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Suppression of *Sclerotium rolfsii* causing foot rot of groundnut by *Bacillus* sp.

S. K. RAY AND NILANGSHU MUKHERJEE

Department of Plant Pthology, Bidhan Chandra Krishi Viswavidyalaya Mohanpur 741252, Nadia, West Bengal

Two selected strains of *Bacillus* sp. viz S_{12} and S_{17} were isolated from rhizosphere soil of groundnut from Nadia district of W. B. Two formulations of bioantagonists like Bacterial Cell Power (BCP) and broth as seed treatment, soil drenching and combination of two before or after pathogen inoculation at different time intervals in *in vivo* pot trials against *S. rolfsii* were tested. Significant reduction of seedling mortality was observed by both strains S_{12} and S_{17} at pre-(20.71%-23.85%) and post-(26.39%-30.31%) inoculation seed treatments when applied at 25 days before or after pathogen inoculation. Drenching with antagonists at pre- (46.54%-57.71%) and post-incoculation (50.55%-61.19%) also effectively reduced seedling mortality when applied 5 or 10 days before or after pathogen inoculation. Combined application of bioantagonist like S_{12} as seed treatment followed by soil drenching also reduced the mortality percentage at pre-(13.34%) and post-application (19.44%) at 5 days before or after pathogen inoculation.

Key words : *Sclerotium rolfsii, Bacillus* sp., suppression

INTRODUCTION

Sclerotium rolfsii Sacc., the incitant of foot rot of groundnut (*Arachis hypogaea* L.) causes considerable damage (upto 59.61 %) and results in loss of production (Ray, 1994). There are few cost effective and eco-friendly control measures available for this pathogen (Papavizas, 1973). Biological control has, thus, become more popular world wide (Weller, 1988) mainly due to non-hazardous effect on nearby ecosystem. *Bacillus* sp. an important antagonist to *Sclerotium rolfsii*, has been successfully used in controlling the pathogen in several crops including groundnut under green house and field conditions Hedge *et al.*, 1980; Keyser and Ferreira, 1989 ; Ray and Mukherjee, 2000). An attempt has been made in this experiment to compare the efficacy of two strains of *Bacillus* sp. viz., S_{12} and S_{17} applied through different formulations vis-a-vis different time of application for controlling foot rot disease of groundnut caused by *Sclerotium rolfsii* under *in vivo* pot experiments in green house condition.

MATERIALS AND METHODS

Preparation of inoculum and application

The test pathogen *Sclerotium rolfsii* was isolated from infected roots and collar regions of groundnut (cv. JL-24) from Teaching Farm, B. C. K. V., Mohanpur, Nadia. The pathogen was maintained in a freshly prepared potato dextrose agar (PDA) medium. The inoculum was multiplied on sand maize medium for future use. The medium was prepared by mixing crushed maize grains (30 g) with white sand (70 g) and required amount of water to moisten the medium. Before mixing, the sand was seived thoroughly, washed with acid and subsequently with deionized water. This mixture was placed in polypropylene bag, plugged and sterilized at 121°C for 30 minutes twice at 12 hrs interval. Generally 6-7 days old cultures of *S. rolfsii* were grown in sand-maize medium and incubated at 30°C for 10-12 days. The inoculum of *S. rolfsii* @ 100 g/pot was incorporated in the earthen pots (9") at a depth of 2-3 cm near the collar region of groundnut seedings (var - JL-24) and covered with 2-3 cm layers of sterilized sandy soil.

FORMULATION OF ANTAGONISTS

Isolation, selection, and *in vitro* antagonism of two strains viz., S_{12} and S_{17} of *Bacillus* sp. were done by

previously standardized procedure of this laboratory (Ray and Mukherjee, 1996, 1997)

Bacterial Cell Powder (BCP)

Bacterial broth (potato-dextrose broth) of 72 h age were contrifuged at 10,000 rpm for 15-30 minutes. After decanting the culture filtrate, the supernatent was transferred with a little amount of sterile water on petriplates. The cellmass was dried at 30±1°C for 5 days and fine dust of Bacterial Cell Powder (BCP) was used for seed treatment.

