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The Impact of Bio and Mineral Fertilization on Plant Growth and Root Nodulation in Soybean *Glycine max* (L.) Merr

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A field experiment was conducted to study the effect of *Bradyrhizobium japonicum* and phosphate solubilizing fungus in combination with graded levels of chemical fertilizers on growth and root nodulation of soybean (*Glycine max* (L.) Merr.). Treatments comprising of: dual inoculation of soybean seeds with *Bradyrhizobium japonicum* @ 25 g/kg seed and phosphate solubilizing fungus @ 10 g/kg seed in conjunction with application of recommended dose of chemical fertilizers; dual inoculation with *Bradyrhizobium japonicum* and phosphate solubilizing fungus in combination with application of 75% of recommended dose of nitrogen and phosphorus and 100% of recommended dose of potash; seed inoculation with phosphate solubilizing fungus in conjunction with recommended dose of fertilizers and; the treatment consisting of seed inoculation with *Bradyrhizobium japonicum* in conjunction with recommended dose of fertilizers had profound effect on growth and root nodulation in soybean. However, the treatment comprising of dual inoculation of seed with *Bradyrhizobium japonicum* and phosphate solubilizing fungus in conjunction with application of 75% of recommended dose of nitrogen and phosphorus and 100% of recommended dose of potash was most economical, since it caused 25% savings in nitrogenous and phosphatic fertilizers.

Keywords: Bradyrhizobium japonicum; phosphate solubilizing fungus; soybean.

1. INTRODUCTION

“Soybean (*Glycine max* (L.) Merr.) is one of the most important pulse crops in the world. Cultivated soybeans was originated in China around 2800 BC. This crop was introduced to India in 1950’s” (Caldwell and Vest, 1970; Suresh Kumar et. al., 2011). In recent years, the area, production and productivity of this soybeans in India have been increasing rapidly. Currently, India ranks fourth in the world of soybean production (Anonymous, 2016) with Madhya Pradesh, Maharashtra and Rajasthan being the major in cultivating this crop, which collectively contributing 92 to 93 % of the area and production. Soybean has emerged as the wonder crop of the 21st century. “Soybean contains about 40% protein, possessing high levels of essential amino acids methionine and cysteine; 20% oil rich in poly unsaturated fatty acids especially omega-6 and omega-3 fatty acids; 6-7 % total minerals; 5-6 % crude fibre and 17-19 % carbohydrates” (Chauhan et al., 2002). Besides, soybean contains good amount of iron, vitamin B-complex and isoflavones such as daidzein, genistein and glycitein. Because of calcium and iron contents, “it is highly suitable for women suffering from osteoporosis and anaemia Sheoran, (2006). The isoflavones of soybean have been found to possess health benefits, as they exhibited properties like cancer prevention, combating menopausal problem and helping to recover from diabetes” (Chauhan et al., 2002).

The foregoing facts explicitly substantiate that there is a need to improve the yield of soybean in order to meet the protein malnutrition and the edible oil needs of our country. In this context, biofertilizers play a major role in increasing nutrient availability and thus eventually in yielding high crop yield. Biofertilizers also topple cost of production by limiting chemical fertilizer application, which at higher doses have harmful effects on plant growth and development and also on environment. *Bradyrhizobium japonicum* is a species of legume root-nodulating, microsymbiotic nitrogen-fixing bacterium. It forms a symbiotic relationship with soybean and increase nodulation, which leads to increase plant fresh weight, seed protein and seed yield. The application of *Bradyrhizobium* culture in legumes has been reported by several researchers to increase grain yield, pods and nodulation (Sahu and Behera, 1972; Dashti et al., 1998; Elkoca et al., 2008; Abbasi et al., 2008; Abitew and Kibret, 2017).

