An antibiotic-producing and hydrogen-cyanide-producing rhizobacteria strain *Bacillus* BS2 showed a wide range of antifungal activity against many *Fusarium* sp. and brinjal wilt disease pathogen *Rhizoctonia solani*. Seed bacterization with the strain BS2 promoted seed germination and plant growth in leguminous plants *Phaseolus vulgaris* and non-leguminous plants *Solanum melongena* L., *Brassica oleracea* var. capitata, *B. oleracea* var. gongylodes and *Lycopersicon esculentum* Mill in terms of relative growth rate, shoot height, root length, total biomass production and total chlorophyll content of leaves. Yield of bacterized plants were increased by 10 to 49% compared to uninoculated control plants. Brinjal sapling raised through seed bacterization by the strain BS2 showed a significantly reduced wilt syndrome of brinjal caused by *Rhizoctonia solani*. Control of wilt disease by the bacterium was due to the production of antibiotic-like substances, whereas plant growth-promotion was due to the activity of hydrogen cyanide. Root colonization study confirmed that the introduced bacteria colonized the roots and occupied 23-25% of total aerobic bacteria, which was confirmed using dual antibiotic (nalidixic acid and streptomycin sulphate) resistant mutant strain. The results obtained through this investigation suggested the potentiality of the strain BS2 to be used as a plant growth promoter and suppressor of wilt pathogen.

**Keywords**: *Bacillus* BS2, Brinjal, *Rhizoctonia* wilt, Seed bacterization

Use of synthetic agro-chemicals to enhance crop productivity and to control fungal plant pathogens and insect pests is popular all over the world. Simultaneously, the broad spectrum activity of agrochemicals, increasing risk of residue toxicity, sky rocketing prices and pathogen resistance urge agriculturists to look for a viable alternative. Use of naturally-occurring, root colonizing beneficial microorganisms may be a safe and alternative approach for they are capable of exerting multiple effects of fertilizers, pesticides and plant growth regulators.

The *Bacillus* genera is capable of stimulating plant growth and controlling soil-borne fungal phytopathogens. The plant growth promoting activity of these beneficial rhizobacteria is due to the production of some plant growth promoting substances, production of enzymes and production of some antifungal and antibacterial secondary metabolites. Any one of these mechanisms have been used by such bacteria. Yet, commercial development of these rhizobacteria for agriculture and forest species have been slow, in large part due to plant growth response variability, because of abiotic and biotic factors associated with the rhizosphere.

The present investigation was taken up to establish the potentiality of a rhizobacterial strain *Bacillus* BS2 to enhance plant growth and crop productivity on five vegetable crops, viz. broad bean (*P. vulgaris*), brinjal (*S. melongena*), cabbage (*B. oleracea* var. capitata), kohlrabi (*B. oleracea* var. gongylodes) and tomato (*L. esculentum*) under field conditions. Investigation was also taken up to control wilt disease of brinjal caused by *Rhizoctonia solani* by seed bacterization.

**Material and Methods**

**Bacterial strains and culture condition**—The bacterial strain *Bacillus* BS2 was taken from Soil Microbiology Division, Regional Research Laboratory, Jorhat originally isolated by Dr Dileep Kumar from rhizosphere soil of Pigeon pea grown in Regional Research Laboratory (RRL), Jorhat and maintained on nutrient agar slant. Inoculum was prepared by incubating the bacterium in nutrient broth for 18 hr at 30°C. From this culture broth, a bacterial lawn was prepared on potato dextrose agar (PDA) plate and incubated for 48 hr at 30±2°C.

Bacterial cells were then harvested in sterile distilled water (20 ml) and optical density of the
bacterial cell suspension was adjusted to 1.5 at 600 nm in a UV-VIS spectrophotometer by adding sterile distilled water and CFU ml⁻¹ was estimated. This cell suspension was used for seed bacterization study.

Fungal pathogens—Fusarium moniliforme (2816), F. semitectum (3803), F. solani (3150) and Rhizoctonia solani (2755) used were obtained from Indian Type Culture Collection, IARI, New Delhi, India.

Soil—Field experiments were conducted at Regional Research Laboratory (RRL), Jorhat. Particle size of the soil was determined by laser diffraction particle size analyzer Model CILAS 1820 using sodium carbonate hexametaphosphate as dispersing agent. The pH of the soil was determined by 1:2 (soil-water mixture) in a automatic glass electrode. Total nitrogen and total organic carbon of the soil was estimated by Kjeldhal digestion methods and potassium dichromate titration respectively.¹⁷,¹⁸

Test plants—Brinjal (Solanum melongena L.), Phaseolus vulgaris L., Brassica oleracea L. var. capitata, Brassica oleracea L. var. gongylodes and Lycopersicon esculentum Mill. were used as test crops and the seeds were obtained from Agrotech Engineering, Jorhat a certified seed distributing agency.

