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EFFECT OF *RHIZOBIUM* STRAINS AND BIOFERTILIZERS ON FOOT, ROOT ROT AND YIELD OF BUSH BEAN IN *SCLEROTINIA SCLEROTIORUM* INFESTED SOIL

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Abstract

A glasshouse experiment was carried out to find out the effect of *Rhizobium* strains and biofertilizers on foot and root rot (*Sclerotinia sclerotiorum*) and yield of Bush bean. Six *Rhizobium* strains and three biofertilizers were used for seed treatment. *Rhizobium* strains (BINAR P36 and BINAR P6) and BINA biofertilizer resulted maximum reduction of seed rot, and foot and root rot of Bush bean in pot. In addition, these treatments increased germination, plant stand, shoot length/plant, root length/plant, shoot weight/plant, root weight/plant, vigour index, plant height, number of green pods/plant, weight of green pods/plant, weight of seeds/plant and healthy looking seeds in pot trials. Among them *Rhizobium* strains (BINAR P36) showed best performance.

Key words: *Rhizobium*, biofertilizer, foot and root rot, *Sclerotinia sclerotiorum,* yield, Bush bean.

Introduction

Bush bean (*Phaseolus vulgaris*) is cultivated popularly in Chittagong, Sylhet, North Bengal, Hill tracts and also many other places of Bangladesh. It is highly nutritious and protein rich legume vegetable. Bush bean is used as tender vegetable, shelled green beans, dry bean and pulses. Now a days, it is considered as one of the most important exporting vegetables of Bangladesh to earn foreign currency and is being exported by Horticultural Export Foundation, Dhaka, Bangladesh. The average green pod yield of this crop in Bangladesh is 13-14 t/ha (Anonymous 2000). The average yield of Bush bean is very low in Bangladesh due to various reasons, where diseases are considered as one of the important factors. Among the diseases, foot and root rot caused by *Sclerotonia sclerotiorum* is the most serious disease (Blum *et al.* 1991, Abo Ellil *et al.* 1998). The highest 38.43% foot and root rot of bush bean was observed in control treatment in the field (Khalequzzaman *et al.* 2003).

Farmers use chemicals for controlling the diseases of crop plants in Bangladesh. Chemicals create environmental pollution and health hazards. On the other hand, poor farmers could not purchase costly chemicals. As an alternative means of avoiding these problems, biological control agents are now being used in many developed countries for combating the disease with the aim of increasing food production. *Rhizobium* inhibit growth of foot and root rot causal pathogens, decrease foot and root rot disease and increase yield of leguminous and nonleguminous crops (Khan *et al*. 1998, Hossain *et al.* 2000, Hossain and Mohammed 2002, Kibria and Hossain 2002). No research work in the discipline of controlling foot and root rot of Bush bean by biological means in Bangladesh has so far been conducted. So, the present study was undertaken to achieve the effect of *Rhizobium* strains and biofertilizers in controlling the foot and root rot (*Sclerotonia sclerotiorum*) and on yield of Bush bean under glasshouse condition.

Materials and Methods

The experiment was laid out at glasshouse of the Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh during 2002–2003. The variety was used BARI Bush bean 1.

