International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; SP-9(12): 936-942
www.biochemjournal.com
Received: 01-09-2025
Accepted: 04-10-2025

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Isolation and characterization of nitrogen-fixing, phosphate and potash solubilizing bacteria and their inoculation effect on sorghum

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i12Sl.6634

Abstract
The current investigation entitled "Isolation and Characterization of Nitrogen Fixing Phosphate and Potash Solubilizing Bacteria and Their Inoculation Effect on Sorghum" was carried out as field experiment during the Rabi 2024 at farm of the Department of Plant Pathology and Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri. The rhizospheric soil samples of orchards of Mango, Tamrind, Fig, Coconut and Sapota were collected for isolation of nitrogen fixer, PSB and KSB. Out of these collected samples seven isolates of nitrogen fixer designated as MRN-1, MRN-2, TRN-1, FRN-1, CRN-1, CRN-2 and SRN-1; four isolates for P -solubilizing such as MRP-1, TRP-1, TRP-2 and SRP-1 and 3 isolates for K solubilizing bacteria named as TRK-1, SRK-1and SRK-2 were isolated by serial dilution and pour plate method. Ashby's, Pikovaskya's and Aleksandrow's media were used for isolation of nitrogen fixing, Phosphate solubilizing bacteria and Potash solubilizing bacteria, respectively. These isolates undertook laboratory testing to determine their efficacy. On the basis of test results isolates were identified as *Azotobacter* spp., *Bacillus* spp. and *Pseudomonas* spp. and used for further field experimental studies.
Among all treatments, *Azotobacter* spp. (Nitrogen fixer), *Bacillus* spp. (PSB) and *Pseudomonas* spp. (KSB) along with 100% RDF found to be highly significant which increased germination percentage (94.03), plant height (236.70 cm at flowering and 241.33 cm at harvesting), number of leaves (14.33), root length (13.33 cm), Panicle length and girth (23.17 cm and 16.67 cm), 1000 grain weight (34.57 g), grain and fodder yield (27.70 and 55.40 q ha^{-1}). While among all treatments there was non-significant differences were observed as regards number of days required for 50% flowering of sorghum. However, the application of 75% RDF + *Azotobacter*, PSB and KSB was found to be statistically at par with 100% RDF + *Azotobacter*, PSB and KSB. From present investigation on the basis of it was concluded that, the seed treatment of *Azotobacter*, PSB and KSB with 75% RDF found to be effective. As this treatment saves the 25% of dose of nitrogen, phosphate and potash fertilizer to obtained maximum growth and yield of Sorghum.

Keywords: Sorghum, *Azotobacter*, *Bacillus*, *Pseudomonas*, RDF

Introduction
Sorghum, (*Sorghum bicolor*), cereal grain plant of the grass family (Poaceae) and its edible starchy seeds. The plant likely originated in Africa, where it is a major food crop, and has numerous varieties, including grain Sorghums, used for food; grass Sorghums, grown for hay and fodder; and broomcorn, used in making brooms and brushes. In India Sorghum is known as Jowar, Cholam, or Jonna, in West Africa as Guinea corn, and in China as Kaoliang. Sorghum is especially valued in hot and arid regions for its resistance to drought and heat. Globally, Sorghum produced about 52.8 million tonnes during 2023-24. United States stands top with 8.07 million tons (14%) followed by Nigeria with 6.7 million tons (11%), Brazil with 4.76 million tons (8%) and India with 4.4 million tons (8%). India ranks fourth in total Sorghum production with 4.4 million tonnes grown in an area of 3.97 million hectares in 2023-24, where majority of Sorghum was produced during rabi season. The kharif Sorghum (36.6%) was grown predominantly in Rajasthan (43.3%), Uttar Pradesh (15.6%), Haryana (10.1%) and Madhya Pradesh (9.8%), while rabi Sorghum grown in Maharashtra (63.5%), Karnataka (22%), Tamil Nadu (7.9%) and Andhra Pradesh (3.2%). According to the 3rd advance estimates for 2023-24 by the Government of India, the Sorghum crop was estimated at 47.42 lakh tonnes, compared to 38.14 lakh tonnes in 2022-23 (Reddy, 2024) [1].

