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Sulphur oxidizing bacteria to boost up mustard (*Brassica juncea* l.) growth and biomass yield

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ABSTRACT

The study aimed to find out the most efficient sulphur oxidizing bacteria (SOB), isolation was attempted from soil, water and biogas slurry samples of AAU, Anand and main rice research station, Nawagam, wherein total 25 isolates were obtained on thiosulphate agar. Further, three most efficient isolates; SOB1, SOB2 and SOB3 were selected on the basis of lowering pH from 8 to 5 in thiosulphate broth and sulphate production (1.13 to 1.76 mM) ability. They were culturally and morphologically characterized. The 3 strains; SOB1, SOB2 and SOB3 were identified as Bacillus tropicus AAUSOB1 (ACCN ON127698), Beijerinckia fluminensis AAUSOB2 (ACCN ON127700) and Klebsiella variicola AAUSOB3 (ACCN ON127847), respectively. All the three cultures were mutually compatible with each other in cross streak assay and a consortium was prepared combining each culture in equal proportion. Inoculation of SOB alone and/or their consortium @ 5 ml kg^{-1} seed, in presence of either elemental or bentonite sulphur @ 20 kg ha^{-1}, increased plant growth and biomass yield of mustard as compared to their respective controls.

Keywords: Isolation, molecular characterization, sulphur oxidizing bacteria, mustard

INTRODUCTION

The transformations of inorganic S compounds in nature have been studied in the S cycle wherein, microorganisms play the most important role in S transformations. Heterotrophic and autotrophic modes of nutrition are involved in S cycle. Bacteria having the capacity to oxidize the reduced forms of inorganic S compounds into SO_4^{-2} as a final product are generally known as Sulphur oxidizing bacteria / microorganisms (SOB/ SOM) which play a vital role in the removal of poisonous hydrogen sulphide (H_2S) from the atmosphere (Das *et al*. 1996) which is then included into S containing amino acids and enzymes (Friedrich *et al*. 2001). The SOB generally belongs to various genus like *Thiobacillus*, *Beggiatoa*, *Thiothrix*, *Thiomicrospira*, *Desulphuromonas, Chromatium etc.*but the oxidation process is unlimited to the true S bacteria as it is found in the bacteria having heterotrophic mode in nature (Das *et al*. 1996).

S is one of the essential plant nutrients for the synthesis of proteins, oils, vitamins and flavoured compounds. Different doses of S have a significant impact on the most of growth parameters and yield attributes of mustard (Rakesh & Banik, 2016). The amino acids methionine, cysteine and cystine contain 21, 26 and 27% S, respectively. Almost 90% of S is present in methionine, cysteine and cystine amino acids only (Tandon and Messick, 2002). The agricultural activities in the African and middle eastern countries are carried out majorly, along the river banks and streams. Most of the farmers in these countries are marginal and produce grains and other products for their own consumption and sell. However, farmers in a few Middle Eastern countries are gradually becoming aware of the benefits of S fertilizers with the help of non-profit and non-governmental organizations. The S status of Indian soils is going down. About 70% of soil samples analyzed by the ICAR system TSI-FAI-IFA project and other programs have been found to be either deficient or marginal in plant available S. (Singh & Kumar, 2013) A soil is considered deficient in S, if its tests the value less than 10 mg S kg^{-1} soil, extractable with 0.15% $CaCl_2$. S is a crucial element for rapeseed-mustard in determining its seed yield, oil content, quality, and resistance to various biotic and abiotic stresses. S increases the seed yield of mustard by 12 to 48% under irrigated and 17 to 124%

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under rainfed conditions (Rathore *et al*. 2015). To appraise the role of SOB, the research work was planned with following objectives like isolation, characterization, screening and selection of efficient SOB isolates. Evaluation of efficient SOB isolates on growth and biomass yield of mustard (*Brassica juncea l.*) under pot condition.

