Response of *Bacillus megaterium* and *Bacillus mucilaginosus* Strains on Growth and Nutrient Uptake of Soybean

Abstract: The current study aimed to investigate the Response of *Bacillus megaterium* and *Bacillus mucilaginosus* strains on growth attributes and uptake 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 study was carried out in randomized block design (RBD) with 8 treatments, viz. Control with 75% RDF (T_1), Control with 100% RDF (T_2), 75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strains as seed treatment (T_3), 100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strains as seed treatment (T_4), 75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strains as foliar application at 30 and 45 DAS (T_5), 100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strains as foliar application at 30 and 45 DAS (T_6), 75% 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 (T_7) and 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 (T_8). Among all the treatments, growth parameters, NPK uptake by grain and straw and total uptake by crop were observed highest with 100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS.

Keywords: Soybean; *Bacillus megaterium*; *Bacillus mucilaginosus*; nutrient uptake

1. Introduction

Soybeans, scientifically known as *Glycine max* (L.) Merrill, are prominent legume crops with high protein and oil content. This remarkable legume also contains a high oil content of 18% to 22.5%, in addition to its protein content of 40.5%. Furthermore, it contains 20-30% extractable substances and is well-balanced in amino acids [1]. In China and Japan, soybeans are called yellow beans and edamame, while in the United States, they are called miracle beans and golden beans [2]. As a result of its ability to adapt to diverse soil conditions and climates, as well as its nutritional value, soybeans have gained popularity in Central India. Soybean occupies approximately 6% of all agricultural land worldwide as the most widely cultivated oilseed crop [3]. As per [4,] soybean cultivation in India covers approximately 11.33 million hectares, and the potential production in 2019-20 is over 13.79 million tonnes. Madhya Pradesh is known as the "Soya State of India" in Central India.

Synthetic fertilizers are used widely, which helps to address some challenges, but also poses risks to the environment and food production, as highlighted by [5, 20]. It is possible to address these concerns by harnessing the potential of bacterial species such as *Azotobacter*, *Azospirillum*, *Bacillus* sp., and *Pseudomonas* sp. In general, these microbes are classified as plant growth-promoting rhizobacteria (PGPR). By utilizing these microorganisms as biofertilizers, synthetic fertilizers can be replaced with an alternative that is sustainable and viable [20]. There are numerous bacteria that exhibit remarkable capabilities, including *Azotobacter* and *Azospirillum*. According to [6] they are capable of fixing atmospheric nitrogen and increasing soil phosphorus solubilization. Through processes like BNF (Biological nitrogen fixation) and phosphate solubilization, plant growth-promoting bacteria (PGPB) promote plant growth as well as reduce stress by modulating 1-aminocyclopropane-1-carboxylate deaminase expression and producing phytohormones and siderophores [7]. In agriculture, microorganisms can be beneficial, and they are now used to produce sustainable food crops. A number of beneficial microorganisms have been shown to play a role in nitrogen fixation, the decomposition of organic wastes, the detoxification of pesticides, the suppression of plant diseases, the enhancement of nutrient cycling, and the production of bioactive compounds that upregulate plant growth [8, 20]. A holistic improvement in agriculture is achieved when organic, inorganic, and bio-fertilizers are combined. By integrating soil productivity, sustainability, reclamation, and crop development, setting, and quality, this integrated approach leads to improvements in soil productivity, sustainability, and reclamation [9]. The production of microbial metabolites, including organic acids, can reduce soil pH, thereby facilitating the solubilization of certain nutrients, thus improving plant availability.

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 growth attributes; (2) To find out the effect of *Bacillus megaterium* and *Bacillus mucilaginosus* strains on nutrient uptake study

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 ^{0}N and longitude of 75.66 ^{0}E. This region enjoys sub-tropical, semi-arid type climate. The maximum temperature varied from 25.36^{0}C - 32.43^{0}C and the minimum temperature varied from 20.4^{0}C - 24.57^{0}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 potassium (410 kg·ha^{-1}).

