot caused by *Macrophomina phaseolina* which is a seed and soil borne pathogen is very difficult to be managed by a single method. Meeting the future challenge for a productive, but sustainable agriculture will require the use of all strategies that are effective, economical, ecofriendly and compatible. The present study was undertaken to investigate the biocontrol potential of *B. cereus* for the successful management of dry root rot disease in groundnut. Seed treatment with *B. cereus* @ 12.5g kg^{-1} of seed recorded the minimum root rot incidence and maximum growth parameters of groundnut. With regard to the delivery system of soil application, the dosage of *B. cereus* at 3.0 kg ha^{-1} recorded the minimum disease incidence and maximum growth parameters of groundnut. The combined delivery system (Seed treatment @ 12.5 g kg^{-1} of seed + soil application @3.0 kg ha^{-1}) of *B. cereus* proved much superior by recording the least incidence of root rot which was on par with the fungicide treatment and also recorded the maximum growth parameters of groundnut.

Key words: Groundnut, *Macrophomina phaseolina*, Seed treatment, Soil application

Introduction

Groundnut (or) peanut (*Arachis hypogaea*) is a short duration oilseed crop having worldwide adaptation and is extensively grown in many parts of the world. It is a cultivated annual crop grown both under irrigated and rainfed conditions (Porter *et al.,* 1984). Major groundnut producers in the world are China, India, Nigeria, USA, Indonesia and Sudan. Developing countries account for 96% of the global groundnut area and 92% of the global production. Groundnut seeds contain high quality edible oil (50%), easily digestible protein (25%) and carbohydrates (20%) (ICRISAT, 2005).

The crop is affected by several fungal pathogens, of which *Macrophomina phaseolina* (Tassi.) Goid, inciting root rot disease is devastating right from the establishment phase of the crop (Muthusamy, 1989). The pathogen *M. phaseolina* is both soil as well as seed borne (Dhingra and Sinclair, 1973) in nature. Though the infection by *M. phaseolina* is found throughout the crop period, the symptoms of the disease are much pronounced during senescence of the host (Dhingra and Sinclair, 1978).

***Author for correspondence :** E-mail : skvivasayam@gmail.com

The fungus can attack all parts of groundnut plants and the disease may appear at any stage of crop development. Water soaked necrotic spots appear on the stem and the dead issue are covered by abundant black sclerotia and then wilting follows. Eradication of this soil borne pathogen is a difficult problem because of its polyphagous nature and its survival in the soil through its resting structure. However, soil has enormous untapped potential antagonistic microbes, which are helpful in reducing pathogen inoculum through different modes of action. Hence, it was thought that the heterotrophic rhizobacteria *viz., Bacillus cereus* could be tested against *M. phaseolina* causing root rot disease of groundnut.

Materials and methods

Isolation of pathogen and bio-control agents

Macrophomina phaseolina

Groundnut plants showing the typical root rot disease symptoms were collected from the farmer's field. The pathogen was isolated by tissue segment method onto potato dextrose agar medium and purified in plain agar medium by single hyphal tip method (Rangaswami, 1972).

The axenic culture of the isolate was maintained on PDA slants and stored at 4°C for further study.

Bacillus cereus isolate

The culture of *B. cereus* was obtained from the culture collection of Department of Plant Pathology, Annamalai University. The culture was re-streaked on NA slants and used for further studies.

Effect of delivery system of *B. cereus* on the incidence of root rot and biometrics of groundnut

Effect of *B. cereus* as seed treatment

Sterilized soil was mixed with the pathogen inoculum @ five cent (w/w) level and filled in 30cm earthen pots. Surface sterilized groundnut seeds were separately treated with the talc based formulations of the antagonists at different levels *viz.,* 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 gm/kg of seed. Five numbers of the treated seeds were sown 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. The pots were maintained in a glass house with need based irrigation and all the agronomic procedures were followed as per the standard protocols. The incidence of root rot disease (%), shoot and root length (cm) and biomass (g) of the plant were recorded at the time of harvest. The biomass of the plant was recorded after drying the plants in hot air oven at 60ÚC 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 in the rhizosphere was estimated at the time of harvest by using suitable selective media and serial dilution technique.