Potato dextrose broth (72 h old) of the two strains of *Bacillus* sp. were also prepared and used for soil drenching.

Seed treatment with antagonists prior to pathogen inoculation

Seeds of groundnut were thoroughly washed in water, air dried and mixed with adhesive 0.5% CMC (carboxy methyl cellulose @ 20-25 ml/100 g seeds) and BCP @ 3 g/kg of seeds in a capped plastic box and shaken thoroughly. The treated seeds were air dried for 24 hrs and then sown in 9" earthen pots containing sterilized sandy loam garden soil. The whole experiment was conducted under glass house condition in completely randomized block design (CRBD) with three replications. Ten to twelve healthy seedlings were maintained in each pot. Pathogen inoculation was done @ 100 g/pot at 10, 15, 20 and 25 days after sowing of BCP-treated seeds. Soil drenching was done with antagonists broth (1.8×10^9 cfu/ml) into root zone (about 4-5 cm depth) @ 150-200 ml/pot at 5 and 10 days before pathogen inoculation i.e., at 14 days after sowing of untreated seedlings. Seed treatment with BCP followed by soil drenching trial was done 5 days before (i.e., 9th days after sowing) pathogen inoculation i.e., 14th day after sowing keeping pots with sterile water as control.

Treatment with antagonists following pathogen inoculation

Seed treatment and soil drenching were done at the same doses as pre-inoculation trial. Soil inoculation wih *S. rolfsii* was done in pots in the same day. The BCP-treated seeds were sown at 10, 15, 20 and 25 days after pathogen inoculation in the pots. Inoculation of pathogen and sowing of untreated groundnut seeds were done simultaneously followed by drenching of soil with antagonist broth after 5 and 10 days respectively. In case of mixed application, treated seeds were sown in the infested soil (inoculation in the same day) and soil drenching of antagonists were done 5 days after sowing. Watering was done as and when required.

Disease assessment was done by counting the number of plants mortality either 30 days after pathogen inoculation (DAI) or 30 days after sowing (DAS) of seeds with the following formula :

$$\text{Mortality}(\%) = \frac{\text{No. of wilted/dead plant/pot}}{\text{No. of plant population/pot}} \times 100.$$

RESULTS AND DISCUSSION

Effect of pre-and-post inoculation seed treatment of antagonist on the mortality of groundnut

The results from this experiment showed that *Bacillus* strain S_{12} always performed better in reducing seeding mortality in both type of treatments viz. pre (20.71 %) and post (26.39 %) application as compared to S_{17} (pre : 23.85 % and post : 30.31 %) over control 64.85 % and 64.16 % respectively. The difference in seedling mortality within the treatments was statistically significant but the interaction between treatments with pre and post application were not significantly different. Pre and post application treatments with antagonist at an interval of 10, 15, 20 and 25 days were effective for minimizing the seedling mortality of groundnut and their difference were also statistically significant. Lowest seedling mortality (26.86 %) was observed when pots were inoculated 25 days after sowing of treated seed as compared to 10, 15 and 20 days. This may be due to sufficient availability of time to maximum build up of suppressive bacteria in the site when infection occurs (Table 1). Mininum mortality of seeding (20.13 %) was also obtained when the bioantagonists were applied 25 days after pathogen inoculation. Such result may be due to decline in pathogen load under natural conditions in absence of the host. Here also the difference in mortality

Table 1. Effect of pre-and post-sowing of BCP-treated seeds against pathogen inoculation at different time interval on the seedling mortality of groundnut and their interactions.