Sorghum (*Sorghum bicolor* L.) is one of the most valuable multipurpose crops used as human food in developing countries. More than 750 million people worldwide depend directly on food from this crop. With its high protein content, sorghum grains also make the prime component in the concentrated diet of poultry, which reaches 12%. It serves the form of green fodder and silage as animal feed (Wilson, 2011) [2].

Sorghum is commonly known as 'Great millet' and popularly known as 'Jowar' in India. It is used the world over as food, feed and staple food of the poor in many countries. Sorghum is the fifth most important annual, cereal crop in the world after wheat, rice, maize and barley. The cereal grain is said to have originated around present-day Ethiopia as a wild grass. India is the leading producer after the USA and Nigeria (Doifode, 2021) [3]. Nutritionally, sorghum is rich in carbohydrates, protein, fibre, vitamins, and minerals, and is gluten-free, making it a valuable food source for many. Additionally, it contains antioxidants, adding to its health benefits (Hariprasanna and Patil, 2015) [4].

According to APEDA (2023) [5], Maharashtra leads the country in both the area under cultivation and total production of sorghum, underscoring its dominant role in sorghum farming. Karnataka ranks second in terms of the area devoted to sorghum, though its production remains behind Maharashtra.

Sorghum is believed to have originated in tropical Africa. From Africa, sorghum spread to other parts of the world through trade and migration. Sorghum is unique in the sense that it is naturally drought, heat, and insect resistant. It thrives in arid areas, which makes sorghum increasingly important globally. The International Water Management Institute (IWMI) warns that, by the year 2025, 25% of the world's population will experience severe water scarcity, and drought tolerant crops such as sorghum will be important in meeting the food demands for those people by (Kumari *et al*., 2016).

The state stands first in terms of area and production of Sorghum (Jowar). Maharashtra produced 381 thousand tonnes of Jowar from an area of 379 thousand hectares during the period 2021-22 as published in the Economic Survey of Maharashtra, 2021-22, Directorate of Economic and Statistics, Planning Department, Government of Maharashtra.

Application of bio-fertilizers is known as a sustainable solution for reducing and eliminating the chemical inputs in sustainable agricultural systems (Rezaei, 2020; Hafez, 2021) [10, 11]. A large number of soil bacterial species that live in the rhizosphere of plants are able to improve plant growth by different mechanisms. These bacteria stimulate plant growth by producing various compounds, facilitating the absorption of elements, stabilizing atmospheric nitrogen, solubilizing minerals such as phosphate, and producing plant hormones such as auxins and gibberellins, which increase plant growth and productivity (Daniel, 2022) [12].

However, in the present energy crises and increased cost of fertilizers, the current emphasis is on the integrated use of different sources of plant nutrients such as organic manure and biofertilizers in combination with chemical fertilizers. Organic manure and biofertilizers being less expensive, easily available and eco-friendly, expected to improve soil fertility crop yield and quality. Therefore, the present investigation was conducted to find out the effectiveness of biofertilizers with organic manure and various levels of chemical fertilizers on growth yield and quality of sorghum tested. The biofertilizers i.e. *Azotobacter*, phosphate-solubilizing bacteria (PSB) and potassium-solubilizing bacteria were used in combination. These bacteria were isolated from rhizosphere soil samples of sorghum, collected from AICRP on Sorghum Project, MPKV, Rahuri (M.S.). On the basis of their effectiveness under laboratory conditions single selected isolate of *Azotobacter*, PSB and KSB further used for field studies, respectively.

