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EFFECT OF BACTERIAL ANTAGONISTS AGAINST ROOT ROT OF COWPEA CAUSED BY *MACROPHOMINA PHASEOLINA* (TASSI.) GOID

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Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important pulse crop grown for food and fodder purposes both in the tropics and sub-tropics. In the present study, we investigated the management of *Macrophomina phaseolina* using *Bacillus subtilis*. The results revealed that Seed treatment with *B. subtilis* @ 10 ml kg^{-1} of seed recorded the minimum root rot incidence and maximum growth parameters of cowpea. Also, among the soil application dosages, *B. subtilis* at 3 lit ha^{-1} recorded the minimum disease incidence and maximum growth parameters of cowpea. Among combination of delivery systems of field studies revealed the supremacy of the integrated treatment combination identified in the study *viz*., the treatment T_6 *B. subtilis* (ST @ 10 ml kg^{-1} of seed + SA @ 3 lit ha^{-1}) plus basal application of FYM @ 10 tonnes/ha which reduced the root rot incidence to the minimum and recorded the maximum biometrics of cowpea. The comparison chemical Carbendazim @ 4.0g/kg of seed as seed treatment recorded a PDI of 22.16 per cent at 90 DAS.

***Keywords*:** Cowpea, *M. phaseolina, B. subtilis*, Dry root rot, Management.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is a poor men's protein source. It is one of the most ancient human food sources (Ng and Marechal, 1985) and an important grain legume and hay crop in many tropical and subtropical regions (Fang *et al*., 2007). Among the various pulse crops, cowpea (southern pea) is one of the important crop and is grown in Tamil Nadu and Andhra Pradesh widely as rainfed crop. The name "Cowpea" probably derived from when it was an important livestock feed for cows in the United States. Cowpea is called the "hungry-season crop" because it is the first crop to be harvested before the cereal crops (Gomez, 2004).

Cowpea is a good source of food, forage, fodder, vegetable and certain snacks (Nirmal *et al*., 2001). Cowpea pod husks obtained after threshing are also used to feed livestock. It is a crop of low and high rainfall regions, an important component of cropping system grown as catch crop, mulch crop, intercrop, mixed crop and green crop. It has the ability to fix atmospheric nitrogen in soil at the rate of 56 kg per ha in association with symbiotic bacteria under favourable conditions through its root nodules (Ahlawat and Shivkumar, 2005). It grows well in poor soils with more than 85% sand and with less than 0.2% organic matter and low phosphorus. The mature cowpea seed contains 24.8 per cent protein, 63.6 per cent carbohydrate, 1.9 per cent fat, 6.3 per cent fiber, 0.00074 per cent thiamine, 0.00042 per cent riboflavin and 0.00281 per cent Niacin (Shaw monica, 2007).

Cowpea is attacked by many diseases caused by viruses, bacteria and fungi. Among the fungal diseases, the charcoal rot caused by *Macrophomina phaseolina* (Tassi.) Goid causes significant loss in yield. Concurrent heat and moisture stress favour development of charcoal or dry root rot caused by *M. phaseolina* often makes cultivation of cowpea uneconomical (Singh *et al*., 2012). 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. Among the bio-control bacteria, *Bacillus* has become the bacterium of the choice for its versatility and ability to contain a large number of plant pathogens in diverse environments (Malleswari, 2014). Therefore the present study was undertaken to develop an ecofriendly management strategy with *B. subtilis* to control root rot of cowpea disease.

Materials of Methods

Isolation of pathogen and biocontrol agents

The pathogen *M. phaseolina* was isolated from the diseased roots of cowpea plants showing the typical root rot symptoms by tissue segment method on potato dextrose agar (PDA) medium. The axenic culture of the different isolates of the pathogen were obtained by single hyphal tip method (Rangaswami, 1972) and these were maintained on PDA slants for subsequent experiments. The native antagonist *B. subtilis* was isolated from rhizosphere soil collected from farmer's field at village. The soil particles loosely adhering to the roots were gently teased out and used for the isolation of *B. subtilis* following serial dilution plate technique with Kings (King *et al.,* 1954) and NA medium respectively.

Preparation of liquid formulation of *B. subtilis*

For the preparation of liquid formulations the method suggested by Manikandan *et al*. (2010) was followed. The most effective isolate of *B. subtilis* identified in the present study was multiplied on Nutrient broth. The log phase culture of *B. subtilis* was inoculated into NA broth and incubated at room temperature (28 ± 2°C) for three days. Further, the broth was added with glycerol at 2 per cent level. After the incubation period, the formulation was assessed for adequate CFU ($1x10^8$) following serial dilution plating technique and the formulation thus prepared was sealed in plastic containers and used for studies.