Phosphorus (P) is abundant in soils in both organic and inorganic forms; “nevertheless, it is unavailable to plants. Consequently, soil becomes P-deficient, making P one of the most important nutrient elements limiting crop productivity. Besides, the recovery of applied P is lower i.e. 10-20% compared to 30-50% for N by the crop. The lower recovery of applied phosphorus is mainly due to slow mobility and quick fixation of phosphorus. Thus, phosphorus applied is not lost from the soil but stored in soil reserves. A substantial component of P in any soil is in the form of sparingly soluble mineral phosphates, which are largely unavailable to plants. To circumvent the P deficiency, phosphate solubilizing microorganisms could play an important role in making P available for plants by dissolving insoluble P. The dissolution of inorganic P by microbial communities, including fungi, is common under *in vitro* conditions. The challenge is how to make use of such biological resources to maintain soil health while increasing the crop productivity by providing P to plants through the application of phosphate-solubilizing fungi" (Malviya et al., 2011). Abd-Alla et al. (2001) has reported yield increase in soybean through simple inoculation of P-solubilizing fungi.

"Reports from numerous workers indicate that the beneficial effects of plant growth-promoting rhizobacteria (PGPR) are often enhanced through dual inoculation. Co-inoculation studies involving *Rhizobium/Bradyrhizobium* species and phosphate-solubilizing fungi have demonstrated significant increase in root and shoot biomass, nodule dry matter, nitrogenase activity, nitrogen fixation and grain yield in legumes" (Abd-Alla et al., 2001).

In view of the specifics portrayed in the foregoing paragraphs, it was thought worthwhile to study the effect of dual inoculation with *Bradyrhizobium japonicum* and phosphate solubilizing fungi on growth and yield of soybean.

2. MATERIALS AND METHODS

A field experiment was conducted at the Agronomy Farm of Rajarshee Chhatrapati Shahu Maharaj College of Agriculture, Kolhapur (Maharashtra, India), during *kharif* season of the year 2018, to study the effect of *Bradyrhizobium japonicum* and phosphate solubilizing fungus on growth, yield and nutrient content in soybean (*Glycine max* L.). The experiment was laid out in RBD (Randomized Block Design) with ten treatments and three replications. The treatments were: T_1: Phosphate solubilizing fungus (PSF) + recommended dose of fertilizers (RDF), T_2: PSF + 75% of recommended dose of P (RDP) + recommended dose of N and K (RDN&K), T_3: PSF + 50% of RDP + RDN&K, T_4: *B. japonicum* (*Bj*) + RDF, T_5: *Bj* + 75% of recommended dose of N (RDN) + recommended dose of P and K (RDP&K), T_6: *Bj* + 50% RDN + RDP&K, T_7: *Bj* + PSF + RDF, T_8: *Bj* + PSF + 75% recommended dose of N and P (RDN&P) + recommended dose of K (RDK), T_9: *Bj* + PSF + 50% recommended dose of N and P (RDN&P) + RDK, T_{10}: Uninoculated control (RDF).

Lignite based inoculum of *B. japonicum* was obtained from Biofertilizer Production Unit of Rajarshee Chhatrapati Shahu Maharaj College of Agriculture, Kolhapur, whereas the efficient culture of phosphate solubilizing fungus *Aspergillus niger*, which we isolated in our previous study was used as a talc based inoculum for seed application. Talc based formulation of the fungus was used @ 10 g/kg seed for the seed treatment. The weight of talc based formulation was taken on weighing balance and mixed with the seeds. The material was slightly moistened with sterilized water, shook slightly so as to cover the entire seed surface by the fungal inoculum. After this, seeds were inoculated with lignite based inoculum of *B. japonicum* @ 25 g/kg seed by adopting the slurry method. After the seed treatment, the seeds were dried in shade and sowed immediately. The soybean cv. KDS-726 (Phule Sangam) was used as a test crop. The crop was sown by maintaining distance of 45 cm between rows and 5 cm between plants. The chemical fertilizers were applied, as per the treatments, at the rate of 50:75:45 kg/ha N, P_2O_5, K_2O. The cultural operations like irrigation, weeding were uniformly carried out to all the treatments. The observations were recorded on seed germination percentage, plant height, number of main branches, dry plant weight, number of nodules and, dry weight of nodules. The statistical analysis was carried out by using OPSTAT statistical analysis tool (*www.hau.ernet.in*).

3. RESULTS AND DISCUSSION

Results of the present investigation pertaining to plant growth and nodulation in soybean crop as influenced by inoculation with *Bradyrhizobium japonicum* and phosphate solubilizing fungus in conjunction with application of graded levels of recommended dose of chemical fertilizers are discussed below under different sub-headings.

