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Response of *Bacillus megaterium* and *Bacillus mucilaginosus* Strains on Yield and Quality of Soybean

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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

At present due to continuous use of phosphatic and potassic fertilizers a deposits of these nutrient have increased in the soil of studied area, and *Bacillus megaterium* and *Bacillus mucilaginosus* solubilize/mobilize P and K in the soil, thus, for increasing yield and quality of soybean, an experiment was planned with the aim to investigate the response of *Bacillus megaterium* and *Bacillus mucilaginosus* strains on yield and quality of soybean [*Glycine max* (L.) Merrill.] at All India Coordinated Research Project (AICRP) on Integrated Farming System Research at College of Agriculture, Indore, Madhya Pradesh, India during *kharif*, 2019 & 20. The research was conducted in randomized block design (RBD) with 8 treatments, viz. Control with 75% RDF (T₁), Control with 100% RDF (T₂), 75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed

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treatment (T₃), 100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment (T₄), 75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas foliar application at 30 and 45 DAS (T₅), 100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas foliar application at 30 and 45 DAS (T₆), 75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strainsas seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strains seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strains (T₈) at 30 and 45 DAS compared to control (786 kg ha⁻¹). Among all the treatments, yield attributes such as pods count plant⁻¹ (29.66), seeds count pod⁻¹ (3.36) and seed yield plant⁻¹ (13.17) were observed highest with T₈ at 30 and 45 DAS.

Keywords: Soybean; Bacillus megaterium; Bacillus mucilaginosus; quality parameters.

1. INTRODUCTION

The soybean, scientifically known as Glycine max (L.) Merrill, is a premier legume crop distinguished by two key qualities: protein and oil content. In addition to having a protein content of 40.5%, this remarkable legume is also high in oil, ranging from 18% to 22.5%. In addition, it contains 20-30% extractable substances and a well-balanced amino acid profile [1]. Soybeans are known internationally by many credentials and local names, including yellow beans and large beans in China, edamame in Japan, and miracle beans and golden beans in the United States [2]. Due to its adaptability to diverse soil conditions and climates, as well as its nutritional content, soybeans are becoming increasingly popular in Central India.As the most widely cultivated oilseed crop in the world, soybean occupies around 6% of all agricultural land under cultivation [3]. According to [4] the soybean cultivation area in India is approximately 11.33 million hectares, with a potential production of over 13.79 million tonnes in 2019-20. In Central India, Madhya Pradesh is known as the "Soya State of India."

The widespread use of synthetic fertilizers, while addressing some challenges, also poses significant environmental and food production risks, as highlighted by [5]. To address these concerns, a promising solution lies in harnessing the potential of various bacterial species such as Azotobacter, Azospirillum, Bacillus sp., and Pseudomonas sp. These microbes fall under the category of plant growth-promoting rhizobacteria (PGPR). Leveraging these microorganisms as biofertilizers offers a viable and sustainable synthetic alternative to fertilizers. Notably. bacterial species like Azotobacter and Azospirillum exhibit remarkable capabilities. They can effectively fix atmospheric nitrogen and enhance soil phosphorus solubilization, as demonstrated by [6,7].

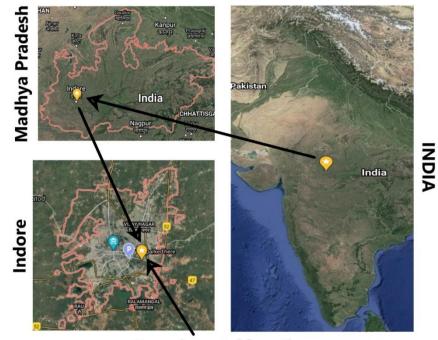
Microorganisms are beneficial in agriculture, and are now being used in the production of sustainable food crops [7]. Beneficial microorganisms have been shown to play a role in atmospheric nitrogen fixation, organic wastes and residues decomposition, detoxification of pesticides, suppression of plant diseases and soil-borne pathogens, enhancement of nutrient cycling, and production of bioactive compounds such as vitamins, hormones, and enzymes that upregulate plant growth [8]. The combined utilization of organic, inorganic, and bio-fertilizers brings about a holistic enhancement in various aspects of agriculture. This integrated approach leads to improvements in soil productivity, sustainability, reclamation, as well as the growth, development, setting, and quality of crops and seeds [7,9]. Notably, the production of microbial metabolites, encompassing organic acids, can induce a reduction in soil pH.

Based on these assumptions, the current study focused on evaluating the effects of *Bacillus megaterium*and *Bacillus mucilaginosus* strains on soybean aiming to achieve the following: (1) Evaluation of the effects of *Bacillus megaterium* and *Bacillus mucilaginosus* strains on yield attributes, yield and harvest index; (2) To find out the effect of *Bacillus megaterium* and *Bacillus mucilaginosus* strains on quality parameters.

