

Original Research Article

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Effect of Dual Inoculation of *Bacillus subtilis* and *Bradyrhizobium japonicum* on Growth Parameters of Soybean (*Glycine max* L.)

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ABSTRACT

Keywords

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Field experiment was conducted during *Kharif*, 2017 at Farm of Agronomy, RCSM College of Agriculture, Kolhapur. To study the effect of dual inoculation of *Bacillus subtilis* and *Bradyrhizobium japonicum* along with graded levels of chemical fertilizers on plant growth parameters of soybean (*Glycine max* L.) The dual inoculation of *Bacillus subtilis* and *Bradyrhizobium japonicum* along with 75 % nitrogen and phosphorus significantly superior over rest of the treatment in ameliorating growth parameters. Which recorded highest germination (86.94 %), height of plant at 50 % flowering (58.32 cm), number of main branches at 50 % flowering (7.66), dry plant weight at 50 % flowering (28.39 g plant⁻¹), number of root nodules plant⁻¹ at 50 % flowering (120.37), dry weight of root nodules (1.60 plant⁻¹) and dry matter yield at harvesting (30.90 q ha⁻¹). However, above treatment did not differ significantly from those recorded with *B. japonicum* and *B. subtilis* along with 100 % recommended dose of fertilizers and un-inoculated control.

Introduction

Soybean (*Glycine max* L.) is one of the most important pulse crops in world. The cultivated soybean originated in china during 2800 BC. It was introduced in India in 1950's (Caldwell, 1970; kumar *et al.*, 2011). In India, the area and productivity have been rapidly increasing over the recent years. It plays major role in oil economy of India. It supplies 43.3 % protein and 19.5 % oil. The production of soybean in India is dominated by Maharashtra and Madhya Pradesh, which contribute 84.08 % of the total production. Currently, India ranks fourth in production of soybean in the world (Anonymous, 2016).

Biofertilizers play a major role in increasing nutrient availability for plant growth and yield. Phosphorus biofertilizer help to increase the availability of phosphate for plant growth by solubilization, promoting the plant growth through production of plant growth promoting substances by increasing the efficiency of biological nitrogen fixation (Kucey *et al.*, 1989). *Bradyrhizobium japonicum* is a species of legume root nodulating, microsymbiotic nitrogen-fixing bacteria. Co-inoculation of *Bacillus* strains on to soybean plants with *Bradyrhizobium japonicum* provided the largest and most consistent increase in nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen and grain

yield (Bai *et al.*, 2003). Co-inoculation studies with *Bacillus subtilis* and *Rhizobium/ Bradyrhizobium spp.* have been shown to increase root and shoot biomass, nodule dry matter, nitrogenase activity, N₂-fixation, and grain yield in legumes (Elkoca *et al.*, 2008).

Materials and Methods

The experiment was carried out during *Kharife*, 2017 at Agronomy Farm, RSCM College of Agriculture, Kolhapur in RBD with 12 treatments and 3 replication. The treatment consist of T₁ = *B. japonicum* alone, T₂ = *B. subtilis* alone, T₃ = *B. japonicum* + 50 % N and RD of P, T₄ = *B. japonicum* + 75 % N and RD of P, T₅ = *B. japonicum* + RDF, T₆ = *B. subtilis* + 50 % P and RD of N, T₇ = *B. subtilis* + 75 % P and RD of N, T₈ = *B. subtilis* + RDF, T₉ = *B. japonicum* + *B. subtilis* + 50 % N & P, T₁₀ = *B. japonicum* + *B. subtilis* + 75 % N & P, T₁₁ = *B. japonicum* + *B. subtilis* + RDF and T₁₂ = Uninoculated control (RDF). Soybean variety named KDS-344 (Phule Agrani) was sown in net plot size 5.40 m x 4.00 m with the row to row spacing 45 cm x 5 cm plant to plant spacing.

The recommended dose of fertilizer was applied 50:75:00 N, P₂O₅, K₂O kg ha⁻¹ with 25 Cartloads of FYM ha⁻¹. All the intercultural operation was carried out as per need. The data recorded on various parameters were subjected to statistical analysis and F- test was applied as per method (Panse and Sukhatme 1985).

Observation recorded

Plant growth parameters

Germination percentage

The seed germination percentage was recorded 10 days after sowing. It was calculated by following formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seed} \times 100}{\text{Total number of seeds}}$$

Height of plant at 50 % flowering

Height of plant in a treatment was observed at 50 % flowering stage of the crop, on randomly selected five plants per treatment.

Number of main branches at 50 % flowering

Number of main branches plant⁻¹ in a treatment was recorded at 50 % flowering stage of the crop, on randomly selected five plants per treatment.

Dry plant weight at 50% flowering (g plant⁻¹)

Five randomly selected plant samples from each treatment were uprooted as destructive samples at 50 % flowering stage of the crop. The samples were oven dried at 70°C for 72 hrs. and average weight was taken as dry matter.

Number of nodules per plant at 50 % flowering

Five randomly selected plants were carefully uprooted from each plot at 50 % flowering stage for nodulation count.

The uprooted plants were washed under running tap water and finally cleaned with a soft camel hair brush to remove soil particles adhering to root surface. The nodules from the root system of each plant were separately collected and counted.

Dry weight of nodules (g plant⁻¹)

The nodules were air dried and then oven dried at 65°C for 72 hrs and recorded their oven dry weights.

Dry matter yield (ha⁻¹)

Dry matter weight per plot was recorded as kg plot⁻¹ and was converted in to q ha⁻¹.