Materials and Methods

A field experiment was carried out during the rabi seasons of 2024 at Department of Plant Pathology and Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri (M.S.) to assess the effect of inoculation with *Azotobacter*, phosphate-solubilizing bacteria (PSB) and potash-solubilizing bacteria (KSB) on the growth and yield of Sorghum. The rhizospheric soil samples were collected from five orchards *viz*. Mango (*Mangifera indica*), Tamrind (*Tamarindus indica*), Fig (*Ficus carica*), Coconut (*Cocos nucifera*) and Sapota (*Manilkara zapota*). The bacterial isolation was carried out by serial dilution and pour plate method using Ashby's, Pikovskaya's and Aleksandrov's medium for Nitrogen fixer, P-solubilizers and K-solubilizes, respectively**.** The bacterial colonies were obtained 3 to 4 days of incubation at 28±2 ^{0}C temperature. Picked up the individual colony and streaked on the respective fresh medium to obtained pure cultures. The pure cultures of bacterial isolates thus obtained were presented in Table 1. The seven bacterial isolates of N-fixers (named as MRN -1, MRN-2, TRN-1, FRN-1, CRN-1, CRN-2 and SRN-1), four of P- solubilizers exhibiting halo zone, thus showing the capability of P-solubilisation (named as MRP -1, TRP-1, TRP-2 and SRP-1) and three of K-solubilizers (named as TRK-1, SRK-1 and SRK-2) were obtained. The selection of efficient N-fixer isolate was done on the basis of Jensen's medium with bromothymol blue (BMB), a colour change in the medium from its initial greenish-blue to yellow around the colonies indicates acid production and a positive result for nitrogen fixation. Where, as for P-solubilizer bacterial isolates exhibiting halo zone, thus showing the capability of P solubilisation were selected as phosphate solubilizers. On Aleksandrov's medium clear halos, indicating K solubilization were selected. Among these isolates, on the basis of morphological, physiological, biochemical and efficiency tests only one most efficient isolate from each category was selected for further studies. The most promising bacterial isolates MRN-2 as N-fixer, TRP-1 as P-solubilizer and SRK-2 as K-solubilizers were selected for further studies. The selected three isolates MRN-2 (*Azotobacter*), TRP-1 (*Bacillus* spp.), and SRK-2 (*Pseudomonas* spp.) were used as an inoculant in the plant growth promotion experiment. In doing so, the suspension of MRN-2, TRP-1, and SRK-2 after being separately cultured in a respective broth medium and were mixed together with a ratio of 1:1:1. The total number of effective bacteria was about $3x10^8$ cfu g^{-1} in this complex inoculant.

The plant growth promotion activity was studied during kharif 2024 which comprised of seven treatments which were replicated three times in a Randomised Block Design. Seven treatments were administered (Table 2): T_1: Untreated control, T_2: 100% RDF, T_3: 100% RDF + ST and SA of

Azotobacter + PSB + KSB, T_4: 75% RDF + ST and SA of *Azotobacter* + PSB + KSB, T_5: 50% RDF + ST and SA of *Azotobacter* + PSB + KSB, T_6: 25% RDF + ST and SA of *Azotobacter* + PSB + KSB, T_7: ST and SA of *Azotobacter* + PSB + KSB alone. The application of complex inoculant was done at the time of sowing through seed treatment (ST) @ 10ml $kg^{-1}$of seed and soil application (SA) @ 10ml $kg^{-1}$of FYM.

Results and Discussions

Isolation of N-fixer, P-Solubilizing Bacteria, K-Solubilizing Bacteria from Rhizospheric Soil Samples Orchards and selection of efficient isolate

The soil samples were collected from orchards as shown in Table 1 and analysed by serial dilution and pour plate method. Total seven isolates of N-fixers, four of p-solubilizers and three of k-solubilizers were obtained. As shown in Table 2 clearly indicated that the maximum nitrogen fixing ability of 12.78 mg of nitrogen/g of sucrose consumed was recorded by isolate MRN-2. Whereas, the maximum clear halo zone of phosphorus and potassium solubilization were shown by bacterial isolate TRP-1 (16 mm) and SRK-2 (15 mm), respectively. On the basis of morphological, physiological, biochemical tests these bacterial isolate MRN-2 was identified as *Azotobacter* spp., isolate TRP-1 as *Bacillus* spp. and SRK-2 as *Pseudomonas* spp. These three bacterial isolates *viz*. MRN-2, TRP-1 and SRK-2 were used for field studies in the form of complex inoculant.