2. MATERIALS AND METHODS

The experiment was conducted at Research Farm of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, College of Agriculture, Indore, Madhya Pradesh, India during *kharif* season (2019–20) with soybean var. "JS 95-60". The topography of field was uniform with gentle slope. Indore is situated at an altitude of 555.5 m above mean sea level (MSL). It is located at latitude 22.43°N and longitude of 75.66°E. This region enjoys sub-tropical, semi-arid type climate. The maximum temperature varied from 25.36°C - 32.43°C and

the minimum temperature varied from 20.4° C - 24.57°C. The soil of experimental site was predominantly clayey in texture, slightly alkaline in reaction (pH 7.5) and low in organic carbon (0.45%) and available nitrogen (210 kg·ha⁻¹), low in available phosphorus (11.5 kg·ha⁻¹) and high in available potash (410 kg·ha⁻¹).



Experimental location

Fig. 1. Experimental location

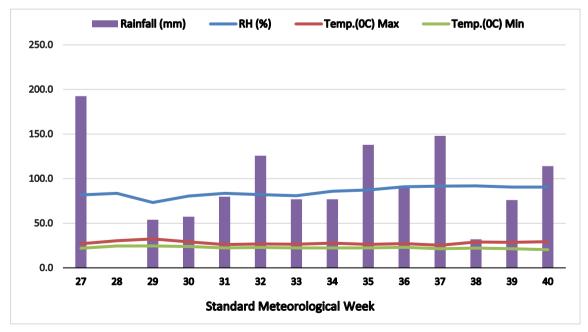


Fig. 2. Rainfall and other weather parameters during crop growing period of experimental crop season

Chemical properties	Values	Method
pН	7.5	pH meter by using glass electrode in 1:2.5 soil water
		suspension [10]
Organic carbon (%)	0.45	Walkley and Black rapid titration method [11]
EC (dSm ⁻¹ at 25 °C)	0.23	Electrical conductivity bridge (1:2.5) soil water suspension)
Available N (kg ha-1)	210	Alkaline permanganate method [12]
Available N (kg ha-1)	11.5	Olsen's method [13]
Available K (kg ha-1)	410	Flame photometer method [10]

Table 1. Different soil properties and methods employed for analysis

2.1 Crop Management

In order to get a good tilth of soil for sowing, the field preparation was started with summer ploughing by tractor drawn plough followed by cross harrowing. Final harrowingwas followed by planking to level the field before sowing. All the fertilizers were applied as basal in the furrows made and mixed with soil before placing theseeds. For ensuring perfect germination, healthyand good quality seeds were used. Seeds weretreated by Bavistin @ 2g kg-1 seeds and after that inoculated with Bacillus megaterium and Bacillus mucilaginosus strains@ 3 g kg-1 seeds at the time of sowing. The treated seeds were sown in plots with 6 m x 4.5 m dimensions maintaining the row and plant distance of 30 cm x 5 cm @ seed rate of 80 kg ha⁻¹. To protect the crop from insects, pests like girdle beetle, stem fly caterpillars, blue beetle etc. in soybean at early stage two sprays of Triazophos 40 EC 600 mlha-1 was doneat 30 and 45 daysof crop growth.

The soil of experimental site was predominantly clayey in texture, slightly alkaline in reaction (pH 7.50) and low in organic carbon (0.45%) and available nitrogen (210 kg·ha⁻¹), low in available phosphorus (11.5 kg·ha⁻¹) and high in available potash (410 kg·ha⁻¹). Table 1 showed different soil methods adopted for analysis.

2.2 Analysis of Quality Parameters

At maturity, seed and stover yield data were recorded using standard methodologies. The protein content (%) of seeds worked out by multiplying nitrogen content of seed by a factor of 6.25 as suggested by [14]. The oil content was calculated by Hexane extraction method [15].

2.3 Statistical Analysis

The analysis of variance will be carried out according to the method given by [16] for

Randomized Block Design (Factorial) and results was tested at 5% probability level of significance.

3. RESULTS AND DISCUSSION

The results obtained from the present investigation are presented in Tables 2 and 3.