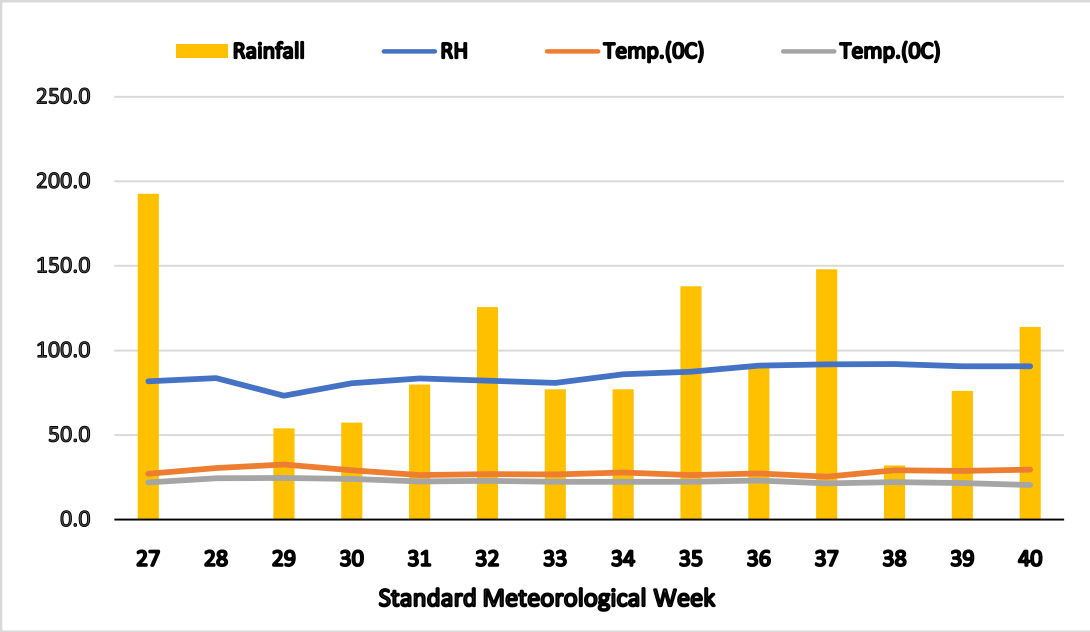

Figure 1. Rainfall and other weather parameters during crop growing period of experiment