Effect of *B. cereus* as soil application

Sterilized soil was mixed with the pathogen inoculum @ five per cent (w/w) level and filled in 30 cm earthen pots. The antagonists meant for soil application were applied at 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 kg/ha level to the sterilized soil in pots and incorporated well. Five numbers of the treated seeds were sown in each pot. Seeds sown in the pathogen alone inoculated pot served as control and the pot drenched with carbendazim (0.1%) was used for comparison. The experiment was conducted with three replications in a randomized block design. The crop was maintained in a glass house and all the observations were recorded.

Effect of *B. cereus* as seed treatment plus soil application

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

Results and Discussion

Efficacies of seed treatment of the *B. cereus* against root rot incidence and plant growth of groundnut

Results of the experiment (Table 1) showed that the seed treatment with *B. cereus* of antagonists enhanced the seed germination, shoot length, root length and reduced the population of the pathogen and root rot incidence of groundnut. Statistically all the treatments were significantly superior over control in biometrics and root rot incidence. Among the treatments, seed treatment with *B. cereus* 15.0g per kg of seed recorded the minimum per

Table 1: Effect of seed treatment with *B. cereus* on the incidence of root rot and rhizosphere population of *M. phaseolina* (pot culture).

S. No	Treatments	Root rot incidence (%)			Population of *M. phaseolina* × 10^{-3}		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
1.	ST @ 2.5 gm/kg	20.40	18.80	16.12	20.62	18.34	17.50
2.	ST @ 5.0 gm/kg	19.90	18.75	15.58	20.57	17.43	16.88
3.	ST @ 7.5 gm/kg	19.05	16.05	15.12	19.82	15.43	14.07
4.	ST @ 10 gm/kg	18.77	14.50	13.78	18.10	13.52	11.58
5.	ST @ 12.5 gm/kg	18.52	14.18	13.42	18.02	13.49	11.43
6.	ST @ 15.0 gm/kg	18.05	14.03	13.11	18.00	13.33	11.34
7.	Carbendazim (0.1%)	18.04	14.68	13.54	18.14	13.44	11.45
8.	Control	20.05	31.24	48.05	28.98	28.48	27.56
	SED	0.039	0.602	0.656	0.164	0.074	0.064
	CD (p=0.05)	0.078	0.210	1.320	0.330	0.149	0.129

Table 2: Effect of seed treatment with *B. cereus* on the biometrics of groundnut var. TMV 13.

S. No.	Treatments	Germination (%)	Shoot length(cm)	Root length(cm)	Biomass (g/plant^{-1})
1	ST @ 2.5 gm/kg	90.02	10.24	9.05	18.15
2	ST @ 5.0 gm/kg	91.74	11.24	10.05	19.05
3	ST @ 7.5 gm/kg	92.75	12.49	13.99	20.00
4	ST @ 10 gm/kg	93.20	16.20	8.50	21.12
5	ST @ 12.5 gm/kg	94.48	16.79	15.25	22.80
6.	ST @ 15.0 gm/kg	94.00	16.88	15.26	22.82
7.	Carbendazim 0.1%	92.20	12.00	11.00	19.25
8.	Control	82.46	4.79	3.12	18.02
	SED	1.36	0.10	0.13	6.119
	CD (p=0.05)	2.15	0.28	0.27	1.300

cent root rot incidence (13.11%) and *M. phaseolina* population (11.34) at 90 DAS (Table 1) while control recorded the maximum root rot incidence of (48.05%) and *M. phaseolina* population (27.56). This was followed by the seed treatment levels with 12.5, 10.0, and 7.5 gm/kg of seed in the decreasing order of merit. The dosage levels at 15.0 and 12.5 gm/kg of seed recorded at par results with each other. The seed treatment with *viz., B. cereus* at 2.5 kg^{-1} of seed was the least effective dose and it proved insufficient to manage the disease.

The antagonist as seed treatment showed significant increase in plants growth parameters (Table 2). Among the different levels the treatment with *B. cereus* at 15.0 g kg^{-1} of seed recorded the maximum germination percent (94.00), root length (15.26 cm), shoot length (16.88 cm) and biomass (22.82) as against the minimum root length and shoot length recorded in control. An increase in the level of dosage resulted in increase in the growth parameters of groundnut.

Similar results on the efficacy of biocontrol agents as seed treatment were reported by several workers. Seed treatment with *P. fluorescens* @ 10g kg^{-1} of seed efficiently reduced the root rot incidence and increased plant growth and yield of groundnut (Meena *et al.,* 2001). Peanut seeds bacterized with *P. fluorescens* showed a significant increase in the germination and decrease in the incidence of root rot caused by *M. phaseolina* (Gupta *et al.,* 2002).