Table 1: Isolation of nitrogen fixing, P-solubilizing and K-solubilizing bacteria from rhizospheric soils of orchard plantation

Sr. No.	Source of rhizospheric soil sample	Nitrogen fixer	P-solubilizer	K-solubilizer
1.	Mango (*Mangifera indica*)	MRN -1, MRN-2 (2)*	MRP -1 (1)	-
2.	Tamarind (*Tamarindus indica*)	TRN-1 (1)	TRP-1, TRP-2 (2)	TRK-1 (1)
3.	Fig (*Ficus carica*)	FRN-(1)	-	-
4.	Coconut (*Cocos nucifera*)	CRN-1, CRN-2(2)	-	-
5.	Sapota (*Manilkara zapota*)	SRN-1 (1)	SRP-1 (1)	SRK-1, SRK-2 (2)

*No. of isolate(s) obtained

Table 2: Selection of efficient nitrogen fixing, P-solubilizing and K-solubilizing bacteria

Sr. No.	N-fixer isolate and their Nitrogen fixing ability (mg of Nitrogen/g of sucrose consumed)		P-solubilizer isolate and their phosphorus solubilizing ability indicated by Clear zone diameter (mm)		K-solubilizer and their potash solubilizing ability indicated by Clear zone diameter (mm)	
1.	MRN -1	8.21	MRP -1	10	TRK-1	12
2.	MRN-2	12.78	TRP-1	16	SRK-1	13
3.	TRN-1	7.12	TRP-2	13	SRK-2	15
4.	FRN-1	6.67	SRP-1	13	--	--
5.	CRN-1	6.00	--	--	--	--
6.	CRN-2	5.13	--	--	--	--
7.	SRN-1	6.19	--	--	--	--

Effect of complex inoculant on germination percentage, plant height at flowering and harvesting stage

The results in respect of seed germination and plant height at flowering and harvesting shown in Table 3, among all the treatment, the treatment resulted that T_3 (100% RDF + *Azotobacter* + PSB + KSB) recorded maximum germination i.e. 94.03% and it was followed by T_4 (75% RDF + *Azotobacter* + PSB + KSB) which recorded 91.90% and found statistically on par with each other. Untreated control (T_1) shown minimum germination of 80.73%. It was found that untreated control has noticeably least germination percentage. T_3 (100% RDF + *Azotobacter* + PSB + KSB) recorded maximum plant height at flowering i.e. 236.70 cm and it was followed by T_4 (75% RDF + *Azotobacter* + PSB + KSB) which recorded 233.50 cm plant height which was statistically at par with treatment T_3. Untreated control (T_1) shown minimum plant height of 216.13 cm.

The present results are in agreement with earlier researcher, Jadhav and Patil (1985) [13] observed that *Azotobacter* as biofertilizer performed better than inorganic fertilizers in relation to seed germination and overall growth parameters of paddy plant. Then after Sajindranath *et al*. (2002) [14] evaluated the growth regulators and biofertilizers in improving seed germination and seedling vigour in okra cv. Parbhani kranti. *Azotobacter* + PSB proved to be better in increasing seed germination and vigour index when compared with separate application. Pathak *et al*. (2013) [15] conducted the experiment on the impact of bio-incoulant PSB, *A. chrooccum* and PGPR on seed germination and plant growth of guava and found the significant increase in its percentage.

Reddy and Lakhdive (1982) [16] found that when the hybrid sorghum variety CSH-5 seeds were inoculated with *Azotobacter*, there was a significant increase in plant height and the number of functional leaves per plant. Additionally, Radhakrishnan and Mallikarjunaiah (1983) [17] reported that, on the 30th day, inoculating with *Azotobacter vinelandii* and *Beijerinckia mobilis* led to an increase in both height and leaf number compared to the control in vegetable crops.

Table 3: Effect of complex inoculants of *Azotobacter*, PSB and KSB on germination percentage, plant height (cm) at flowering and at harvest

Tr. No.	Treatment Details	Germination percentage	Plant height (cm) at flowering stage	Plant height (cm) at harvesting stage
T_1	Untreated control	80.73	216.13	221.47
T_2	100% RDF	89.93	231.13	235.30
T_3	100% RDF + *Azotobacter* + PSB + KSB	94.03	236.70	241.33
T_4	75% RDF + *Azotobacter* + PSB + KSB	91.90	233.50	237.63
T_5	50% RDF + *Azotobacter* + PSB + KSB	87.80	227.80	231.77
T_6	25% RDF + *Azotobacter* + PSB + KSB	85.93	224.40	228.10
T_7	*Azotobacter* + PSB + KSB alone	82.77	220.57	225.33
	S.Em. ±	1.22	1.20	1.38
	CD at 5%	3.76	3.71	4.28