MATERIALS AND METHIODS

Representative soil / muddy water samples were collected from various fields of AAU, Anand and main rice research station, Nawagam, and biogas digestate / slurry samples from college of food processing technology and bio energy, Anand agricultural university, Gujarat. Sulphur oxidizing bacteria (SOB) was isolated by taking 10 g soil sample/ 10 ml water or slurry mixed with 90 ml sterilized distilled water and considered as 10^{-1} dilution and shaken at 200 rpm for 30 min at 28°C. One ml of this suspension was transferred to 9 ml of sterile distilled water to get 10^{-2} dilution and similarly stepwise final dilution of 10^{-3} to 10^{-4} was attained. About 100 µl of final dilution of 10^{-1} to 10^{-4} was spread with a sterile glass spreader on different media such as thiosulphate agar, starkey agar, NCL agar supplemented with cyclohexamide 50 ppm to avoid fungal contamination. All culture plates were incubated at 28 ± 2°C for 5 days. After single colony observed on agar plates picked up aseptically, transferred to same growth medium, pure colony was sub-cultured on thiosulphate agar slants and preserved in nutrient agar at 4°C for further use. All the pure bacterial cultures grown on nutrient agar were evaluated for various cultural (colony size, margin, texture, opacity and pigmentation) and morphological (shape, arrangement and gram reaction) characterization as procedure give in Bergey's manual of determinative bacteriology (Holt *et al*. 1994). For selection of efficient SOB isolates, screening was done through pH reduction test and sulphate production assay. In pH reduction test, bacterial isolates were inoculated into thiosulphate broth with an initial pH of 8.0. The pH of the broth was measured using a pH meter after 3 and 6 days of incubation. The bacterial isolates that reduced BCP dye colour on thiosulphate agar plates also passed the broth test and reduced dye colour was due to oxidation of thiosulphate to sulphate that showed decrease in pH from 8.0 to around 4.0 and were selected for further studies. All the SOB isolates were then screened for the production of the sulphate ion (SO_4^{-2}). During growth on thiosulphate broth, the amount of SO_4^{-2} produced by SOB was measured by using of UV-visible spectrophotometer. A loopful of 48 h old culture was inoculated into 10 ml thiosulphate broth tubes and kept at 28 ± 2ºC in a BOD incubator. The supernatant was separated from the cell growth by centrifugation at 15000 rpm for 10-15 minutes. After adding barium chloride (10% w/v) to the supernatant in a 1:1 ratio, the amount of sulphate produced was measured. The suspensions were vigorously mixed and the white turbidity caused by barium sulphate formation was measured with a UV-visible spectrophotometer at 420 nm. Potassium sulphate standard curve was used to compare the amount of sulphate produced by bacterial cultures. Many biochemical tests were carried out for characterization of SOB such as: catalase production, oxidase test, methyl red test, Voges-Proskauer reaction, indole production, citrate utilization test, cellulose hydrolysis, starch hydrolysis, H_2S production, acid production and mortality test by using the kits available with HiMedia (HiPure Bacterial Identification Kit). Molecular characterization of all SOB bacterial isolates was done with their genomic DNA extracted using the protocol described by Nour *et al*. (1995) and Sambrook *et al*. (1989). The three isolates found compatible with each other on nutrient agar in cross streak plate.

Selected 3 isolates and their consortium were further studied in presence of elemental and bentonite sulphur to check efficacy on growth of mustard. Mustard seeds were sown in test tube with treated SOB broth. The best three isolates and their combinations in presence of two S sources were studied for efficacy to enhance growth and biomass of mustard in *rabi* 2021-22 in pot.

RESULTS AND DISCUSSION

Isolation and Characterization of Sulphur Oxidizing Bacteria

A total of 25 isolates were obtained from different samples like soils, muddy water samples and biogas slurry. Behera *et al*. (2014) isolated SOB from six different locations of

mangrove forest soil of Maha river delta, Odisha at Indian Farmers Fertilisers Corporation (IFFCO) on thiosulphate medium and only 4 isolates were selected based on differential tests in thiosulphate media. Table 1 shows the cultural and morphological characteristics of bacterial isolates wherein they had shown different colony characters like large, medium, small, entire, smooth, opaque, transparent, off white and gram positive etc. Bacterial isolates were grown for 48 h at 28 ± 2ºC on nutrient agar plate. Medina *et al*. (2021) studied the SOB genomes of 22 diazotrophs from colombian sugarcane fields and were sequenced to investigate potential biofertilizers. Moreover, all the isolates were studied for morphological and biochemical characteristics.