Seed treatment would have helped in the effective colonization of the antagonists in the rhizosphere which could have resulted in enhanced plant growth and reduced incidence of root rot. PGPR's were also reported to produce amino acids, salicylic acid and IAA (Sivamani and Ganamanickam, 1988). Similar such plant growth promoting substances produced by *B. cereus* might have improved the plant growth and seedling vigour of groundnut. These earlier reports lend support to the present findings.

Efficacy of soil application of *B. cereus* on the root rot incidence and plant growth of groundnut

The results of the study showed that the different levels of treatments tested by soil application varied in their efficacy in reducing the population of *M. phaseolina* and root rot incidence of groundnut (Table 3). The minimum disease incidence of (14.18%) and pathogen population (13.86) was recorded at a level of 4.0 kg ha^{-1}. This was followed by the soil application of *B. cereus* at 3.5, 3.0 and 2.5 kg ha^{-1} in the decreasing order of merit. A similar trend was observed with regard to the reduction in the pathogen population. However, the levels 3.0 kg/ha and above recorded statistically at par values in reducing the disease incidence and pathogen population.

The treatments with soil application of antagonists showed substantial increase in plants growth parameters. Among the different levels the treatment with *B. cereus* at 4.0 kg ha^{-1} recorded the germination percent (94.15 %), maximum root length (10.10 cm), shoot length (12.99 cm) and biomass (22.95 g) as against the minimum root length and shoot length recorded in control (Table 4). This was followed by the soil application of *B. cereus* at 3.5, 3.0 and 2.5 kg ha^{-1} in

Table 3: Effect of seed treatment with *B. cereus* on the incidence of root rot and rhizosphere population of *M. phaseolina* (pot culture).

S. No	Treatments	Root rot incidence (%)			Population of *M. phaseolina* × 10^{-3}		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
1.	SA @ 1.5 kg/ha	21.54	19.94	17.48	20.88	19.45	18.20
2.	SA @ 2.0 kg/ha	20.28	19.68	16.78	20.64	17.58	16.92
3.	SA @ 2.5 kg/ha	20.06	17.44	16.28	19.98	17.02	16.48
4.	SA @ 3.0 kg/ha	19.84	15.42	14.52	19.02	16.64	15.52
5.	SA @ 3.5 kg/ha	18.84	15.54	14.26	18.76	15.50	14.26
6.	SA @ 4.0 kg/ha	18.60	15.48	14.18	18.02	14.90	13.86
7.	Carbendazim(0.1% as soil drench)	18.95	15.82	14.46	18.74	15.68	13.92
8.	Control	20.05	31.24	48.05	28.98	28.48	27.56
	SED	0.39	0.06	0.25	0.164	0.074	0.24
	CD (p=0.05)	0.78	0.21	0.52	0.330	0.149	0.52

Table 4: Effect of soil application of *B. cereus* on the biometrics of groundnut var. TMV 13.

S. No.	Treatments	Germination (%)	Shoot length(cm)	Root length(cm)	Biomass (g/plant^{-1})
1.	SA @ 1.5 kg/ha	84.75	9.02	8.00	18.99
2.	SA @ 2.0 kg/ha	87.50	10.12	8.12	19.57
3.	SA @ 2.5 kg/ha	90.12	11.05	8.99	20.05
4.	SA @ 3.0 kg/ha	92.22	11.69	9.65	21.25
5.	SA @ 3.5 kg/ha	93.40	12.18	9.85	22.87
6.	SA @ 4.0 kg/ha	94.15	12.99	10.10	22.95
7.	Carbendazim 0.1%	90.15	10.22	8.88	17.05
8.	Control	82.46	4.79	3.12	18.02
	SED	1.119	0.970	0.522	0.623
	CD (p=0.05)	2.300	1.960	1.050	1.242

pressmud base reduced the root rot of blackgram. Soil application of biocontrol agents including *B. subtilis* is reported to effectively reduce root rot caused by soil borne pathogens in several crops (Saravanakumar *et al*., 2007; Thilagavathi *et al*., 2007; Loganathan *et al*., 2010; Senthilraja *et al*., 2010). These earlier reports are in line with the present findings.

Efficacy of seed plus soil application of *B. cereus* on the root rot incidence and plant growth of groundnut

The results obtained on the efficacy of *B. cereus* as seed + soil application in a pot culture

Table 5: Effect of seed treatment plus soil application of *B. cereus* on the incidence of root rot and rhizosphere population of *M. phaseolina* (Pot culture).