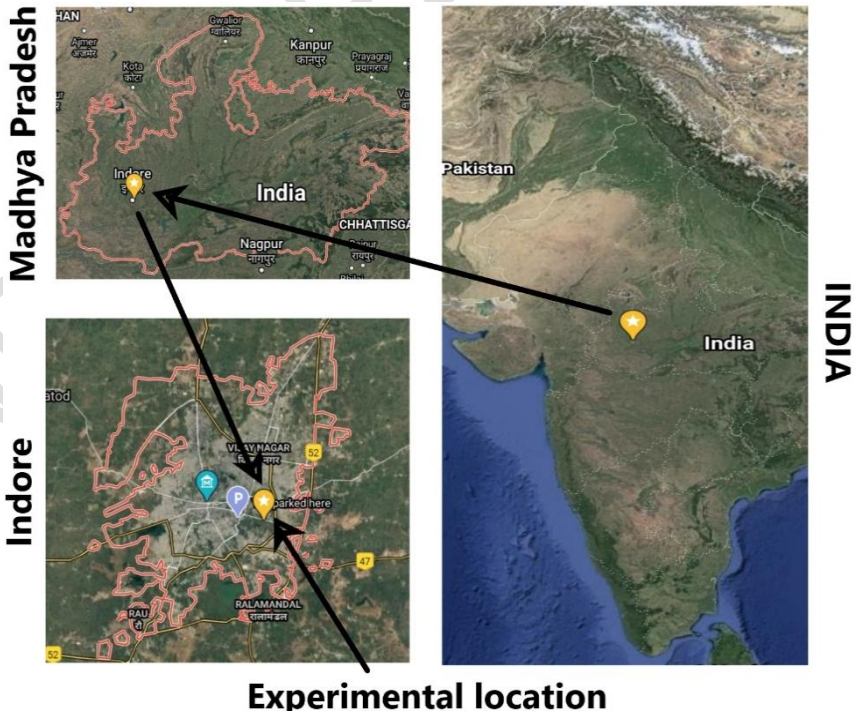

Fig. 2. Experimental location

2.1. Crop Management

Field preparation began with summer plowing with a tractor-drawn plough followed by cross harrowing to prepare the soil for sowing. After harrowing, the field was leveled by planking before sowing. Before planting the seeds, all fertilizers were applied as basal in the furrows and mixed with soil. The seeds used were healthy and of good quality to ensure perfect germination. At the time of sowing, seeds were treated with Bavistin @ 2 g kg^{-1} seeds and then inoculated with *Bacillus megaterium* and *Bacillus mucilaginosus* strains @ 3 g kg^{-1} seeds. Plants were spaced 30 cm x 5 cm apart in rows with a seed rate of 80 kg ha^{-1} in plots with dimensions of 6 m x 4.5 m. In soybean at early stages, two sprays of Triazophos 40 EC 600 ml ha^{-1} were applied at 30 and 45 days of crop growth to protect from insects and pests such as girdle beetles, stem fly caterpillars, blue beetles, etc.

2.2. Collection and preparation of soil sample

We collected soil samples randomly before sowing and after harvesting from each plot, mixed them thoroughly, dried them in air, crushed them, and sieved them through a 2 mm sieve to determine their fertility status.

2.3. Nutrient uptake estimation

The final soil samples were collected from the experimental plot with the help of Augur to the depth of 15 cm. The soil samples were composited and preserved in clean polythene bag with proper labeling for the determination of the followings.

2.3.1. Nitrogen uptake (kg ha^{-1}): The nitrogen content in plant was determined by Kjeldahl's method. The grain and straw were separated and then grinded. The powered plant sample of 0.5 g was digested in concentrated sulphuric acid using copper sulphate and potassium sulphate mixture as catalyst in digestion unit for 3 hrs and temperature was maintained at 410^{o}C. The digested material was then distilled with 40 percent sodium hydroxide and distilled was collected in boric acid containing the mixture indictor. The content was estimated by titrating the distilled against N/20 sulphuric acid. The nitrogen uptake was calculated by multiplying the dry weight with nitrogen content. In order to get total uptake of nitrogen, the uptake values for grain and straw were added together.

2.3.2. Phosphorous uptake (kg ha^{-1}): Total phosphorus uptake was determined in the extract by Vandomolybe yellow colour method. The optical density was measured with photoelectric

colorimeter at 470 nm. The content was estimated with calibration curve. The phosphorous uptake by grain and straw per hectare was calculated with the help of percent value of phosphorus and yield of grain and straw. In order to get uptake of phosphorus, the uptake value for grain and straw was added together plot wise.

Digestion of plant sample: 0.5 g powdered plant samples were weighed and digested with Diacid (HNO_3: $HClO_4$) mixture at 9:4 ratios in hot plate till clear solution was observed or till white fumes cease to come out. Then the contents were cooled and transferred to 50 ml volumetric flask and volume was made up to the mark by adding distilled water. Then the contents were filtered through Whatman No. 1 filter paper and a known quantity of aliquot were used for further analysis of phosphorus and potassium.

2.3.3. Potassium uptake (kg ha^{-1}): The potassium uptake was determined with the help of Flame photometer [12] and was estimated with calibration curve. Total uptake of potassium by grain and straw was calculated by multiplying their relative contents with yield and values were added to know the total uptake of potassium in kg ha^{-1}.

The uptake of N, P and K at harvest in seed and straw was estimated by using following formula:

$$\text{Nutrient uptake (kg/ha)} = \frac{\text{Nutrient content (\%) in seed x seed yield (kg/ha)} + \text{Nutrient content (\%) in straw x straw yield (kg/ha)}}{100}$$

2.4. Analysis of available nutrients in soil

Available nitrogen (kg ha^{-1}): An alkaline permanganate solution was used to determine nitrogen availability [10]

Available phosphorus (kg ha^{-1}): As referred to by [11], Olsen's extract (0.5 N sodium bicarbonates solution of pH (8.5) was used to estimate available P

Available potassium (kg ha^{-1}): In order to determine potassium availability, N-neutral ammonium acetate was used, as described by [12]

2.5. Statistical Analysis

According to [13], an analysis of variance will be performed using a Randomized Block Design and the results will be analyzed at a level of significance of 5%

3. Result and Discussion

A summary of the results of the present investigation is presented in Tables 1, 2 and 3

3.1. Growth parameters

The data on plant height at 60 DAS was differed significantly among the treatments. The significantly higher plant height was obtained in T_8 (31.33) which was found at par T_7 (30.55).