Table 1: Cultural and Morphological characteristics of SOB isolates on nutrient agar

Sr. No.	Name of isolate	Colony characters					
		Size	Margin	Texture	Opacity	Pigment	Gram's Reaction
1.	SOB1	Large	Entire	Smooth	Opaque	Off white	Gram +ve
2.	SOB2	Medium	Entire	Smooth	Transparent	-	Gram –ve
3.	SOB3	Large	Entire	Smooth	Transparent	-	Gram –ve
4.	SOB4	Small	Entire	Smooth	Opaque	Off white	Gram –ve
5.	SOB5	Small	Entire	Smooth	Opaque	Red white	Gram +ve
6.	SOB6	Medium	Entire	Smooth	Opaque	Creamy	Gram +ve
7.	SOB7	Small	Entire	Smooth	Opaque	Off white	Gram –ve
8.	SOB8	Medium	Entire	Smooth	Opaque	Creamy	Gram –ve
9.	SOB9	Medium	Entire	Smooth	Opaque	White Cream	Gram +ve
10.	SOB10	Large	Entire	Smooth	Opaque	Creamy	Gram +ve
11.	SOB11	Medium	Entire	Smooth	Opaque	White Cream	Gram –ve
12.	SOB12	Small	Entire	Smooth	Opaque	Off white	Gram –ve
13.	SOB13	Small	Entire	Smooth	Opaque	White Cream	Gram +ve
14.	SOB14	Medium	Entire	Smooth	Opaque	White Cream	Gram +ve
15.	SOB15	Medium	Entire	Smooth	Opaque	White Cream	Gram –ve
16.	SOB16	Medium	Entire	Smooth	Opaque	Red white	Gram +ve
17.	SOB17	Large	Entire	Smooth	Opaque	Off white	Gram +ve
18.	SOB18	Medium	Entire	Smooth	Opaque	Red white	Gram +ve
19.	SOB19	Small	Entire	Smooth	Opaque	White Cream	Gram –ve
20.	SOB20	Large	Entire	Smooth	Opaque	Off white	Gram +ve
21.	SOB21	Medium	Entire	Smooth	Opaque	Red white	Gram +ve
22.	SOB22	Medium	Entire	Smooth	Opaque	White Cream	Gram –ve
23.	SOB23	Small	Entire	Smooth	Opaque	Creamy	Gram +ve
24.	SOB24	Medium	Entire	Smooth	Opaque	Brown	Gram +ve
25.	SOB25	Small	Entire	Smooth	Opaque	Off white	Gram +ve

Screening and Selection of Efficient SOB Isolates

Cultural and Morphological characteristics were completed after screening of SOB isolates. All the twenty five isolates were screened based on pH reduction (BCP dye) and sulphate production to find out most efficient SOB. The yellow colour zone around the bacterial colony indicated the reduction of bromocresol purple dye from purple to yellow and the oxidation of thiosulphate to sulphuric acid (Fig.1). All the bacterial isolates were inoculated in thiosulphate broth with BCP dye. The bacterial isolates that reduced dye color on thiosulphate agar plates also passed the broth test and reduced dye color due to lowering of pH (Fig.1). Joshi *et al.* (2020) isolated total 40 different bacteria from soil samples on Starkey and thiosulphate agar from agricultural soils of Indore, Madhya Pradesh and total 14 isolates screened on the basis of their efficacy to reduce pH of growth medium from 8.0 to ≤ 5.0. The maximum pH reduction was shown by isolates $IS_2$9GA, S12ND. All the bacterial isolates showed sulphate production. These bacterial isolates were considered as efficient sulphur oxidizers. The amount of sulphate produced by different bacterial isolates ranged from 0.22 mM to 1.76 mM concentration. Ashraf *et al*. (2018) screened SOB by using thiosulphate medium from different ecological samples were screened on the basis of pH reduction, sulphate ions production up to 800 mg l^{-1} by *Bacillus* sp. strain SS-16.

Three most effective isolates which showed pH reduction (BCP dye reduction), maximum zone, sulphate production and growth on Starkey and NCL agar media were studied and selected for further testing of their efficacy (Table 2).