Effect of complex inoculant on number of leaves, number of days required for 50% flowering and root length (cm)

The data presented in Table 4 revealed that the T_3 treatment (100% RDF + *Azotobacter* + PSB + KSB) recorded highest number of leaves i.e. 14.33 per plant. It was significantly superior over other treatments. The treatment T_4 (75% RDF *Azotobacter* + PSB + KSB) which recorded 13.30 leaves per plant and which is statistically at par with treatment T_3. Untreated control T_1 showed minimum number of leaves i.e. 9.27 per plant. The data perusal to mean number of days required to attain 50% flowering as influenced by different treatments were found to be statistically non-significant.

As regards the root length treatment T_3 (100% RDF + *Azotobacter* + PSB + KSB) recorded maximum root length i.e. 13.33 cm. It was followed by treatment T_4 (75% RDF + *Azotobacter* + PSB + KSB) which recorded 12.27 cm root length per plant which is statistically at par with treatment T_3. Untreated control T_1 showed minimum root length i.e. 6.27 cm per plant. As biofertilizer and inorganic fertilizers were added in treatment T_3 which ultimately increase the number of leaves, were observed, whereas less number of leaves where shown by absolute control due to non-addition of inputs. The bacteria are considered to be rhizobacteria (PGPR) that promote plant growth as they produce compounds that help plants growth and development. Similar findings were demonstrated by Koley and Pal (2011) [18] in tuberose and Prajwal and Godse *et al*. (2006) [19] in gladiolus. Tilak, K. V. B. R. *et al*. (2005) [20] highlighted the synergistic effects of *Azotobacter*, PSB, and KSB in promoting overall root development in cereals including sorghum. Later Bharath, S., & Reddy, Y. N. (2016) [21] reported that increased root length in sorghum with combined application of *Azotobacter* and PSB due to improved nutrient uptake. Also Sahu, P. K., & Brahmaprakash, G. P. (2016) [22] Showed that KSB inoculation significantly increased root and shoot length of sorghum compared to uninoculated control.

Table 4: Effect of complex inoculants of *Azotobacter*, PSB and KSB on number of leaves, number of days required for 50% flowering and root length (cm)

Tr. No.	Treatment Details	No. of leaves at 50% flowering stage	No. of days require for 50% flowering	Root length (cm)
T_1	Untreated control	9.27	76.83	6.27
T_2	100% RDF	12.17	79.07	11.33
T_3	100% RDF + *Azotobacter* + PSB + KSB	14.33	80.17	13.33
T_4	75% RDF + *Azotobacter* + PSB + KSB	13.30	79.70	12.27
T_5	50% RDF + *Azotobacter* + PSB + KSB	11.87	78.80	9.30
T_6	25% RDF + *Azotobacter* + PSB + KSB	11.03	78.11	8.03
T_7	*Azotobacter* + PSB + KSB alone	10.47	77.03	7.10
	S.Em. ±	0.45	0.56	0.45
	CD at 5%	1.41	NS	1.41

Effect of complex inoculant of *Azotobacter, PSB* and KSB on panicle length and girth (cm) and 1000 grain weight (g)

The data in respect of panicle length, its girth and 1000 grain wt. of sorghum is presented in Table 5 revealed that the statistically significant differences were observed for panicle length, panicle girth (cm) and 1000 grain weight (g) observed among all the treatment. From the data it was observed that the panicle length and girth was highest in treatment T_3 (100%RDF + *Azotobacter* + PSB + KSB) i.e. 23.17 cm and 16.67 cm., respectively and significantly at par with treatment T_4 **(**75% RDF + *Azotobacter* + PSB + KSB**)** with panicle length and girth are 21.23 cm and 14.83 cm. The uncontrol treatment T_1 shown minimum panicle length and girth of 14.07 cm and 6.20 cm, respectively. As regards the 1000 grain weight (g) treatment T_3 (100% RDF + *Azotobacter* + PSB + KSB) shown highest 1000 grain weight (g) i.e.34.57 g. The next best treatments where T_4 (75% RDF + *Azotobacter* + PSB + KSB) which recorded 32.47 g. This treatment where statistically at par with treatment T_3. Theuntreated control recorded least 25.13 g grain weight among all the treatments

Arangarasan *et al*. (1998) [23] reported that the inoculated treatments with two phosphate solubilizing bacterial cultures increased in shoot and root length, 1000 grain weight and grain yield were recorded over uninoculated control in rice. However, performance of diazotrophic bacteria *Herbaspirillum* treated plots was marginally better than that with *Azospirillum* and the differences were not statistically significant. Arora *et al*. (2001) [24] had shown that *Azotobacter* inoculation increases sorghum's nitrogen content, which results in improved photosynthesis and better nutrient utilization. This leads to an increase in overall plant growth, including larger panicles (both in length and girth).