3.1 Effect on Seed Germination

The data presented in Table 1 regarding germination percentage of soybean seeds, as influenced by inoculation with *B. japonicum* and PSF, were statistically significant. All the treatments had significantly profound effect on germination percentage of soybean seeds, recorded at 10 days after sowing. However, plots which had integrated application of *B. japonicum* + PSF+ RDF (T_7) had the highest mean germination percentage of 86.87%. However, the germination percentage recorded with this treatment did not differ significantly from those recorded with the treatments consisting of *B. japonicum* + PSF + 75% of RDN&P + RDK (T_8); PSF + RDF (T_1); *B. japonicum* + RDF (T_4) and;

the treatment of RDF (T_{10}). Thus, these five treatments were found to be significantly superior over rest of the treatments in ameliorating seed germination. Significantly lowest germination percentage of 76.43% and 76.90% was observed in the plots, which had treatment of PSF + 50% of RDP + RDN&K (T_3) and, the treatment of PSF + 75% of RDP + RDN & K (T_2). The present results of increase in soybean germination due to inoculation of *B. japonicum* and PSF are in agreement with those of Kucey et al. (1989), Rudresh et al. (2005), Deshwal et al. (2003) and Mathivanan et al. (2014).

3.2 Effect on Plant Height

The data presented in Table 1 regarding plant height at 50% flowering as influenced by inoculation with *B. japonicum* and PSF, were statistically significant. However, the plots wherein inoculation with *B. japonicum* + PSF was followed along with application of RDF (T_7) had the tallest soybean plants (58.77 cm). Seed treatment with *B. japonicum* + PSF along with application of 75% of RDN&P and RDK (T_8); the treatment of PSF along with application of RDF (T_1); the treatment of *B. japonicum* along with RDF (T_4) and; the treatment of RDF alone (T_{10}) were the next best treatments, which enhanced the plant height to the tune of 58.57 cm, 58.27 cm, 58.20 cm and 58.17 cm respectively. However, these four treatments did not differ statistically from the former and thus, all these five treatments were equally effective and significantly superior over rest of the treatments.

Mittal et al., (2008) reported that the dual inoculation of PSF significantly increases plant height. Verma et al. (2010) reported that high proportion of rhizo-microorganisms are able to produce plant growth hormone, which acts to stimulate plant growth. In some PGPR, plant growth promotion dominates. The mechanisms that are involved in this process can include nitrogen fixation, phosphate solubilization and the production of phytohormones and volatile growth stimulants (Vessey 2003; Ryu et al., 2003). Increased plant height in the present investigation due to inoculation with *B. japonicum* and PSF may be explained on these bases. Results of the present investigation concur with observations of the foregoing researchers.

3.3 Effect on Number of Main Branches

The data presented in Table 1 in regard to number of main branches/plants, as influenced by inoculation with *B. japonicum* and PSF, were statistically significant. All the treatments tried in the present investigation gave significantly varied response to production of number of main branches per plant, which ranged between 3.07 and 7.67. However, plants which received integrated inoculation of *B. japonicum* + PSF along with application of RDF (T_7), produced the highest number of main branches to the tune of 7.67/plant. Seed treatment of soybean with *B. japonicum* + PSF along with application of 75% RDN&P and RDK (T_8); the treatment consisting of PSF along with application of RDF (T_1); the treatment of *B. japonicum* with RDF (T_4) and, the treatment of RDF (T_{10}) were the next best treatments, which enhanced the mean number of branches to the tune of 7.57, 7.37, 7.23 and 7.20 /plant, respectively. However, the later treatments did not differ significantly from the former and thus, these five treatments unveiled the excel performance and were found to be significantly superior over rest of the treatments. Verma et al., (2010) found that high proportion of rhizo-microorganisms are able to produce plant growth hormone, i.e. Indole acetic acid, which acts to stimulate plant growth and provides it with more branching and larger surface area. In some PGPR, plant growth promotion dominates. The mechanisms that are involved in this process can include nitrogen fixation, phosphate solubilization and the production of phytohormones (such as auxin and cytokinin) and volatile growth stimulants (such as ethylene and 2,3-butanediol) (Vessey 2003; Ryu et al.,2003). Increased branching in the present investigation due to inoculation with *B. japonicum* and PSF may be explained on these bases.