Hydrogen cyanide and antibiotic assay—Production of hydrogen cyanide by the strain BS2 was tested by the change of colour of a sodium carbonate and picric acid amened filter paper attached to lid of the culture plate grown in PDA media containing glucose (4 g l⁻¹) and ferric chloride 100 µM.¹⁹ Antibacterial activity against the bacterial strains Mycobacterium smegmatis ATCC 11758 and M. phlei ATCC 19420 obtained from Professor G Winklemann, University of Tubingen, Germany were evaluated by circular paper disc agar dissolution technique.

In vitro antibiosis against fungal pathogens—In vitro antagonism of Bacillus strain BS2 against Fusarium moniliforme, F. semitectum, F. solani and Rhizoctonia solani was examined by dual culture technique on PDA and nutrient agar (NA) media by spot inoculation. The culture plates were incubated at 28°±2°C and the zone of inhibition (distance between the edges of the bacterial spot and the fungal mycelium) was scored after 7 days of growth. Control plate was inoculated with fungal strain only.

Seed bacterization—It was performed by dipping the seeds in the suspension of Bacillus BS2 having OD of 1.5 at 600 nm². Before treating with the bacterial suspension, the seeds were surface sterilized by sodium hypochlorite solution (2.4%) for 3 min followed by rinsing in 3% aqueous hydrogen peroxide for 30 min and then dried under sterile conditions. These gave approximately 1.2×10⁷ CFU seed⁻¹. Seeds treated with sterile distilled water only served as uninoculated control.

BS2 treated and control seeds were then sown in a small field beds (3 x 2.5 m) with a spacing 20 cm between rows and 15 cm between plants, in a completely randomized block design. Each experiment was replicated four times. The relative growth rate (RGR) of the treated and control plants was recorded at 15 days interval for 60 days with a sampling of three plants each time. Total chlorophyll content of the leaves were estimated after 30 days.

The yield, shoot height, root length, leaf size and total biomass (fresh weight) was measured after 90 days.

Disease suppression—Suppression of wilt disease caused by Rhizoctonia solani in brinjal was evaluated under controlled condition.² The study was conducted in four separate sets under natural photoperiod. The first set had non bacterized seeds of brinjal grown in artificial small field beds (30 x 25 cm) prepared with the above described soil infested with 0.45% (w/w) of R. solani culture mycelium. The second set contained the bacterized seeds (1.2×10⁷ CFU/seed) grown in soil infested with R. solani. The third set contained the bacterized seeds grown in pathogen free soils whereas the fourth set had non bacterized seeds grown in pathogen free soil as control. The incidence of disease syndrome and other growth parameters was recorded after 28 days.

Root colonization—To enumerate the colonization of introduced bacterial strain BS2, a spontaneous antibiotic resistant mutant strain, resistant to nalidixic acid and streptomycin sulphate (designated as BS2⁶) was developed. For this, 14 days old plants were uprooted, removed soil particle of the root by gentle shaking and weighed. The root was then cut (0.5-1 cm) and placed in sterile distilled water (20 ml) containing glass beads and agitated for 5 min to release the rhizoplane bacteria. Serial dilution of the root washings was made and plated on PDA (amended with nalidixic acid and streptomycin sulphate) for enumeration of the total introduced bacteria and in NA media for enumeration of total aerobic bacteria.
The CFU g⁻¹ of fresh root weight was enumerated after 48 hr of incubation at 28°±2°C.

Statistical analysis—Data was analysed by analysis of variance (ANOVA) and the significance was calculated at P<0.05. Treatment means were only compared with control. The experiment was repeated twice having four replications for each treatment.

Results and Discussion

The bacterial strain, *B. subtilis*, promoting plant growth and suppressing plant pathogens has been reported earlier. In the present study, the bacterial strain *Bacillus* BS2 showed *in vitro* antagonism against all the test fungal plant pathogens. More or less similar inhibition zone was recorded both in NA and PDA against all the test pathogens. Growth inhibition of *Fusarium solani* and *F. semitectum* in NA was found the most, whereas the lowest was against *F. moniliforme* in both the media. The zone of inhibition produced by BS2 was found significantly different from the control. *Bacillus* BS2 also weakly inhibited the growth of *M. phlei* and *M. smegmatis*.

Soil of the experimental field was sandy loam (23.1% silt, 55.5% sand and 21.3% clay) with slightly acidic reaction (pH 6.4). The total organic C and N content of the soil were 0.268 and 0.065%, respectively. Plantlets raised from bacterized seeds with *Bacillus* BS2 had significantly higher shoot growth and leaf size over the non-bacterized control plants (Table 1). Best enhancement of shoot height was found in brinjal (59.6%), followed by *P. vulgaris*, *B. oleracea* var capitata (24.2% each), *B. oleracea* var. gongylodes (14.3%) and *L. esculentum* (13.1%), respectively.

The plantlets raised from bacterized seeds had significantly more root growth compared to control plants. The overall increment in root length ranged from 15.0 to 45.1%. Total biomass production in terms of fresh weight basis was found to be significantly higher in all the BS2 treated plants (Table 1). The order of enhancement of total biomass production was *P. vulgaris* > *L. esculentum* > *S. melongena* > *B. oleracea* var. gongylodes > *B. oleracea* var capitata.