Similarly, Prakash *et al*. (2018) [25], reported inoculation with a combination of *Azotobacter***,** PSB**,** and KSB has been shown to have a synergistic effect on sorghum growth. The nitrogen-fixing properties of *Azotobacter*, coupled with the phosphorus and potassium mobilizing capabilities of PSB and KSB, result in overall better plant nutrition and growth. This, in turn, leads to significant improvements in panicle size (length and girth), resulting in increased grain yield.

Table 5: Effect of inoculants of *Azotobacter*, PSB and KSB on 1000 grain weight (g), Panicle length and girth (cm)

Tr. No.	Treatment Details	Panicle length (cm)	Panicle girth (cm)	Grain weight ((g)
T_1	Untreated control	14.07	6.20	25.13
T_2	100% RDF	19.60	11.90	30.47
T_3	100% RDF + *Azotobacter* + PSB + KSB	23.17	16.67	34.57
T_4	75% RDF + *Azotobacter* + PSB + KSB	21.23	14.83	32.47
T_5	50% RDF + *Azotobacter* + PSB + KSB	17.37	10.23	29.27
T_6	25% RDF + *Azotobacter* + PSB + KSB	16.00	9.03	28.40
T_7	*Azotobacter* + PSB + KSB alone	15.00	7.70	26.97
	S.Em. ±	0.72	0.67	0.95
	CD at 5%	2.24	2.06	2.94

Effect of complex inoculants of *Azotobacter*, ***PSB*** **and KSB on grain yield (q/ha) and fodder yield(q/ha)**

The yield is the ultimate goal of all the crops and the results presented in Table 6 revealed that the highest grain and fodder yield of 27.70 q/ha and 55.40 q/ha. respectively recorded by the treatment T_3 (100% RDF + *Azotobacter* + PSB + KSB). However, treatment T_4 (75% RDF + *Azotobacter* + PSB + KSB) which was statistically at par with treatment T_3 which recorded grain and fodder yield likewise 25.50 and 53.57 q/ha, respectively. The untreated control T_1 shown lowest grain and fodder yield i.e. 61.05 and 40.87 q/ha.

The current findings align with previous studies. Narayan *et al*. (2007) [26] reported that applying biofertilizers under specific temperature conditions improved the growth, yield, and quality of tomatoes. Similarly, Hernando *et al*. (2011) [27] found that biofertilizer treatments enhanced the weight, diameter, and overall yield of lettuce and cabbage. In addition, Scherer (2006) [28] noted a positive impact of foliar biofertilizer application on the growth and yield of common beans. Furthermore, Dhankhar *et al*. (2013) [29] demonstrated that the use of phosphate-solubilizing bacteria (PSB) as inoculants increased phosphorus availability to plants, thereby boosting crop yields. Singh *et al*. (2015) [30] found that biofertilizer inoculation improved nutrient uptake efficiency, which directly influenced the yield parameters of sorghum. Chandravanshi *et al*. (2017) [31] reported that the combined application of *Azotobacter*, PSB, and KSB significantly enhanced the grain and fodder yield of sorghum compared to control treatment.