S. No	Treatments	Root rot incidence (%)			Population of *M. phaseolina* × 10^{-3}		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
1.	ST @ 2.5 gm/kg + SA @ 1.5 kg/ha	10.18	16.20	19.70	17.20	9.05	8.25
2.	ST @ 5.0 gm/kg + SA @ 2.0 kg/ha	9.35	15.15	17.20	10.12	10.15	5.77
3.	ST @ 7.5 gm/kg + SA @ 2.5 kg/ha	7.85	12.88	15.65	9.00	6.88	6.18
4.	ST @ 10 gm/kg + SA @ 3.0 kg/ha	5.65	11.00	16.17	8.15	6.79	6.98
5.	ST @ 12.5 gm/kg + SA @ 3.5 kg/ha	5.62	9.78	11.05	8.12	5.89	6.00
6.	ST @ 15.0 gm/kg + SA @ 4.0 kg/ha	5.70	8.52	9.05	8.02	5.23	5.98
7.	Carbendazim 50% WP @ 0.1 % as (ST+SD)	5.69	7.48	8.68	6.83	4.92	5.32
8.	Control	20.05	31.24	48.05	28.98	28.48	27.56
	SED	0.522	0.577	0.776	0.333	0.268	0.004
	CD (p=0.05)	1.050	1.160	1.560	0.670	0.540	0.008

Table 6: Effect of seed treatment plus soil application of *B. cereus* on the biometrics of groundnut var. TMV 13.

S. No.	Treatments	Germination (%)	Shoot length(cm)	Root length(cm)	Biomass (g/plant^{-1})
1.	ST @ 2.5 gm/kg + SA @ 1.5 kg/ha	91.15	10.05	10.12	19.07
2.	ST @ 5.0 gm/kg + SA @ 2.0 kg/ha	92.06	10.10	10.15	19.68
3.	ST @ 7.5 gm/kg + SA @ 2.5 kg/ha	92.78	11.12	10.16	21.10
4.	ST @ 10 gm/kg + SA @ 3.0 kg/ha	93.52	11.15	11.00	22.94
5.	ST @ 12.5 gm/kg + SA @ 3.5 kg/ha	94.44	11.60	11.12	24.06
6.	ST @ 15.0 gm/kg + SA @ 4.0 kg/ha	94.98	12.05	11.67	26.59
7.	Carbendazim 50% WP	89.72	13.52	17.89	19.03
8.	Control	67.85	19.20	9.80	17.12
	SED	0.262	0.656	0.263	0.121
	CD (p=0.05)	0.526	1.320	0.530	0.251

experimental are furnished in Table 5. Among the various treatments, the T_6 (seed treatment 15.0 g/kg + soil application @ 4.0 kg/ha) application, of *B. cereus* recorded the minimum disease incidence (9.05) and least population of *M. phaseolina* (5.98). This was followed by the treatments T_5 and T4 in the decreasing order of merit. The maximum root rot incidence (30.15) and *M. phaseolina* population (27.56) was observed in control. The treatment T_5 (ST @ 12.5 gm/kg + SA @ 3.5 kg/ha) also recorded statistically similar results as that of T_6.

Among the various levels of *b. cereus* tested by seed treatment plus soil application, the treatment with *B. cereus* (seed treatment @ 15.0 g/kg + soil application @

the decreasing order of merit. Here again the levels at 3.0 kg/ha and above recorded statistically at par values in promoting the biometrics of groundnut.

Introduction of biocontrol agents into the soil for disease control is becoming a common proactive in recent years. According to Jacob (1989) reported that introduction of *B. subtilis* to soil through peat soil or

4.0 kg/ha) recorded the maximum germination (94.98%), shot length (12.05 cm), root length (11.67) and plant biomass (26.59 gm). the least values in the biometrics were observed in control. The treatment T_5 (ST @ 12.5 gm/kg + SA @ 3.5 kg/ha) also recorded statistically similar results as that of T_6. (Table 6). Similar results were reported by several earlier workers in various crops (Thilagavathi *et al*., 2007 in chickpea, pigeon pea and groundnut). The superiority of combined delivery system of antagonist was also reported by Harman (1991) in maize and Subramaniam *et al.,* (2003) in grams.

Seed treatment plus soil application of *P. fluorescens* resulted in significantly the lowest root rot incidence of blackgram (Sethuraman *et al.,* 2003). These reported are in line and lend support to the present findings.

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