Effect of seed treatment with different doses of *B. subtilis* on the incidence of dry root rot of cowpea (Pot culture)

Sterilized soil (3.0 kg) was mixed with the pathogen inoculum @ five per cent level (multiplied on sand maize medium) and filled in 15 x 30 cm dia. earthen pots. Surface sterilized cowpea seeds were separately treated with the liquid formulation of the antagonist as per the treatment schedule.

The treatment schedule:

T_1 - Seed treatment with *B. subtilis* @2.5 ml / kg of seed

T_2 - Seed treatment with *B. subtilis* @5.0 ml / kg of seed

T_3 - Seed treatment with *B. subtilis* @7.5 ml / kg of seed

T_4 - Seed treatment with *B. subtilis* @10.0ml / kg of seed

T_5 - Seed treatment with Carbendazim 50WP @4g/kg of seed

T_6 - Control

The experiment was conducted in a randomized block design and replicated thrice. The treated seeds were sown in pathogen inoculated soil @ 5 seeds per pot and maintained with need based irrigation following all standard agronomic practices. Pathogen alone inoculated pots served as control and carbendazim @ 4 g/kg of seed was used for comparison. The observations on the incidence of dry root rot disease (%) (Assessed at 30, 60 and 90 DAS), germination (% - assessed at 10 DAS), root length (cm) and shoot length (cm), vigour index and yield were recorded at the time of harvesting following standard procedures.

Effect of soil application with different doses of *Bacillus subtilis* on the incidence of dry root rot of cowpea (Pot culture)

Sterilized soil (3.0 kg) was mixed with the pathogen inoculum @ @ five per cent level (multiplied on sand maize medium) and filled in 15 x 30 cm dia. earthen pots. Liquid formulation of the antagonist was applied to the soil 10 days before sowing. The treatment schedule followed is mentioned below.

T_1 - Soil application with *B. subtilis* @1.25 lit / ha

T_2 - Soil application with *B. subtilis* @2.5 lit / ha

T_3 - Soil application with *B. subtilis* @3.0 lit / ha

T_4 - Soil application with *B. subtilis* @ 3.5 lit / ha

T_5 - Soil application with Carbendazim50 WP @ 0.1% / ha

T_6 - Control

The experiment was conducted in a randomized block design and replicated thrice. Soil drenching with carbendazim @ 0.1% was used for comparison and pathogen alone inoculated pots served as control. The observations on the incidence of dry root rot disease (%) (Assessed at 30, 60 and 90 DAS), germination (% - assessed at 10 DAS), root length (cm) and shoot length (cm), vigour index and yield were recorded at the time of harvesting following standard procedures.

Field trial

Based on the best results obtained from the pot culture experiments, a field trial under rainfed conditions was conducted in a farmer's field where root rot of cowpea is endemic at Ramanatham village of Cuddalore district. The field experiment was laid out in a randomized block design with eight treatments and three replications in a plot size 5x4 sq. meters per treatment. The predominant local variety available in that village was used for the study.

The treatment schedule followed is mentioned below.

T_1 - Seed treatment with *B. subtilis* @ 10.0 ml / kg of seed

T_2 - Soil drenching with *B. subtilis* @ 3.0lit/ha

T_3 - T_1 +T_2

T_4 - T_1 + FYM

T_5 - T_2 + FYM

T_6 - T_3 + FYM

T_7 - Carbendazim 50 WP as ST @ 4g/Kg of seed

T_8 - Control

The treatments were given as per the schedule and all the agronomic practices were followed as per standard procedures as recommended by the State Agricultural Department. The observations on the germination (% - assessed at 10 DAS), root length (cm) and shoot length (cm) incidence of dry root rot disease (%) (Assessed at 30, 0 and 90 DAS) and yield q/ha of cowpea crop was recorded at the time of harvesting following standard procedures.