3.1 Yield and Yield Attributes

The increased grain output, biological yield, and harvest index may be due to enhanced nutrient availability. The treatment that received 100% RDF with Bacillus megaterium and Bacillus mucilaginosus strains as seed treatment + foliar application of Bacillus megaterium and Bacillus mucilaginosus strains at 30 and 45 DAS (T₈) produced the most pods per plant (29.66), the most seeds per pod (3.36), and the highest seed and straw vield i.e. 1139 kg ha-1 and 1567 kg harespectively (Table 2). By boosting P solubilization and the synthesis of growthpromoting chemicals and hormones. the combination of 100% RDF and microbial strains improved crop development. It also enhanced microbial activity in the rhizospheric zone, balancing the crop's supply of critical nutrients. These findings are in line with what was discovered in the past by other studies [17]. The harvest index exhibited a consistent trend owing to negligible fluctuations in the physical characteristics of the grain. The various treatments had no major influence on the harvest index and seed index. However, the application of 100% RDF with Bacillus megaterium and Bacillus mucilaginosus strains as seed treatment, along with foliar application of the same strains at 30 and 45 DAS, showed the highest harvest index (42.08). Similar findings were reported by [18] and [7].

3.2 Quality Parameters

Data indicated that various treatments caused significant variation in protein and oil yield (Table 3). Data revealed that application

Symbol	Treatment	Pods count plant ⁻¹	Seeds count pod ⁻¹	100 Seed weight	Seed yield (kg ha ⁻¹)	Straw yield (kg ha⁻¹)	Harvest index (%)
T ₁	Control with 75% RDF	21.66	2.71	11	786	1167	40.24
T ₂	Control with 100% RDF	22.55	2.81	11	807	1263	38.9
Τ ₃	75% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as seed treatment	23.44	2.82	11.33	843	1288	39.52
T 4	100% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as seed treatment	23.77	3.02	11.67	890	1350	39.68
T5	75% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as foliar application at 30 and 45 DAS	24.66	3.08	11.67	900	1410	38.9
T ₆	100% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as foliar application at 30 and 45 DAS	26.33	3.17	11.67	958	1432	40.1
Τ7	75% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as seed treatment + foliar application of <i>Bacillus</i> <i>megaterium</i> and <i>Bacillus mucilaginosus</i> strains at 30 and 45 DAS	26.89	3.25	12	1083	1537	41.3
Τ8	100% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as seed treatment + foliar application of <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains at 30 and 45 DAS	29.66	3.36	12	1139	1567	42.08
SEm (±)		0.92	0.21	1.09	49	33	1.32
C.D. at 59	%	2.8	0.64	NS	149	102	NS

Table 2. Impact of Bacillus megaterium and Bacillus mucilaginosus strains on yield attributes, yield and harvest index of soybean:

Symbol	Treatment	Protein (%)	Protein yield (kg ha ⁻¹)	Oil (%)	Oil yield (kg ha ⁻¹)
T ₁	Control with 75% RDF	34.4	27066	18.2	143349
T ₂	Control with 100% RDF	37.5	30262	18.3	14880
T ₃	75% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as seed treatment	33.5	28383	18.4	15472
T 4	100% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as seed treatment	38.1	33954	18.4	16345
T₅	75% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as foliar application at 30 and 45 DAS	39.6	35791	18.5	16617
Τ ₆	100% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as foliar application at 30 and 45 DAS	39.8	38162	18.5	17729
T ₇	75% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as seed treatment + foliar application of <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains at 30 and 45 DAS	39.9	43251	18.6	20092
T ₈	100% RDF with <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains as seed treatment + foliar application of <i>Bacillus megaterium</i> and <i>Bacillus mucilaginosus</i> strains at 30 and 45 DAS	41.0	46609	18.7	21251
SEm±		0.92	1.27	2385	0.09
C.D. at 59	%	2.8	3.86	7324	0.27

Table 3. Impact of Bacillus megaterium and Bacillus mucilaginosus strains on quality parameters of soybean

of 100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strains as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* strains at 30 and 45 DAS (T₈) recorded significantly higher protein and oil yield (46609 kg/ha) and (21251 kg/ha). The protein and oil yield were increased with 64 and 70% respectively as compared to RDF. The protein content in seed is essentially a manifestation of N content. Increased N content due to seed inoculation with bio-inoculants and foliar spray resulted in higher protein content because of their beneficial role in enhancing N content in seed. Similar, findings have also been reported by [19] and [20] in soybean.

4. CONCLUSION

The research analysis confirms that the application of both inorganic fertilizers and biofertilizers has found significant variations in yield and yield-associated traits, along with biochemical features of soybean. The treatment denoted as T8, which involved the application of the recommended dosage of fertilizers (100%), seed treatment, and foliar application of Bacillus megaterium and Bacillus mucilaginosus strains, was determined to be the most efficacious approach for augmenting crop yield (1139 kg ha-1) and associated characteristics (i.e. 29.66 pods plant⁻¹, 12 seeds pod⁻¹, 41% protein content and 18.7% oil content). Thus, it can be suggested that the T_8 module presents a viable and economical solution for attaining increased output and enhanced quality over an extended period of time.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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