3.4 Effect on Dry Plant Weight

The data presented in Table 2 in regard to dry plant weight at 50% flowering, as persuaded by inoculation with *Bradyrhizobium japonicum* and PSF, were statistically significant. All the treatments tried in the investigation recorded significantly varied response to plant biomass production of soybean, which ranged between 17.90 and 28.37 g/plant. However, plots wherein integrated inoculation with *B. japonicum* + PSF was followed along with application of RDF (T_7) recorded the highest biomass production to the tune of 28.37 g/ plant. Seed treatment of soybean with *B. japonicum* + PSF along with application of 75% RDN&P and RDK (T_8); the treatment consisting of PSF along with application of RDF (T_1); the treatment of *B. japonicum* with RDF (T_4) and, the treatment of RDF (T_{10}) were the next best treatments, which enhanced the dry plant weight per plant to the tune of 27.90 g, 27.63 g, 27.53 g, and 27.30 g, respectively. However, these treatments did not differ significantly from the former and thus, these five treatments were found to be statistically at par with each other and found to be significantly superior over rest of the treatments in enhancing dry plant weight of soybean. The lowest dry plant weight of 17.90 g/plant was recorded in the plots inoculated with PSF along with application of 50 % of RDP and RDN&K. PSF have high competitive and wide metabolic capability and exert plant growth-promoting activity. It has been shown that these fungi rapidly colonize plant roots and cause at the plant level significant shoot increases. Downey and Kessel (1990) reported that the dual inoculation with *Rhizobium* and PSF significantly increased dry plant weight as compared with single inoculation of individual organisms in soybean. Gleddie (1993) reported increased dry plant weight in soybean due to inoculation with PSF. Zaidi and Khan (2008) as well reported increase in dry plant weight due to inoculation with *B. japonicum* + PSF. Increased dry plant weight in the present investigation due to inoculation with *B. japonicum* and PSF may be explained on these bases.

3.5 Effect on Number of Root Nodules

The data presented in Table 2 pertaining to number of root nodules per plant as influenced by inoculation with *Bradyrhizobium japonicum* and PSF, were statistically significant. All the treatments experimented in the investigation had significantly profound effect on number of root nodules per plant. However, plots wherein seeds inoculated with *B. japonicum* + PSF were sown along with application of RDF (T_7), had the highest number of nodules per plant, which was evident from mean number of nodules to an extent of 123.10 as compared to rest of the treatments. However, seed treatment of soybean with *B. japonicum* + PSF along with application of 75% RDN&P and RDK (T_8) (122.87 nodules/plant); the treatment consisting of PSF along with application of RDF (T_1) (122.30 nodules/plant); the treatment of *B. japonicum* with RDF (T_4) (122.27 nodules/plant) and, application of only RDF (T_{10}) (122.20 nodules/plant) did not differ significantly from the former treatment in enhancing number of nodules per plant. Thus, all these five treatments were found to be significantly superior over rest of the treatments in ameliorating number of root nodules in soybean. The lowest number of nodules to the tune of 110.80/plant was observed in the plots wherein PSF was inoculated along with application of 50 % of RDP and RDN&K (T_3). The present results of increase in soybean nodulation due to inoculation of *B. japonicum* and PSF are in agreement with those of Abd-Alla et al. (2001) who found that the combined application of a *Bradyrhizobium* along with *Aspergillus niger* in bean 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. (2008) reported that seed inoculation with *Rhizobium* and phosphate fertilizers had positive effect on number and weight of nodules. Solomon et al. (2012) reported that the nodule number per plant in soybean was significantly influenced by *B. japonicum* strains alone. Results of the present investigation concur with observations of the foregoing researchers.