Effect of seed treatment with different doses of *B. subtilis* on the dry root rot incidence and plant growth promotion of cowpea (pot culture)

The data depicted in table 1& 2 revealed that the seed treatment with different doses of *B. subtilis* showed significant influence on the incidence of root rot and plant growth promotion of cowpea. Among the various treatments, *B. subtilis* with 10ml/kg of seeds recorded the minimum root rot incidence (24.95%) at 90 DAS and the same treatment was recorded the maximum growth parameters with 19.9 and 9.0 cm of shoot and root length and 93.43 per cent of germination. In general the treatment with a dosage level of 2.5 ml kg^{-1} of seed was proved to be the least effective as it recorded higher level of disease incidence. Seed treatment with Carbendazim 50 WP @ 4 g/kg of seed recorded the root rot incidence of 26.65 per cent and 20.8 and 10.8 cm of shoot and root length and 94.03 per cent of germination. The untreated control recorded the maximum disease incidence of 61.12 per cent (90 DAS) and minimum growth parameters of 9.8 and 4.2 cm of shoot and root length and 60.25 per cent of germination.

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. Seed treatment with *B. subtilis* is effective for management of root rot fungus *M. phaseolina* in chickpea (Selvarajan, 1990), charcoal rot of soybean (Krishnaveni, 1991), sunflower (Muhammad anis *et al.*, 2010), cowpea (Killani *et al.*, 2011). These earlier reports lend support to the present findings.

Effect of soil application with different doses of *B. subtilis* on the dry root rot incidence and plant growth promotion of cowpea (pot culture)

The results presented in table 3 & 4 showed that the treatments with different doses of *B. subtilis* antagonists as soil application differed in their efficacy in reducing the root rot incidence of cowpea. Among the treatments *B. subtilis* with 3 lit/ha recorded the least disease incidence of 19.06 per cent incidence of root rot (90 DAS). Carbendazim as soil drenching (0.1%) recorded the incidence of 18.84 per cent (90 DAS). The untreated control recorded the maximum disease incidence of 58.05 per cent (90 DAS) (table 3). The treatments with different doses of *B. subtilis* antagonists as soil application differed in their efficacy in increasing the plant growth promotion of cowpea Among the treatments *B. subtilis* with 3 lit/ha recorded the also recorded the maximum growth parameters of cowpea with 22.7, 12.3 cm of shoot and root length and 94.35 per cent of germination(table 4).

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 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; Loganathan *et al*., 2010; Senthilraja *et al*., 2010). These earlier reports are in line with the present findings.

Effect of combined application of *B. subtilis* + FYM on the dry root rot incidence of cowpea (Field trial - Rainfed)

The results obtained in the field studies are furnished in table 5. In general the root rot incidence showed an increasing pattern with an increase in the age of the crop in all the treatments and also control plots. Observations taken at harvest revealed that the treatment, T_6 *B. subtilis* ST @ 10 ml/kg of seeds + SA @ 3 lit/ha + FYM recorded 12.20% of root rot incidence at 90 DAS which was followed by the treatment, T_3 *B. subtilis* ST @ 10 ml/kg of seeds + SA @ 3 lit/ha which recorded an incidence of 12.86 per cent. The treatments with integration of FYM showed enhanced disease suppression when compared to individual antagonistic treatments. The chemical treatment with carbendazim (ST @ 4 g kg^{-1}) recorded 13.70 per cent root rot incidence and the untreated control recorded 37.25 per cent root rot incidence at 90 DAS of cowpea.

Santos *et al*. (2006) reported that *Bacillus* sp. is the most numerous rhizobacteria in the soil. This high presence in the soil reveals the great competitive potential, when it is present in the soil environment. In the present study, the dual delivery system followed could have ensured adequate population of the antagonist in the soil which resulted in enhanced disease suppression. *B. subtilis* is an example of antagonistic bacteria that usually act through antibiosis and eventually by parasitism and competition for space and nutrients (Nagorska *et al*., 2007). Thus it could be assumed that the combination delivery system of *B. subtilis* might have increased bacterial colonization in the rhizosphere and the various antifungal metabolites and combined action of such antibiotics, and ISR with the induction of PR proteins and defense enzymes may have caused the greater suppression of the root rot of cowpea as observed in the present study. Seed plus soil application of *B. subtilis* resulted in significant reduction in the damping off diseases in tomato (Kavitha *et al*., 2005).

In the present study application of *B. subtilis* along with FYM further enhanced the disease suppression. Earlier workers also have recorded similar results. Lukhade and Rane (1993) have reported that application of farmyard manure gave the lowest incidence of the root-rot disease. According to Lumsden *et al*. (1983) incorporation of FYM with antagonists destroyed the propagules of *M. phaseolina.* These earlier reports add value to the present findings.