Table 6: Effect of inoculants of *Azotobacter*, *PSB* and KSB on grain and fodder yield (q/ha)

Tr. No.	Treatment Details	Grain yield (q/ha)	Fodder yield (q/ha)
T_1	Untreated control	14.80	40.87
T_2	100% RDF	23.63	50.57
T_3	100% RDF + *Azotobacter* + PSB + KSB	27.70	55.40
T_4	75% RDF + *Azotobacter* + PSB + KSB	25.50	53.57
T_5	50% RDF + *Azotobacter* + PSB + KSB	21.07	48.20
T_6	25% RDF + *Azotobacter* + PSB + KSB	19.70	45.17
T_7	*Azotobacter* + PSB + KSB alone	17.53	43.77
	S.Em. ±	0.81	0.79
	CD at 5%	2.52	2.44

Effect of complex inoculants of *Azotobacter*, PSB and KSB on Microbial count at harvesting stage

The result shown in Table 7 revealed that the *Azotobacter* count ranged from 11.87 to 23.93 $CFUx10^6$, PSB from 13.13 to 33.83 $CFUx10^6$ and KSB from 15.97 to 35.47 $CFUx10^6$. In the treatment T_3 (100% RDF + *Azotobacter* + PSB + KSB) recorded highest microbial count i.e. 23.93$CFUx10^6$, 33.83 $CFUx10^6$ and 35.47 $CFUx10^6$ of *Azotobacter*, PSB and KSB, respectively. It was followed by treatment T_4 (75% RDF + *Azotobacter* + PSB + KSB) which recorded 21.20 $CFUx10^6$, 30.00 $CFUx10^{-6}$ and 32.87 $CFUx10^{-6}$ of *Azotobacter*, PSB and KSB, respectively. The untreated control recorded 11.87, 13.13 and 15.97 $CFU\times10^{-6}$ of *Azotobacter*, PSB and KSB, respectively which was least as compare to rest of the treatments.

Similar findings have been reported in previous studies. Sornalatha *et al*. (2016) [32] investigated the distribution of microbial communities, including diazotrophic *Azotobacter*, in five different rhizosphere soils. Their results showed that the total microbial count was highest for general bacterial populations, with *Azotobacter* populations being relatively lower across the various rhizosphere samples.

Comparable results have been documented by other researchers. Toukhy and Abdel (2000) [33] observed that the combined application of inorganic nitrogen and biofertilizers significantly enhanced microbial activity in the barley rhizosphere, along with an increase in the total microbial population. Similarly, Sundara *et al*. (2002) [34] reported that the use of phosphate solubilizing bacteria (PSB) led to a rise in the PSB population within the rhizosphere and improved the availability of phosphorus in the soil for plant uptake.

Table 7: Effect of inoculants of *Azotobacter*, *PSB* and KSB on Microbial count at harvesting stage

Tr. No.	Treatment Details	*Azotobacter* CFU X 10^6	PSB CFU X 10^6	KSB CFU X 10^6
T_1	Untreated control	11.87	13.13	15.97
T_2	100% RDF	19.43	27.13	30.07
T_3	100% RDF + *Azotobacter* + PSB + KSB	23.93	33.83	35.47
T_4	75% RDF + *Azotobacter* + PSB + KSB	21.20	30.00	32.87
T_5	50% RDF + *Azotobacter* + PSB + KSB	17.77	24.00	27.10
T_6	25% RDF + *Azotobacter* + PSB + KSB	15.93	20.53	22.97
T_7	*Azotobacter* + PSB + KSB alone	13.33	16.70	19.17
	S.Em. ±	1.03	1.53	1.09
	CD at 5%	3.20	4.72	3.37

Conclusion

Among all the treatments evaluated, the application of *Azotobacter* spp. (nitrogen-fixing bacteria), *Bacillus* spp. (phosphate-solubilizing bacteria), and *Pseudomonas* spp. (potassium-solubilizing bacteria) as an inoculant along with 100% of the recommended dose of fertilizers (RDF) proved to be the most effective. This treatment significantly enhanced germination percentage, plant height, number of leaves, panicle length and girth, 1000 grain weight, root length and grain and fodder yield of Sorghum. However, number of days required for 50% flowering in Sorghum showed no significant variation across treatments. Notably, the treatment involving 75% RDF combined with the inoculation of *Azotobacter* + PSB + KSB was found to be statistically at par for all the characters studied. This treatment found to be effective as it saved 25% of inorganic fertilizers without hampering the morphological characters as well as yield of sorghum. Based on the present study, it can be concluded that seed treatment with soil application of Azotobacter, PSB, and KSB along with 75% of the recommended dose of fertilizers, is an efficient and sustainable option. This approach reduces chemical fertilizer usage by 25% without compromising the yield of Sorghum.

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