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Inoculum of *Sclerotonia sclerotiorum* was prepared following the method of Khan (1998). The soil was mixed uniformly with cow dung (2:1). The dried soil was sterilized with formalin (40%) at the rate of 5 ml formalin diluted with 20 ml of water for 4 kg soil (Dasgupta 1988, Hossain 2000). The oat culture of *Sclerotium rolfsii* was then mixed with pot soil at the rate of 0.1% weight of dry soil before seed sowing (Khan *et al* 1998, Hossain *et al*. 1999, Hossain 2000, Kibria and Hossain 2002). The inoculum was applied only up to a depth of 6 cm in the pot soils i.e. 2 g of oat culture was mixed with each pot soil. A total of 50 pots were inoculated with the oat culture for five replications. Seeds were counted, weighed and packed in separate polyethylene bags as per treatment and replication. Six *Rhizobium* strains (BAUR 424, BAUR 979, BARIR 1000, BARIR 7029, BINAR P6 and BINAR P36) and three biofertilizers (BAU biofertilizer, BARI biofertilizer and BINA biofertilizer) were used for treating seeds. Composition of biofertilizers was peat soil and *Rhizobium* strains. Seeds were treated with *Rhizobium* strains and biofertilizers following the method of Somasegaran and Hoben 1994 and Sattar 1997. For seed coating with *Rhizobium* broth, broth containing 10^9 *Rhizobium* cells/ml was taken in sterilized 500 ml Erlenmeyer flasks. The seeds were dipped in broth for 15 minutes. In case of biofertilizers, seeds were initially moistened with molasses at the rate of 50 g per kg of seeds. Then the seeds were thoroughly mixed with biofertilizer (50 g/kg of seed), where each biofertilzer contained 10^8 *Rhizobium* cells/mg. In case of control, seeds were not treated with *Rhizobium* strains or biofertilizers. The inoculant-coated seeds were placed in a cool and dry place under shade for drying. The *Rhizobium* treated seeds and non-treated seeds for control were sown in previously prepared soil in pots. Five seeds were sown in each pot.

The pots were inspected at every day in the morning to take observations on foot and root rot diseased plants. Intercultural operations were done to maintain the normal hygienic condition of crop growth in pots. No plant protecting chemicals (insecticides or fungicides) were used for controlling the pests and diseases of the crop. The data were recorded on germination (%), seed rot (%), foot and root rot (%), plant stand (%), seedling vigour, number of nodules/plant, length of green pod (cm), breadth of green pod (cm), no. of green pods/plant, wt. of green pods/plant (g), plant height (cm), no. of seeds/pod, wt. of seeds pod (g), wt. of seeds/plant (g), apparently healthy looking seeds (%) and discoloured seeds (%). For vigour index (VI) or seedling vigour, data were recorded on germination up to 15 days of sowing. Five plants were randomly selected and uprooted carefully from pot and washed in tap water. Then root length and shoot length were recorded. Vigour index (VI) was calculated by using the formula of Baki and Anderson (1973). Recorded data were subjected to statistical analysis for mean values and test of significance. The variations among the respective data were compared following the Duncan's New Multiple Range Test (Gomez and Gomez 1984).

Results and Discussion

Germination, seed rot, foot and root rot and plants stand were significantly affected by *Rhizobium* strains and biofertilizer (Table 1). The highest germination (90.47%) was recorded when seeds were treated with BINAR P36 which was statistically similar to BINAR P6 (87.05%) and BINA biofertilizer (88.79%). The lowest germination (70.55%) was recorded in untreated control. The highest seed rot (29.45%) was observed in untreated control, while the lowest (9.53%) was found in case of BINAR P36 which was followed by BINAR P6 (12.95%) and BINA biofertilizer (11.21%). The highest foot and root rot (49.33%) was observed in untreated control and the lowest (11.05%) was recorded in BINAR P36 which was statistically similar to BINAR P6 (14.67%) and BINA biofertilizer (13.95%). The highest plant stand (88.95%) was recorded in BINAR P36 which was statistically similar to BINAR P6 (85.33%) and BINA biofertilizer (86.05%). The lowest plant stand (50.67%) was recorded in untreated control.