Effect of combined application of *B. subtilis* + FYM on the biometrics of cowpea (Field trial - Rainfed)

Generally, the antagonistic treatments with integration of FYM, showed enhanced growth and yield attributes when compared to other treatments and control. However, among the treatments the treatment, T_6 *B. subtilis* ST @ 10 ml/kg of seeds + SA @ 3 lit/ha + FYM recorded 60.16 cm of shoot length, 15.20 cm of root length and 97.62 per cent of seed germination, and 2.09 kg $plot^{-1}$ pod yield. This was followed by the treatment, T_3 *B. subtilis* ST @ 10 ml/kg of seeds + SA @ 3 lit/ha which recorded 58.23 cm of shoot length,14.91 cm of root length, 96.42 per cent of seed germination and 1.65 kg $plot^{-1}$ pod yield (Table 6).

Linderman (2000) reported that shifts in the microbial community structure and the resulting microbial equilibria can influence the growth and health of plants. PGPR's increased plant growth directly by mediating the production of secondary metabolites and phytohormones such as IAA, auxins, cytokinins or gibberellic acid (Beyeler *et al.,* 1999). Also, PGPR's are known to be involved in N_2 fixation (Hong *et al*., 1991) and solubilizing nutrients such as P (Whitelaw, 2000).

Bacillus spp. have potent plant growth promoting traits such as IAA production, phosphate solubilization and nitrogen fixation (Senthilkumar *et al*., 2009). *Bacillus* spp. have also been known to produce compounds which promote plant growth directly or indirectly *viz.,* siderophores, indole acetic acid (IAA), solubilize phosphorous and have antifungal activity (Wahyudi *et al*., 2011). All these mechanisms exerted by the combination treatment of *B. subtilis* would have resulted in enhanced plant growth parameters and higher yield of cowpea observed in the present study.

Addition of organic amendments to soil has been demonstrated to have beneficial effects on plant growth and plant health. Accordingly, in the present study also the integration of FYM along with *B. subtilis* showed enhanced growth and yield parameters of cow pea. Earlier workers have also confirmed that *T. viride* combined with soil application of FYM decreased the root rot disease incidence and increased the yield of sunflower (Theradimani and Juliet Hebziba, 2003).

Table 1 : Effect of seed treatment with different doses of *B. subtilis* on the root rot incidence of cowpea (Pot culture)

Tr. No.	Treatments	Root rot incidence (%)			Per cent inhibition over control		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
1	*B. subtilis* for ST @ 2.5ml/kg of Seeds	17.18(24.49)	25.98(30.65)	27.88(31.88)	70.64	56.35	54.38
2	*B. subtilis* for ST @ 5.0ml/kg of Seeds	16.20(23.74)	26.10(30.73)	27.00(31.31)	72.31	56.14	55.82
3	*B. subtilis* for ST @ 7.5ml/kg of Seeds	15.54(23.22)	23.15(28.77)	25.67(30.45)	73.95	61.10	58.00
4	*B. subtilis* for ST @ 10.0ml /kg of Seeds	14.27(22.20)	22.01(27.98)	24.95(29.97)	75.61	63.02	59.17
5	Carbendazim 50 WP @ 4g/kg of Seeds	16.28(23.80)	24.90(29.94)	26.65(31.09)	72.18	58.16	56.39
6	Control	58.52(49.91)	59.52(50.48)	61.12(51.43)	-	-	-
	S.Ed.	0.25	0.29	0.31	-	-	-
	C.D. (p=0.05)	0.55	0.66	0.71	-	-	-

Data in parentheses indicate arcsine transformed values
DAS – Days after sowing

Table 2 : Effect of seed treatment with different doses of *B. subtilis* on the growth parameters of cowpea (Pot culture)

Tr. No.	Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
1	*B. subtilis* for ST @ 2.5ml/kg of Seeds	67.93	10.6	6.9	1188.77
2	*B. subtilis* for ST @ 5.0ml/kg of Seeds	72.22	14.8	7.5	1610.50
3	*B. subtilis* for ST @ 7.5ml/kg of Seeds	92.57	18.7	8.9	2554.93
4	*B. subtilis* for ST @ 10.0ml/kg of Seeds	93.43	19.9	9.0	2700.12
5	Carbendazim 50 WP @ 4g/kg of Seeds	94.03	20.8	10.8	2971.34
6	Control	60.25	9.8	4.2	843.50
	S.Ed.	0.34	0.43	0.02	-
	C.D. (p=0.05)	0.77	0.97	0.05	-

Table 3 : Effect of soil application with different doses of *B. subtilis* on the root rot incidence of cowpea (Pot culture)