Table 2: Selection of the most efficient isolates

Sr. No	Isolates name	pH reduction in Thiosulphate broth	Clearance zone (mm) on Thiosulphate agar	Sulphate production in Thiosulphate broth (mm)	Growth on Starkey and NCL agar
SOB1	*Bacillus tropicus*	5.0	14.41	1.76	+
SOB2	*Beijerinckia fluminensis*	5.2	12.52	1.14	+
SOB3	*Klebsiella variicola*	5.4	13.23	1.13	+

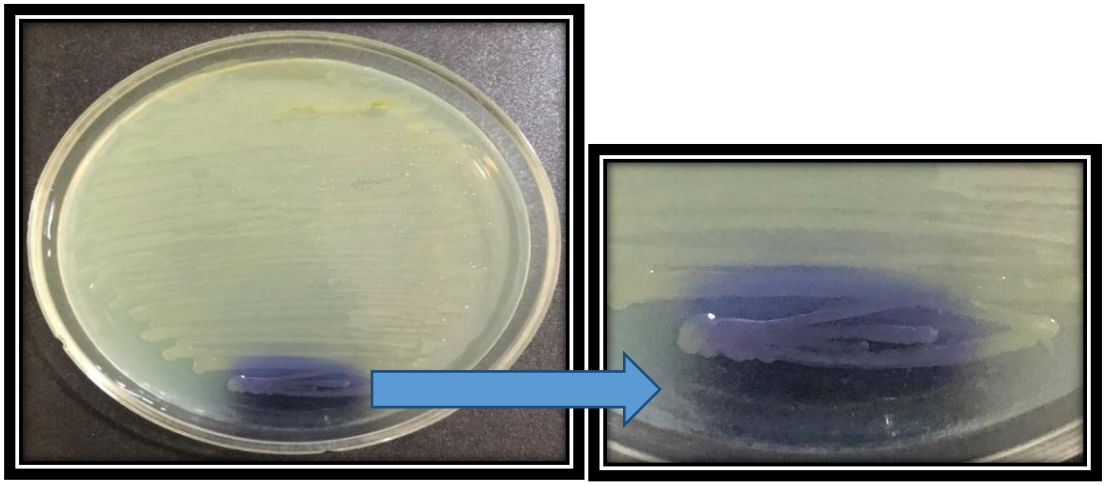

Fig. 1: pH reduction by SOB isolates on thiosulphate agar plates having BCP dye which is purple at 8 pH and yellow at 5 to 5.5 pH

Biochemical characterization of SOB isolates

Various biochemical characteristics of selected bacterial isolates are described in Table 3. All the 3 SOB isolates showed variable specific breakdown product-patterns and utilization of various carbon compounds. All the 3 isolates (SOB1, SOB2, SOB3) were positive for oxidase test, starch hydrolysis and acid production. All the isolates were negative for Voges Proskauer (VP) test and hydrogen sulphide gas. Chaudhary *et al*. (2017) isolated three potential SOB strains SSF7, SSA21 and SSS6 from the rhizosphere of mustard, that were identified based on molecular and biochemical characterization *viz*. cellulose hydrolysis, acid production, H_2S production, methyl red test, oxidase test *etc*.

Table 3: Biochemical characteristics of selected SOB isolates

Sr. No.	Biochemical Tests	SOB1	SOB2	SOB3
1.	Catalase production	+	-	+
2.	Oxidase test	+	+	+
3.	Methyl red test	-	-	+
4.	Voges Proskauer test	-	-	-
5.	Indole production	-	-	+
6.	Citrate utilization test	+	+	-
7.	Cellulose hydrolysis	-	+	+
8.	Starch hydrolysis	+	+	+
9.	H_2S production	-	-	-
10.	Acid production	+	+	+
11.	Motility test	+	+	-

Molecular characterization

SOB1, SOB2 and SOB3 isolates were identified as *Bacillus tropicus*, *Beijerinckia fluminensis* and *Klebsiella variicola* with different percent similarity and different percent query coverage show in Table 4. The phylogenetic tree revealed that SOB1, SOB2 and SOB3 are members of the *Firmicutes, Proteobacteria* and *Proteobacteria* phylum, respectively.