Treatments/ Antagonists	Mortality (%)									
	Pre-sowing					Post-sowing				
	Pathogen inoculation after					Pathogen inoculation before				
	10 days	15 days	20 days	25 days	Mean	10 days	15 days	20 days	25 days	Mean
T_1 (S_{17})	37.78	31.67	14.17	11.79	23.85	53.33	41.08	20.95	5.89	30.31
	(37.91)	(34.15)	(21.90)	(20.03)	(28.49)	(46.93)	(39.86)	(27.03)	(10.73)	(31.14)
T_2 (S_{12})	33.61	28.33	12.22	8.67	20.71	49.05	34.17	17.85	4.50	26.39
	(35.41)	(32.14)	(20.32)	(12.70)	(25.14)	(44.44)	(35.66)	(24.80)	(9.60)	(28.63)
T_3 (Control)	74.17	63.49	61.63	60.11	64.85	82.50	71.39	52.77	50.00	64.16
	(59.70)	(52.85)	(51.77)	(50.84)	(53.79)	(65.61)	(58.27)	(46.59)	(45.00)	(53.87)
Mean	48.52	41.16	29.34	26.86	36.47	61.63	48.88	30.52	20.13	40.29
	(44.34)	(39.71)	(31.33)	(27.86)	(35.81)	(52.33)	(44.59)	(32.80)	(21.78)	(37.88)

		SEm±	C. D. 5%
1.	Day (D)	1.39	3.96
2.	Treatment (T)	1.20	3.43
3.	Pre and Post (Tp) sowing	0.98	NS
4.	Day × Treatment : (D × T)	2.40	6.86
5.	Day × Pre & Post sowing (D × Tp)	1.97	5.60
6.	Treatment × Pre & Post sowing (T × Tp)	1.70	NS
7.	Day × Treatment × Pre & Post sowing (D × T × TP)	3.40	NS

BCP : Bacterial Cell Powder
Figures in the parenthesis indicate angular transformed values.

Table 2. Effect of pre-and post-drenching of antagonist broth against pathogen inoculation at different time interval on the seedling mortality of groundnut and their interaction.

Treatments/ Antagonists	Mortality (%)					
	Pre-denching			Post-denching		
	Drenching day before inoculation			Drenching day after inoculation		
	5 days	10 days	Mean	5 days	10 days	Mean
T_1 (S_{17})	53.34	62.10	57.71	57.94	64.44	61.19
	(46.92)	(52.02)	(49.47)	(49.61)	(53.41)	(51.51)
T_2 (S_{12})	42.50	50.59	46.54	44.44	56.67	50.55
	(40.66)	(45.44)	(43.05)	(41.75)	(48.85)	(45.30)
T_3 - Control	79.17	79.17	79.16	90.47	90.47	90.47
	(63.20)	(63.20)	(63.20)	(72.26)	(72.26)	(72.26)
Mean	58.33	63.95	61.14	64.28	70.53	67.40
	(50.26)	(53.55)	(51.90)	(54.54)	(58.17)	(56.36)

Figure in the parenthesis indicate angular transformed value.

		SEm±	C. D. 5%
1.	Day (D)	1.24	NS
2.	Treatment (T)	1.53	4.45
3.	Pre and Post (Tp) drenching	1.25	3.64
4.	Day × Treatment : (D × T)	2.15	NS
5.	Day × Pre & Post sowing (D × Tp)	1.76	NS
6.	Day × Treatment × Pre & Post sowing (D × T × TP)	3.05	NS

Table 3. Effect of seed and soil treatment of antagonist bacteria against pathogen inoculation on seedling mortality of groundnut.

Treatments/ Antagonists	Mortality (%)	
T_1 (S_{17})	20.64 (26.94)	23.34 (28.86)
T_2 (S_{12})	13.34 (21.16)	19.44 (25.55)
T_3 (Control)	80.24 (64.29)	85.00 (67.40)
Mean	38.24 (37.46)	42.59 (40.60)

Figures in the parenthesis indicate the transformed angular value.

	SEm±	C. D. 5%
Treatment (T)	2.077	6.40
Pre and Post (T_p)	1.69	NS
T × Tp	2.93	NS

was highly significant when BCP-treated seeds were sown at different time intervals (Table 1).