A higher content of chlorophyll was recorded in the leaves of all the bacterized plants over their respective controls (Table 1). This was almost double in case of *P. vulgaris* followed by *S. melongena* > *B. oleracea* var. gongylodes > *L. esculentum* > *B. oleracea* var capitata.

A significantly higher number of fruits/pods was observed in bacterized *L. esculentum*, *S. melongena* and *P. vulgaris* and an increase in weight and size of the edible portion was recorded in *B. oleracea* var. capitata and *B. oleracea* var. gongylodes over their respective non-bacterized control plants (Table 1). The yield (on fresh weight basis) observed in

<table>
<thead>
<tr>
<th>Plant</th>
<th>Shoot height (cm)</th>
<th>Root length (cm)</th>
<th>Leaf size (ctm²)</th>
<th>Fresh biomass (g plant⁻¹)</th>
<th>Edible part of the plant Number/plant</th>
<th>Yield in fresh wt. (g plant⁻¹)</th>
<th>% Total chlorophyll</th>
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<tr>
<td>Bean</td>
<td>C 38.2±0.9</td>
<td>19.2±1.2</td>
<td>214.4±4.1</td>
<td>126.5±2.0</td>
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<td>T 50.4±0.7*</td>
<td>33.4±2.5*</td>
<td>255.1±3.2*</td>
<td>143.0±3.8*</td>
<td>17.7±1.9*</td>
<td>16.9±1.7*</td>
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<td>Brinjal</td>
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<td>20.9±1.3</td>
<td>134.3±3.5</td>
<td>335.2±2.3</td>
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<td>84.7±2.3</td>
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<td></td>
<td>T 24.3±2.7*</td>
<td>38.1±1.6*</td>
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<td>339.5±4.2</td>
<td>791.5±12.7</td>
<td>—</td>
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<td></td>
<td>T 24.6±2.6</td>
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<td>424.9±6.0*</td>
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<td></td>
<td>T 14.6±2.2</td>
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<td>Tomato</td>
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*Values are statistically significant compared to control (P<0.05); C = Control; T = Treated.

Table 1—Effect of seed bacterization with *Bacillus* BS2 on shoot height, root length, biomass and yield of five vegetable crop plants grown under field conditions [Values are mean ± SD of 4 replications]
**Fig. 1**—Effect of seed bacterization with rhizobacteria strain *Bacillus BS2* on relative growth rate. (A)—Broad bean (*Phaseolus vulgaris* L.); (B)—Brinjal (*Solanum melongena* L.); (C)—Cabbage (*Brassica oleracea* var. capitata); (D)—Kohlrabi (*B. oleracea* var. gongylodes); and (E) Tomato (*Lycopersicon esculentum* Mill). [Error bar represents standard error means of observed value].

*L. esculentum*, *S. melongena* and *P. vulgaris* was significantly higher compared to control plants. The overall yield increment was 16.7 to 67.4%. The data on growth and yield parameters demonstrate that relatively greater dry matter was partitioned for vegetative parts such as root, leaf area resulting in greater plant biomass. Dry matter partitioning played a determinative role in pod yield of *P. vulgaris* and *L. esculentum* yield.

An increased relative growth rate due to seed bacterization was noticed in brinjal and tomato (Fig. 1). In beans and cabbage no differences were noted up to 30 days but became marked afterwards. The greater RGR may be the effect of leaf area ratio. Overall increase in RGR ranged from 23.3 to 56%. Enhancement in RGR with the bacterial inoculation signifies the quick establishment of the canopy with greater partitioning to leaf area growth.

It is evident from Fig. 2, that the drug resistant mutant strain of BS2, designated as BS2\textsuperscript{NS}, was able to colonize and multiply along the roots of the introduced plants. Total CFU g\textsuperscript{-1} of root in treated plant varied between 23 to 25% of the total aerobic bacteria.

Plantlets raised from bacterized seeds reduced the number of infected plants than those raised from the non bacterized plants in soil infested with *R. solani*. On 28\textsuperscript{th} day, after bacterization the incidence of diseased plants was 24 and 48% in treated and the control plants, respectively (Table 2). An increased shoot height, root length and total biomass in terms of fresh weight was also observed with the seedlings emerging from the bacterized one over their control non bacterized plants in pathogen infested soils. This reduction in disease incidence may be due to the antimicrobial substances produced by the strain BS2. Regarding germination, it was noticed that against 80% germination in control, BS2 treated seeds...
showed 92.5% germination. More experiments at large scale field level are required to confirm the present result.

Acknowledgement

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References


4 Dileep Kumar B S, Fusarial wilt suppression and crop improvement through two rhizobacterial strains in chickpea growing in soils infested with *Fusarium oxysporum f. sp. ciceria*, *Biol Fert Soils*, 29 (1999) 87.