Similarly, plant dry weight also differed significantly and the maximum plant dry matter associated with T_8 (10.40) which was significantly maximum over other treatments but found at par with T_7. The data on CGR at 40-60 DAS revealed that significantly higher crop growth rate was found at T_8 (0.0019) which was found at par with T_7 and T_6. This might be due to higher availability and uptake of N, P and other nutrients by the crop and as these inoculations plays important role in improving availability of N and P. Seed inoculation with bio-inoculants also produce fungistatic and growth promoting substances which influenced the plant growth. Similar results were found by [14], [15] and [20].

3.2. Nutrient Uptake study (kg ha^{-1}): Seed inoculation with *Bacillus megaterium* and *Bacillus mucilaginosus* as well as spray *Bacillus megaterium* and *Bacillus mucilaginosus* significantly improved the N, P and K uptake by seed and straw.

Nitrogen uptake by seed and straw

It is clear from the data that nitrogen uptake by seed as well as straw differed significantly due to various treatments of RDF and microbial strains. Application of T_8 treatment recorded higher nitrogen uptake by grain (74.57) and straw (18.80) which was statistically at par with T_7 and these treatments were found significantly superior over rest of the treatments. This increase may be related to the supply of P necessary for Rhizobium to fix relatively more nitrogen from soil, resulting in increased plant growth and N uptake by roots and shoots. In plants, phosphorus plays an important role in physiological and developmental processes, and its beneficial effect might have accelerated the growth process that increases N uptake. [16] also found similar results.

Phosphorus uptake by seed and straw

The data pertaining to P uptake by seed and straw as affected significantly by different treatments. Application of 100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS recorded significantly higher P uptake by seed (4.11) which was found at par with T_7, T_6, T_5, T_4, T_3 and T_2. Similarly. significantly higher P uptake by straw (4.38) was recorded at T_8. The principal mechanism for mineral phosphate solubilization is the production of organic acids and acid phosphatases play a major role in the mineralization of organic phosphorous in soil. Similar results were concluded by [17] and [18].

Potassium uptake by seed and straw

The analysis results on potassium uptake by seed and straw showed significant variation due to various RDF and microbial strains treatments. Among the various treatments, the significantly highest potassium uptake in seed was recorded in T_8 (21.04) which was found at par with T_7, T_6, T_5, T_4 and T_3. However, straw uptake was recorded significantly higher T_8 (21.15) which was

found at par with T_7. This might be due to the reason that potash mobilizing bacterial strains (*Bacillus mucilaginosus*) produces plant growth promoting substances which benefits the plant growth and development by mobilizing potash and making it available to crops. It also enhances the efficiency of chemical fertilizer as well. Similar findings were reported by [19].

3.3. Total uptake study (kg/ha):

The data on total NPK uptake by crop revealed that NPK uptake differed significantly among different treatments. Among all the treatments, Application of T_8 recorded significantly higher N uptake (93.37) which was found at par with T_7. Similarly, P uptake was also recorded significantly higher in T_8 (8.49) but was found at par with all treatments except control with 75% and 100% RDF. The K uptake was significantly higher in T_8 (42.19) which was found at par T_7, T_6 and T_5.

3.4. Nutrient available in soil after harvesting of crop

The data revealed that available N did not vary significantly among the treatments. However, the maximum value of available N was found in T_8 (210.0 kg ha^{-1}). The available P showed significant variation among the treatments and the significantly higher value was obtained in T_8 (15.9 kg ha^{-1}) which was found at par with T_7 and T_5. Available K also varied significantly and the significantly higher value was recorded in T_8 (480.5 kg ha^{-1}) which was found at par with T_7.

Conclusion

According to the research analysis, both organic and biofertilizers applied to soybean plants result in significant variations in nutrient uptake features. It was determined that the most effective approach to increase crop yield and associated characteristics was to apply 100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* strains as seed treatment and to apply *Bacillus megaterium* and *Bacillus mucilaginosus* strains at 30 and 45 DAS foliarly. In conclusion, the T_8 module offers a feasible and economical way to increase output and enhance quality over the long term.