Soil drenching

In case of soil drenching, both the strains of *Bacillus* sp. reduced seedling mortality over control. Among the strains, S_{12} showed minimum mortality (46.54 % & 50.55 % at pre-and post-drenching respectively) as compared to S_{17} (57.71 % & 61.19 % respectively) and their difference was statistically significant (Table 2). In pre-drenching treatment, the minimum mortality (42.50 %) was obtained at 5 days as compared to 10 days by strain S_{12} but their difference in mortality was not statistically significant. In case of post drenching application, similar type of results were obtained in reducing the seedling mortality in groundnut. Here also strain S_{12} showed better efficacy as compared to S_{17} which was statistically significant. The difference in mortality in the pre-and post-dreching were also statistically significant among themselves. But the application at different days before or after pathogen inoculation showed no statistical difference in reducing the seedling mortality (Table 2). The treatments with strains S_{12} or S_{17} and their type of application (pre & post) also showed no significant difference among themselves in reducing seedling mortality. The interactions between application at different days (5 & 10 days) treatments (S_{12}, S_{17} & control) and type of application (pre & post) were also statistically insignificant in reducing the mortality. Closer the time of drenching application and pathogen inoculation better was the results in reducing plant mortality. Soil drenching probably helps a large population of antagonists to reach quickly to the site of action thus becoming effective.

Combined application of seed treatment and soil drenching

Results showed that all the treatments were statistically significant in reducing seedling mortality in both types (t_p) of application and antagonist S_{12} 13.34 % & 19.44 % respectively performed better as compared to S_{17} (20.64 % & 23.34 % respectively). Pre-application of antagonist showed minimum mortality (38.24 %) as compared to post application (42.59 %) but their difference was not statistically significant. Interaction effect of treatments and type of application were also statistically insignificant in reducing seedling mortality. Hedge *et al.* (1980) also reported that seed treatment followed by soil drenching of *Bacillus subtilis* reduced the pathogen of *S. rolfsii* in wheat. However, pre-and post-infection treatment with *Bacillus subtilis* alone or in combination with fungicide zineb controlled the soil-borne pathogen like *Fusarium oxysporum* f. sp. *dianthi* in carnation where as zineb alone was ineffective in reducing the disease (Jacob *et al.*, 1988).

REFERENCES

Hedge, R. K.; Kulkarni, S.; Siddaramaiah, A. L. and Krishnaprasad, K. S. (1980). Biological control of *Sclerotium rolfsii* Sacc. causal agent of foot rot of wheat. *Current Research* **9**(4) : 67-69.

Jacob, M.; Junge, H.; Werner, E.; Obiegio, U. (1988). Use of Microbial antagonists for biological control of Fusarium wilt in carnation. *Nachrichtenblatt fur den pflanzenschutz in der DDR*. **42**(912) : 246-250.

Keyser, H. A. and Ferreira, J. H. S. (1989). Chemical and biological control of *Sclerotium rolfsii* in grapevine nurseries. *South African Journal of Ecology and Viticulture* **9(1)** : 43-44.

Papavizas, G. C. (1973). Status of applied biological control of soil borne plant pathogens. *Soil Biology and Biochemistry* **5** : 709-720.

Ray, S. K. (1994). *Biological control of soil borne diseases and crop health by inhibitor micro-organisms.* Ph. D thesis, pp-187, Bidhan Chandra Krishi Viswavidyalaya. West Bengal, India.

Ray, S. K. and Mukherjee, N. (1996). Isolation, selection and purification of some antagonistic bacteria from different agroecosystems of West Bengal. *Indian Agric.* **40(2)** : 125-135.

Ray, S. K. and Mukherjee, N. (1997). Studies on *in vitro* antagonism of some bacterial isolates against *Sclerotium rolfsii* Sacc. causing foot rot of groundnut and sugarbeet. *J. Mycopathol. Res.* **35(2)** : 99-105.

Ray, S. K. and Mukherjee, N. (2000). Ecologically acceptable microbiosuppressor for soil borne plant disease control. Paper presented in the National Seminar on Development & Use of Biofertilizers, Biopesticides and Organic manures, held at B.C.K.V. Nov-10-12, 2000, Abstract pp-39.

Weller, D. M. (1988). Biological control of soil borne pathogens in the rhizosphere with bacteria. *Ann. Rev. Phytopath.* **26** : 379-407.

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