Tr. No.	Treatments	Root rot incidence (%)			Per cent inhibition over control		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
1	*B. subtilis* for SA @ 1.25lit/ha	17.48(24.72)	19.94(26.53)	35.54(36.60)	63.62	59.39	38.77
2	*B. subtilis* for SA @ 2.5lit/ha	15.78(23.41)	18.68(25.61)	21.28(27.48)	67.15	61.96	63.34
3	*B. subtilis* for SA @ 3.0lit/ha	13.12(21.24)	16.42(23.91)	19.84(26.46)	72.69	66.56	65.82
4	*B. subtilis* for SA @ 3.5lit/ha	13.28(21.38)	16.44(23.93)	19.06(25.89)	72.36	66.52	67.16
5	Carbendazim 50 WP @ 0.1%	13.99(21.97)	15.64(25.73)	18.84(25.73)	70.88	68.15	67.54
6	Control	48.05(43.89)	49.11(44.50)	58.05(49.64)	-	-	-
	S.Ed.	0.06	0.04	0.30	-	-	-
	C.D. (p=0.05)	0.13	0.09	0.67	-	-	-

Data in parentheses indicate arcsine transformed values
DAS – Days after sowing

Table 4 : Effect of soil application with different doses of *B. subtilis* on the growth parameters of cowpea (Pot culture)

Tr .No.	Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
1	*B. subtilis* for SA @ 1.25lit/ha	80.37	11.2	5.7	1358.25
2	*B. subtilis* for SA @ 2.5lit/ha	85.17	17.4	8.4	2197.38
3	*B. subtilis* for SA @ 3.0lit/ha	94.35	22.7	12.3	3302.25
4	*B. subtilis* for SA @ 3.5lit/ha	93.07	21.8	12.1	3155.07
5	Carbendazim 50 WP @ 0.1%	95.02	21.3	13.2	3278.19
6	Control	60.63	9.1	4.5	824.57
	S.Ed.	0.52	0.35	0.07	-
	C.D. (p=0.05)	1.18	0.80	0.15	-

Table 5 : Effect of combined application of *B. subtilis* + FYM on the root rot incidence of cowpea (Field trial – Rainfed)

Tr. No.	Treatments	Root rot incidence (%)			Inhibition over control		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
1	Seed treatment with *B. subtilis* @ 10 ml/kg of Seeds	14.25(22.18)	20.31(26.79)	29.52(32.91)	26.96	19.24	20.75
2	Soil application with *B. subtilis* @ 3lit/ha	12.03(20.30)	16.21(23.75)	20.31(26.79)	38.33	35.54	45.47
3	$T_1 + T_2$	6.50(30.33)	8.90(17.36)	12.86(21.02)	66.68	64.61	65.47
4	T_1 + FYM	10.36(18.78)	12.02(20.29)	18.71(25.62)	46.89	52.20	49.77
5	T_2 + FYM	9.52(17.98)	11.20(19.56)	13.95(21.94)	51.20	55.46	62.71
6	T_3 + FYM	8.50(16.96)	10.70(19.09)	12.20(20.45)	56.43	57.45	67.24
7	Carbendazim 50 WP as ST @ 4g/kg of Seeds	7.87(16.30)	9.32(17.78)	13.70(21.72)	59.66	62.94	65.90
8	Control	19.51(26.22)	25.15(30.09)	37.25(37.62)	-	-	-
	S.Ed.	0.08	0.09	o.10	-	-	-
	C.D. (p=0.05)	0.18	0.20	0.23	-	-	-

Data in parentheses indicate arcsine transformed values
DAS – Days after sowing

Table 6 : Effect of combined application of *B. subtilis* + FYM on the growth parameters of cowpea (Field trial - Rainfed)

Tr. No.	Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Grain Yield (kg/ha.)
1	Seed treatment with *B. subtilis* @ 10 ml/kg of Seeds	87.60	50.29	9.96	80.09
2	Soil application with *B. subtilis* @ 3lit/ha	90.80	51.20	10.04	80.25
3	$T_1 + T_2$	96.42	58.23	14.91	100.35
4	T_1 + FYM	91.51	55.10	11.09	82.29
5	T_2 + FYM	93.01	56.14	12.42	89.50
6	T_3 + FYM	97.62	60.16	15.20	112.09
7	Seed treatment with Carbendazim 50 WP @ 4g/kg of Seeds	95.15	57.14	13.24	87.65
8	Control	78.32	23.12	9.82	51.03
	S.Ed.	0.51	0.41	0.36	0.06
	C.D. (p=0.05)	1.09	0.90	0.78	0.14

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