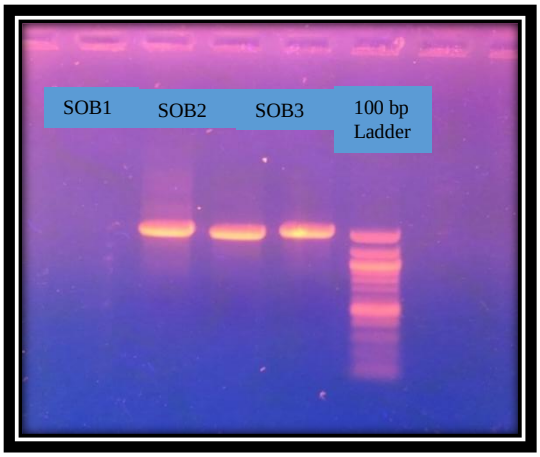

Fig. 2: Gel showing amplified 16S *r*RNA of selected 3 bacterial isolates

The partial 16S *r*RNA gene sequences of isolate SOB1, SOB2 and SOB3 has been deposited in NCBI's GenBank® under accession numbers ON127698, ON127700 and ON127847 and were identified *Bacillus tropicus*, *Beijerinckia fluminensis* and *Klebsiella variicola*, respectively. Guerrieri *et al*. (2021) reported a novel PGPR *Klebsiella variicola* UC4115, an isolate from tomato field and was screened *in-vitro* for different activities related to plant nutrition and growth regulation as well as for antifungal traits. The most consistent data for *K. variicola* UC4115 were observed under organic management, with seed application.

Table 4: Identification of SOB isolates by 16S *r*RNA sequencing

Isolates	Gen Bank® ACCN	Most closely related organisms*			
		Species	Accession description	% Gene identity	% Query coverage
SOB1	ON127698	*Bacillus tropicus* MCCC 1A01406	NR_157736.1	97.61	98
SOB2	ON127700	*Beijerinckia fluminensis* UQM1685	NR_116306.1	99.17	99
SOB3	ON127847	*Klebsiella variicola*F2R9	NR_025635.1	99.18	99

**Data obtained after BLAST analysis from NCBI database*

Compatibility testing of selected SOB isolates

After identification, the selected 3 SOB cultures were cross streaked on nutrient agar plate to verify their compatibility and observed that all the 3 SOB did not inhibit each other (Fig. 3).

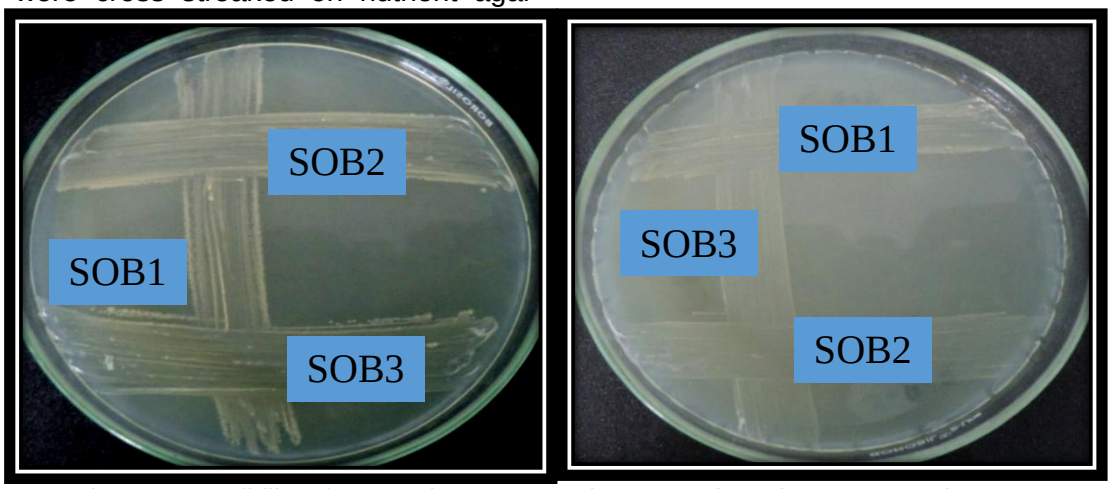

Fig 3: Compatibility of SOB cultures on Nutrient agar plates by cross streak assay

Evaluation of efficient SOB isolates and their combinations on growth of mustard *in vitro* butt agar (test tube assay)

The observations on shoot and root length measured at 15 DAI are narrated in table 5. The results revealed significant differences in shoot and root lengths at 15 DAI. T_7 receiving 20 kg S/ha through elemental sulphur as well as seed treatment of *Bacillus tropicus* + *Beijerinckia fluminensis* + *Klebsiella variicola* consortium had the highest shoot length among all the treatments.