Shoot length/plant, root length/plant, shoot weight/plant, root weight/plant, seedling vigour, number of nodules/plant and plant height were significantly affected by *Rhizobium* strains and biofertilizer (Table 2). The highest shoot length/plant was 16.47 cm when seeds were treated with BINAR P36 which was statistically similar to BINA biofertilizer (14.75 cm) and the lowest shoot length/plant (10.00 cm) was recorded in untreated control. The highest root length/plant (8.33 cm) was found in BINAR P36 which was followed by BARIR 7029 (6.80 cm), BINAR P6 (7.00 cm) and BINA biofertilizer (7.75 cm g), and the lowest root length/plant (5.79 cm) was recorded in plants under untreated control. The highest shoot weight/plant was 6.33 g in BINAR P36 which was statistically similar to BINAR P6 (5.75 g) and BINA biofertilizer (6.00 g), and the lowest shoot weight/plant (3.33 g) was recorded in untreated control. The highest root weight/plant (0.79 g) was found in BINAR P36 which was followed by BARIR 1000 (0.61 g), BARIR 7029 (0.69 g), BARI biofertilizer (0.65 g), BINAR P6 (0.70 g) and BINA biofertilizer (0.74 g). The lowest root weight/plant (0.48 g) was recorded in untreated control. The highest vigour index of seedling (2243.66) was obtained from BINAR P36 and the lowest (1113.98) in untreated control. The highest number of nodules/plant (22.37) were observed in case of BINAR P36 which was statistically similar to BINA biofertilizer (20.85) and but no nodule/plant was observed in untreated control. The tallest plant (41.33 cm) was observed in BINAR P36 which was statistically identical to BARIR 7029 (37.38 cm), BINAR P6 (38.67 cm) and BINA biofertilizer (39.37 cm), and the shortest plant (32.00 cm) was found in untreated control.

Length, breadth, number and weight of green pods/plant; number of seeds/pod, weight of seeds/pod, weight of seeds/plant, healthy looking seeds and discoloured seeds were significantly affected when seeds were treated with *Rhizobium* strains and biofertilizer (Table 3). The highest length of green pod (12.36 cm) was recorded in BINAR P36 and the lowest (6.67 cm) in untreated control. Though the treatments did not show any significant influence on breadth of green pod but the results for all treatments ranged from 0.50 to 0.80 cm, where the highest and the lowest breadth of green pod was found in BINAR P36 and in untreated control, respectively. The highest number of green pods/plant (13.05) was recorded in BINAR P36 and the lowest (6.75) in untreated control. The highest weight of green pods/plant (59.95 g) was recorded in BINAR P36 which was statistically similar to BARIR 7029 (55.67 g), BINAR P6 (57.33 g) and BINA biofertilizer (58.03 g), and the lowest weight of green pods/plant (42.60 g) was recorded in untreated control. Number of seeds/pod ranged from 3.00 to 5.00, where the highest number of seeds/pod was found in BINAR P36 and the lowest number of seeds/pod was recorded in plants under untreated control. The highest weight of seeds/pod (1.20 g) was obtained in BINAR P36 which was followed by BARIR 7029 (0.96 g), BINAR P6 (1.05 g) and BINA biofertilizer (1.13 g), and the lowest weight of seeds/pod (0.72 g) was recorded in untreated control. The highest weight of seeds/plant (15.66 g) was obtained from BINAR P36 which was followed by BINA biofertilizer (14.24 g) and the lowest (4.90 g) was recorded in untreated control. The highest healthy looking seeds (93.79%) was obtained from BINAR P36 and the lowest (83.45%) was recorded in untreated control. The highest discoloured seeds (16.55%) were observed in untreated control and the lowest (6.21%) were observed in BINAR P36 which was statistically identical to BINAR P6 (9.67 %) and BINA biofertilizer (8.44 %).

Table 1. Effect of seed treatment with *Rhizobium* strains and biofertilzers on germination and foot and root rot of BARI Bush bean 1 when soil inoculated with *Sclerotinia sclerotiorum* in pot.

Rhizobium strains and biofertilizers	Germination (%)	Seed rot (%)	Foot and root rot (%)	Plant stand (%)
BAUR 424	80.56 def	19.44 bc	21.70 cd	78.30 cd
BAUR 979	78.05 f	21.95 b	27.63 b	72.37 e
BAU biofertilizer	79.02 ef	20.98 b	23.55 c	76.45 de
BARIR 1000	83.25 cde	16.75 cd	19.50 de	80.50 bcd
BARIR 7029	85.69 bc	14.31 def	16.71 efg	83.29 abc
BARI biofertilizer	84.33 bcd	15.67 de	18.53 def	81.47 bcd
BINAR P6	87.05 abc	12.95 efg	14.67 fgh	85.33 ab
BINAR P36	90.47 a	9.53 g	11.05 h	88.95 a
BINA biofertilizer	88.79 ab	11.21 fg	13.95 gh	86.05 ab
Control	70.55 g	29.45 a	49.33 a	50.67 f
LSD ($P \geq 0.01$)	4.306	3.329	3.673	5.382

Means having similar letters do not differ significantly at 1% level of probability.