Table 1. Effect of *Bradyrhizobium japonicum* and phosphate solubilizing fungus on seed germination, plant height and number of main branches at 50 % flowering

Treatment	Germination (%)	Height of plant at 50% flowering (cm)	No. of main branches at 50 % flowering
T_1 - PSF + RDF	86.33	58.27	7.37
T_2 - PSF + 75% RDP + RDN & K	76.90	49.03	3.13
T_3 - PSF + 50% RDP + RDN & K	76.43	48.77	3.07
T_4 - *Bj* + RDF	86.23	58.20	7.23
T_5 - *Bj* + 75% RDN + RDP & K	83.00	54.03	5.67
T_6 - *Bj* + 50% RDN + RDP & K	79.97	51.93	4.20
T_7 - *Bj* + PSF + RDF	86.87	58.77	7.67
T_8 - *Bj* + PSF + 75% RDN & P + RDK	86.47	58.57	7.57
T_9 - *Bj* + PSF + 50% RDN & P + RDK	80.00	52.03	4.33
T_{10} - Uninoculated control (RDF)	86.20	58.17	7.20
SE (m) ±	0.95	0.62	0.32
CD at 5%	2.82	1.85	0.95

Table 2. Effect of *Bradyrhizobium japonicum* and phosphate solubilizing fungus on dry plant weight and, number and dry weight of root nodules at 50% flowering

Treatment	Dry plant weight at 50 % flowering (g/plant)	No. of root nodules/plant at 50% flowering	Dry weight of root nodules at 50% flowering (g/plant)
T_1 - PSF + RDF	27.63	122.30	1.57
T_2 - PSF + 75% RDP + RDN & K	18.20	111.70	0.84
T_3 - PSF + 50% RDP + RDN & K	17.90	110.80	0.77
T_4 - *Bj* + RDF	27.53	122.27	1.56
T_5 - *Bj* + 75% RDN + RDP & K	24.33	119.03	1.33
T_6 - *Bj* + 50% RDN + RDP & K	21.00	115.70	1.02
T_7 - *Bj* + PSF + RDF	28.37	123.10	1.61
T_8 - *Bj* + PSF + 75% RDN & P + RDK	27.90	122.87	1.59
T_9 - *Bj* + PSF + 50% RDN & P + RDK	21.30	115.97	1.13
T_{10} - Uninoculated control (RDF)	27.30	122.20	1.55
SE (m) ±	0.68	0.96	0.06
CD at 5%	2.02	2.85	0.17

3.6 Effect on Dry Weight of Root Nodules

The data presented in Table 2 in regard to dry weight of root nodules recorded at 50 % flowering, as persuaded by inoculation with *Bradyrhizobium japonicum* and PSF, were statistically significant. All the treatments tested in the investigation gave significantly varied response to dry weight of root nodules of soybean, which ranged between 0.77 g/plant and 1.61 g/plant. However, plots wherein integrated inoculation with *B. japonicum* + PSF was done along with application of RDF (T_7), had the highest dry weight of nodules to the tune of 1.61 g/plant. However, dry weight of nodules recorded in the treatments of seed treatment with *B. japonicum* + PSF along with application of 75% of RDN&P and RDK (T_8) (1.59 g/ plant); PSF along with application of RDF (T_1) (1.57 g/plant); *B. japonicum* with RDF (T_4) (1.56 g/plant) and; application of only RDF (T_{10}) (1.55 g/plant) did not differ significantly from that of the former treatment. Accordingly, all these five treatments were found to be significantly superior over rest of the treatments in ameliorating dry weight of root nodules. The lowest dry weight of nodules to the tune of 0.77 g/plant was observed in the plots wherein PSF was inoculated along with application of 50 % of RDP and RDN&K (T_3). Abd-Alla et al. (2001) reported that the combined application of a *Bradyrhizobium* along with *A. niger* in bean significantly enhanced nodule dry weight. Abbasi et al. (2008) reported that seed inoculation with *Rhizobium* and phosphate fertilizers had positive effect on weight of nodules. Results of the present investigation are in harmony with observations of the foregoing researchers.

4. CONCLUSIONS

From the results of the present investigation, it can be concluded that dual inoculation of soybean seeds with *Bradyrhizobium japonicum* at a rate 25 g/kg seed and *Aspergillus niger* at a rate of 10 g/kg seed in conjunction with the application of 75% of the recommended dose of nitrogen and phosphorus and 100% of the recommended dose of potash, is most economical approach for enhancing plant growth and root nodulation. This method resulted in a 25% saving in nitrogenous and phosphatic fertilizers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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