Table 5: Effect of SOB on shoot and root length of mustard in butt agar test tube at 15 DAI

S. No	Treatments	Shoot length (cm)	Root length (cm)
T_1	Control (without SOB and sulphur)	8.67^{f}	8.00^{g}
T_2	20 kg S/ha through elemental sulphur	10.60^{e}	9.90^{f}
T_3	20 kg S/ha through bentonite sulphur	12.33^{cd}	10.00^{ef}
T_4	T_2 + *Bacillus tropicus*	12.50^{cd}	10.93^{bcd}
T_5	T_2 + *Beijerinckia fluminensis*	12.43^{cd}	10.80^{cde}
T_6	T_2 + *Klebsiella variicola*	13.23^{bc}	10.17^{def}
T_7	T_2 + *Bacillus tropicus* + *Beijerinckia fluminensis* + *Klebsiella variicola* consortium	14.83^{a}	12.27^{a}
T_8	T_3 + *Bacillus tropicus*	13.83^{ab}	11.50^{abc}
T_9	T_3 + *Beijerinckia fluminensis*	13.73^{ab}	11.17^{bc}
T_{10}	T_3 + *Klebsiella variicola*	12.03^{d}	10.90^{cd}
T_{11}	T_3 + *Bacillus tropicus* + *Beijerinckia fluminensis* + *Klebsiella variicola* Consortium	14.00^{ab}	11.83^{ab}
	S.Em. ±	0.36	0.27
	C.D. at 5%	Sig.	Sig.
	C.V. %	4.97	4.32

Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5% level of significance

Fig. 4: Evaluation of efficient SOB isolates and their combinations on growth of mustard *in vitro* using butt agar at 15 DAI

Pot study

An experiment was conducted in pot condition after completion of test tube experiment during *Rabi* 2021-22 to test the effect of SOB on mustard growth.

Effect of SOB on plant growth parameters of mustard *cv.* GDM 4 under pot condition

The observations of plant growth parameters measured in different time like 30, 60 and 75 DAS are narrated in Table 6. Plant growth parameters (plant height, root length,

Table 6: Effect of SOB on mustard growth

S. No	Treatments	Plant height (cm)			Root length (cm)	Number of branches per plant		Number of siliqua per plant	Plant fresh weight (g/plant)	Plant dry weight (g/plant)	Dry shoot weight (g/plant)	Dry root weight (g/plant)
		30 DAS	60 DAS	75 DAS		30 DAS	60 DAS					
T_1	Control (without SOB andsulphur)	15.44^{f}	84.67^{e}	88.00^{e}	32.67^{e}	4.67^{f}	7.11^{f}	2.11^{i}	81.67^{f}	17.53^{j}	15.47^{h}	2.07^{i}
T_2	20 kg S/ha through Elemental Sulphur	18.33^{e}	87.33^{de}	90.11^{e}	32.78^{e}	5.22^{e}	7.67^{ef}	2.44^{hi}	86.33^{e}	22.00^{h}	19.80^{g}	2.20^{h}
T_3	20 kg S/ha through Bentonite Sulphur	19.78^{e}	88.67^{de}	91.44^{de}	32.89^{e}	5.56^{de}	8.33^{de}	3.11^{h}	85.33^{e}	22.93^{g}	20.60^{fg}	2.33^{g}
T_4	T_2 + *Bacillus tropicus*	23.68^{d}	93.67^{cd}	102.22^{abc}	37.56^{bcd}	6.44^{b}	9.22^{b}	9.11^{e}	106.00^{c}	28.67^{d}	25.67^{c}	3.00^{e}
T_5	T_2 + *Beijerinckia fluminensis*	23.67^{d}	94.00^{cd}	101.32^{bc}	36.89^{bcd}	6.33^{bc}	9.11^{bc}	6.67^{f}	103.33^{c}	26.87^{e}	24.27^{d}	2.60^{f}
T_6	T_2 + *Klebsiella variicola*	23.65^{d}	93.33^{cd}	97.00^{cd}	35.44^{de}	5.78^{cd}	8.56^{bcd}	5.89^{g}	95.33^{d}	24.80^{f}	22.27^{e}	2.53^{f}
T_7	T_2 + *Bacillus tropicus* + *Beijerinckia fluminensis* + *Klebsiella variicola* Consortium	44.44^{a}	108.67^{a}	109.22^{a}	42.22^{a}	7.56^{a}	11.44^{a}	15.67^{a}	130.00^{a}	32.93^{a}	28.80^{a}	4.13^{a}
T_8	T_3 + *Bacillus tropicus*	35.44^{cd}	99.00^{bc}	107.11^{ab}	38.78^{bc}	6.78^{b}	10.78^{a}	14.00^{bc}	114.67^{b}	29.80^{bc}	26.60^{bc}	3.20^{c}
T_9	T_3 + *Beijerinckia fluminensis*	36.11^{b}	97.33^{c}	106.78^{ab}	36.00^{cd}	6.56^{b}	9.00^{bcd}	13.78^{c}	95.33^{d}	28.97^{cd}	25.87^{c}	3.10^{d}
T_{10}	T_3 + *Klebsiella variicola*	26.33^{c}	91.67^{cde}	98.89^{c}	34.89^{de}	6.33^{bc}	8.44^{cd}	10.67^{d}	103.00^{c}	23.80^{g}	21.40^{ef}	2.40^{g}
T_{11}	T_3 + *Bacillus tropicus* + *Beijerinckia fluminensis* + *Klebsiella variicola* Consortium	42.11^{a}	107.00^{ab}	107.67^{ab}	39.11^{b}	7.44^{a}	11.11^{a}	14.67^{b}	115.33^{b}	30.40^{b}	27.00^{b}	3.40^{b}
	S.Em. ±	0.75	2.52	2.05	0.98	0.171	0.229	0.22	0.93	0.30	0.29	0.03
	C.D. at 5%	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.	Sig.
	C.V. %	4.65	4.59	3.55	4.57	4.74	4.33	4.25	1.59	1.94	2.16	1.63