Table 1. Impact of *Bacillus megaterium* and *Bacillus mucilaginosus* strains on growth parameters of soybean:

	Treatment	**Plant height (cm)**	**Dry matter accumulated (g $plant^{-1}$)**	**CGR (g m^{-2} day^{-1})**
		60 DAS	**60 DAS**	**40-60 DAS**
T_1	Control with 75% RDF	30.33	8.58	0.0014
T_2	Control with 100% RDF	29.66	8.90	0.0015
T_3	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment	30.44	9.02	0.0015

T_4	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment	30.66	9.53	0.0017
T_5	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as foliar application at 30 and 45 DAS	30.44	8.68	0.0014
T_6	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as foliar application at 30 and 45 DAS	29.89	9.58	0.0017
T_7	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS	30.55	10.23	0.0017
T_8	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS	31.33	10.40	0.0019
	SEm (±)	0.33	0.10	0.00004
	CD at 5%	**0.99**	**0.32**	**0.00013**

Table 2. Impact of *Bacillus megaterium* and *Bacillus mucilaginosus* strains nutrient uptake of soybean:

Treatment		**Nutrient uptake (kg ha^{-1})**					
		Grain			**Straw**		
		N	**P**	**K**	**N**	**P**	**K**
T_1	Control with 75% RDF	43.31	2.22	12.90	11.92	2.52	13.30
T_2	Control with 100% RDF	48.42	2.71	14.04	14.56	3.28	16.23
T_3	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment	45.41	2.93	15.61	15.16	3.48	16.74
T_4	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment	54.33	3.00	16.40	15.88	3.42	17.01
T_5	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as foliar application at 30 and 45 DAS	57.27	3.02	16.35	15.46	3.57	17.62
T_6	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as foliar application at 30 and 45 DAS	61.06	3.37	17.78	16.90	3.60	18.80
T_7	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS	69.20	4.01	19.48	17.82	3.54	20.50
T_8	100% RDF with *Bacillus megaterium* and *Bacillus*	74.57	4.11	21.04	18.80	4.38	21.15

	mucilaginosus as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS						
SEm (±)		3.82	0.58	2.22	0.60	0.15	0.58
CD at 5%		**11.58**	**1.74**	**6.74**	**1.83**	**0.44**	**1.77**

Table 3. Impact of *Bacillus megaterium* and *Bacillus mucilaginosus* strains on total uptake of soybean:

Treatment		**Total uptake crop (kg ha^{-1})**		
		N	**P**	**K**
T_1	Control with 75% RDF	55.23	4.74	26.20
T_2	Control with 100% RDF	62.98	5.99	30.27
T_3	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment	60.57	6.41	32.35
T_4	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment	70.21	6.42	33.41
T_5	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as foliar application at 30 and 45 DAS	72.73	6.59	33.97
T_6	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as foliar application at 30 and 45 DAS	77.96	6.97	36.58
T_7	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS	87.02	7.55	39.98
T_8	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS	93.37	8.49	42.19
	SEm (±)	4.42	0.73	2.80
	CD at 5%	**13.41**	**2.18**	**8.51**

Table 4. Impact of *Bacillus megaterium* and *Bacillus mucilaginosus* strains on nutrient availability after harvest of soybean:

Treatment		**Nutrient after crop harvest (kg ha^{-1})**		
		N	**P**	**K**
T_1	Control with 75% RDF	202.5	12.1	416.6

T_2	Control with 100% RDF	205.6	12.2	421.6
T_3	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment	204.6	12.3	430.0
T_4	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment	205.0	12.3	441.6
T_5	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as foliar application at 30 and 45 DAS	205.2	12.4	453.3
T_6	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as foliar application at 30 and 45 DAS	205.6	12.3	465.0
T_7	75% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS	205.8	12.6	476.6
T_8	100% RDF with *Bacillus megaterium* and *Bacillus mucilaginosus* as seed treatment + foliar application of *Bacillus megaterium* and *Bacillus mucilaginosus* at 30 and 45 DAS	206.0	12.7	485.0
	SEm (±)	2.9	0.1	5.3
	CD at 5%	**NS**	**0.4**	**16.2**

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