Table 2. Effect of seed treatment with *Rhizobium* strains and biofertilzers on plant growth of BARI Bush bean 1 when soil inoculated with *Sclerotinia sclerotiorum* in pot

Rhizobium strains and biofertilizers	Shoot length/ plant (cm)	Root length/ plant (cm)	Shoot weight/ Plant (g)	Root weight/ plant (g)	Seedling vigour	Number of nodules/ plant	Plant height at maturity (cm)
BAUR 424	10.70 c	6.20 bc	4.00 de	0.57 bcd	1361.46 f	7.29 d	34.65 b-e
BAUR 979	10.00 c	6.00 bc	3.50 e	0.50 cd	1248.96 h	0.00 e	33.77 de
BAU biofertilizer	10.39 c	6.11 bc	3.90 de	0.53 cd	1303.99 g	5.00 d	34.41 cde
BARIR 1000	11.39 c	6.55 bc	4.59 cd	0.61 a-d	1493.51 e	15.60 c	35.53 b-e
BARIR 7029	12.05 bc	6.80 abc	5.00 bc	0.69 abc	1615.26 d	16.95 c	37.38 a-d
BARI biofertilizer	11.74 bc	6.33 bc	4.75 cd	0.65 a-d	1523.64 e	15.67 c	36.40 b-e
BINAR P6	13.33 bc	7.00 abc	5.75 ab	0.70 abc	1769.73 c	18.45 bc	38.67 abc
BINAR P36	16.47 a	8.33 a	6.33 a	0.79 a	2243.66 a	22.37 a	41.33 a
BINA biofertilizer	14.75 ab	7.75 ab	6.00 a	0.74 ab	1997.78 b	20.85 ab	39.37 ab
Control	10.00 c	5.79 c	3.33 e	0.48 d	1113.98 i	0.00 e	32.00 e
LSD ($P \geq 0.05$)* LSD ($P \geq 0.01$)	3.003	1.595	0.8921	0.1808*	47.00	2.894	4.300

Means having similar letters do not differ significantly at 1% level of probability.

Table 3. Effect of seed treatment with *Rhizobium* strains and biofertilzers on green pod and seed yield, and yield attributes of BARI Bush bean 1 when soil inoculated with *Sclerotinia sclerotiorum* in pot.

Rhizobium strains and biofertilizers	Length of green pod (cm)	Breadth of green pod (cm)	No. of green pods/ plant	Wt. of green pods/ plant (g)	No. of seeds/ pod	Wt. of seeds/ pod (g)	Wt. of seeds/ plant (g)	Apparently healthy looking seeds (%)	Discoloured seeds (%)
BAUR 424	8.00 bcd	0.57	8.75 cde	52.30 cde	3.44 bc	0.83 cd	7.26 ef	86.77 bcd	13.23 abc
BAUR 979	7.00 d	0.51	7.95 de	50.11 e	3.09 c	0.74 d	5.95 f	85.15 cd	14.85 ab
BAU biofertilizer	7.70 cd	0.54	8.45 cde	51.29 de	3.23 c	0.78 d	6.79 ef	86.39 bcd	13.61abc
BARIR 1000	8.30 bcd	0.60	9.50 b-e	54.01 b-e	3.67 abc	0.88 bcd	9.05 de	87.15 bcd	12.85 abc
BARIR 7029	9.47 a-d	0.67	10.33 a-d	55.67 a-d	4.00 abc	0.96 a-d	10.70 cd	88.85 a-d	11.15 bcd
BARI biofertilizer	8.69 bcd	0.64	9.78 b-e	54.50 b-e	3.90 abc	0.93 bcd	9.55 cde	87.65 bcd	12.35 a-d
BINAR P6	10.75 abc	0.71	11.47 abc	57.33 abc	4.36 abc	1.05 abc	12.04 bc	90.33 abc	9.67 cde
BINAR P36	12.36 a	0.80	13.05 a	59.95 a	5.00 a	1.20 a	15.66 a	93.79 a	6.21 e
BINA biofertilizer	11.09 ab	0.76	12.61 ab	58.03 ab	4.77 ab	1.13 ab	14.24 ab	91.56 ab	8.44 de
Control	6.67 d	0.50	6.75 e	42.60 f	3.00 c	0.72 d	4.90 f	83.45 d	16.55 a
LSD ($P \geq 0.01$)	2.957	NS	2.948	4.841	1.352	0.2419	2.677	5.382	3.868