Results and Discussion

The data recorded in table 1, all the treatments dual inoculation of *Bradyrhizobium japonicum* and *Bacillus subtilis* along with 75% of the recommended dose of nitrogen and phosphorus recorded highest germination per cent (86.94), height of plant at 50 % flowering (58.32 cm), number of main branches at 50 % flowering (7.66), dry plant weight g plant⁻¹ at 50 % flowering (28.39), number of root

nodules plant⁻¹ at 50 % flowering (120.37), dry weight of root nodules plant⁻¹ (1.60) and dry matter yield q ha⁻¹ at harvesting (30.90) at par with treatment T₁₁ (*B. japonicum* + *B. subtilis* + RDF) and T₁₂ (Uninoculated control).

The lowest values were recorded in treatment T₂ (*B. subtilis* alone) viz., germination (65.30 %), height of plant at 50 % flowering (35.45 cm), number of main branches at 50 % flowering (2.03), dry plant weight at 50 % flowering (10.27 g plant⁻¹), number of root nodules plant⁻¹ at 50 % flowering (80.03), dry weight of root nodules (0.73 plant⁻¹) and dry matter yield at harvesting (15.18 q ha⁻¹).

Table.1 Dual effect of *Bacillus subtilis* and *Bradyrhizobium japonicum* on yield and yield attribute of soybean

Tr. No	Treatment details	Germination (%)	Height of plant at 50 % flowering (cm)	No. of main branches at 50 % flowering	Dry plant weight at 50 % flowering (g plant ⁻¹)	No. of root nodules plant ⁻¹ at 50 % flowering	Dry weight of root nodules plant ⁻¹	Dry matter yield at harvesting (q ha ⁻¹)
T ₁	<i>B. japonicum</i> alone	67.20	39.18	2.17	12.37	82.40	0.81	17.48
T ₂	<i>B. subtilis</i> alone	65.30	35.45	2.03	10.27	80.03	0.73	15.18
T ₃	<i>B. japonicum</i> + 50 % N and RDP	73.35	45.73	4.13	18.34	90.35	0.92	21.26
T ₄	<i>B. japonicum</i> + 75 % N and RDP	78.84	52.41	6.33	24.35	115.6	1.35	27.28
T ₅	<i>B. japonicum</i> + RDF	78.17	52.05	6.21	23.92	115.2	1.33	26.80
T ₆	<i>B. subtilis</i> + 50 % P and RDN	69.15	42.19	3.51	13.97	84.34	0.76	20.23
T ₇	<i>B. subtilis</i> + 75 % P and RDN	75.43	49.51	5.35	21.38	95.22	1.25	24.26
T ₈	<i>B. subtilis</i> + RDF	75.13	49.31	5.22	20.90	94.89	1.24	23.73
T ₉	<i>B. japonicum</i> + <i>B. subtilis</i> + 50 % N & P	71.28	42.86	3.10	16.26	86.40	0.83	20.50
T ₁₀	<i>B. japonicum</i> + <i>B. subtilis</i> + 75 % N & P	86.94	58.32	7.66	28.39	120.37	1.60	30.90
T ₁₁	<i>B. japonicum</i> + <i>B. subtilis</i> + RDF	85.33	57.95	7.50	27.90	120.11	1.59	30.56
T ₁₂	Uninoculated control (RDF)	84.18	56.97	7.15	27.21	119.55	1.57	30.21
	S.E±	0.57	0.71	0.19	0.22	0.40	0.05	0.51
	C.D. at 5 %	1.67	2.09	0.57	0.64	1.17	0.15	1.48

Note: RDN= Recommended dose of nitrogen, RDP= Recommended dose of phosphorus and RDF= Recommended dose of nitrogen and Phosphorus

The present results of increase in soybean germination due to inoculation of *Bacillus subtilis* and *B. japonicum* are similar to those Singh *et al.*, (2011) stated that the bacterial species increased the germination due to dual inoculation, including *B. subtilis*. Tilak *et al.*,

(2006) reported that the dual inoculation of *Bacillus subtilis* and *B. japonicum* significantly increase plant height of soybean plant. Rajendran *et al.*, (2008) reported growth promoting bacteria *Bacillus* shown to promote plant height. Bai *et al.*, (2003) found

that the combined application of a *B. subtilis* along with *Bradyrhizobium* in soybean significantly enhanced nodule number and nodule dry weight. Moreover, Sajid *et al.*, (2010) also found significant effect on number of nodules per plant due to inoculation with *Rhizobium*. Abbasi *et al.*, (2011) reported that seed inoculation with *Rhizobium* and PGPR had positive effect on number and weight of nodules. Whereas, at high Phosphorus conditions, coinoculation with *Rhizobium spp.* and *B. subtilis* significantly increased bean nodulation per plant (Roseline *et al.*, 2007). Solomon *et al.*, (2012) reported that the nodule number per plant in soybean were significantly influenced by *Bradyrhizobium japonicum* strains alone. Dashti *et al.*, (1998) showed that the dual inoculation *B. subtilis* and *B. japonicum* significantly increased dry matter yields as compared with single inoculation of individual organisms in soybean. Patra *et al.*, (2012) reported that response of soybean to inoculation with rhizobial strains increase plant dry matter of soybean over control due to inoculation with strain *Bacillus spp.*

To abridge, the results explicitly unveil that the inoculation of soybean seeds with *Bacillus subtilis* and *Bradyrhizobium japonicum* in conjunction with application of 75 % of recommended dose of nitrogen and phosphorus had influential effect on grain yield of soybean.

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