NS = Not significant. Means having similar letters do not differ significantly at 1% level of probability.

Application of *Rhizobium* strains and biofertilizers showed profound effect in reducing foot and root rot of BARI Bush bean 1. *Rhizobium* strain BINAR P36 treated seeds showed highest germination and plant stand and the lowest seed rot, foot and root rot which was followed by BINA biofertilizer. The lowest germination, highest seed rot and lowest plant stand were recorded in case of untreated control. Blum *et al.* (1991) reported that *Rhizobium* strains (573-127K14 and 576-2535) with *Phaseolus vulgaris* seeds decreased the incidence of damping off and the index of disease severity caused by *Rhizoctonia solani*. *Rhizobium meliloti*, *R. leguminosarum* and *Bradyrhizobium japonicum* reduced infection by *Fusarium* spp., and *Rhizoctonia solani* in soybean, mungbean, sunflower and okra plants (Haque and Ghaffar 1993, Ghaffar 1993). Bhattacharyya and Mukherjee (1990) found that soil inoculation with *Rhizobium* reduced the population of *Sclerotium rolfsii* in groundnuts which is in accordance with the findings of Khan *et al.* (1998) and Hossain *et al.* (1999). Hossain (2000) found that treatment of seeds with biofertilizer also showed 76.67 and 86.96% reduction in death of plants of lentil and chickpea, respectively due to infection by *Sclerotium rolfsii*.

Rhizobium strain BINAR P36 gave the highest shoot and root length per plant and vigour index followed by BINAR P6 and BINA biofertilizer which is supported by Kumar *et al*. (2001), and Kibria and Hossain (2002). *Rhizobium* strain BINAR P36 treated seeds resulted highest length and breadth of green pod which was followed by BINAR P6 and BINA biofertilizer in pot which is in accordance with the findings of Mozumder (2004). The highest number and weight of green pods per plant were recorded by using BINAR P36 which was followed by BINAR P6 and BINA biofertilizer. Boiko and Shevchenko (1980) tested three strains of *Rhizobium* on the varieties Dnepr 8 and Khar'kov 7 of French bean and found that strains 695 and 696 were the most effective on yield. Hossain *et al.* (1999) reported that *Rhizobium* inoculants increased significantly pods/plant of lentil up to 42.1% over the untreated control. Rai and Singh (1979) reported that inoculation with *Rhizobium* bacteria led to a significant increase in seed yield of chickpea compared with the uninoculated control, but Harnadez and Hill (1983) observed that inoculation with *Rhizobium* strain CC 1192 increased seed yield of chickpea by 29% and Sandhu (1984) reported that lentil seed inoculation with *Rhizobium* culture improved yield by 8–22%, but Pal and Ghosh (1986) reported that seed inoculation with *Rhizobium leguminosarum* strain L 25 and L 20 increased seed yield by 59.8% in lentil and up to 38.87% in chickpea. *Rhizobium* strains BINAR P36 produced maximum healthy looking and minimum discoloured seeds.

Overall results of the present study indicate that among the *Rhizobium* strains and biofertilizers, BINAR P36, BINAR P6 and BINA biofertilizer resulted maximum reduction of seed rot, and foot and root rot and increased yield and yield attributes of Bush bean. BINAR P36 showed best performance.

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