number of branches per plant, number of silique per plant, plant fresh weight, plant dry weight) at different time showed significant differences. T_7 receiving soil application of 20 kg S/ha through elemental sulphur as well as seed treatment of *Bacillus tropicus* + *Beijerinckia fluminensis* + *Klebsiella variicola* consortium showed significantly higher plant growth parameter as compared to control. Chaudhary *et al*. (2022) reviewed that application of SOB in legume crops improve yield and symbiotic nitrogen fixation. Sulphur is an important nutrient in addition to influencing growth and yield of legumes.

Effect of SOB on soil parameters

The observations of soil parameters measured at harvesting time (75 DAS) are narrated in Table 7. Soils parameter (microbial count, SOB count, soil available sulphur) at harvesting time showed significant differences. T_7 receiving soil application of 20 kg S/ha through elemental sulphur as well as seed treatment of *Bacillus tropicus* + *Beijerinckia fluminensis* + *Klebsiella variicola* consortium showed significantly higher plant growth parameter as compared to control.

Table 7: Effect of SOB on soil properties

S. No	Treatments	Total Microbial count (log cfu/g)	SOB count (log cfu/g)	Soil available sulphur (ppm)
T_1	Control (without SOB andsulphur)	5.34^{c} (2.4×10^{5})	3.29^{e} (2.0×10^{3})	18.33^{g}
T_2	20 kg S/ha through Elemental Sulphur	5.47^{c} (3.4×10^{5})	3.45^{e} (2.8×10^{3})	46.77^{f}
T_3	20 kg S/ha through Bentonite Sulphur	5.47^{c} (3.7×10^{5})	3.33^{e} (2.1×10^{3})	53.35^{e}
T_4	T_2 + *Bacillus tropicus*	6.76^{ab} (5.9×10^{6})	5.58^{abc} (4.0×10^{5})	64.84^{d}
T_5	T_2 + *Beijerinckia fluminensis*	6.46^{b} (3.8×10^{6})	5.39^{bc} (2.8×10^{5})	64.69^{d}
T_6	T_2 + *Klebsiella variicola*	6.16^{b} (2.9×10^{6})	5.16^{cd} (1.4×10^{5})	56.17^{e}
T_7	T_2 + *Bacillus tropicus* + *Beijerinckia fluminensis* + *Klebsiella variicola* Consortium	7.20^{a} (1.6×10^{7})	5.98^{a} (1.0×10^{6})	79.72^{a}
T_8	T_3 + *Bacillus tropicus*	6.80^{ab} (7.5×10^{6})	5.86^{ab} (9.2×10^{5})	74.49^{abc}
T_9	T_3 + *Beijerinckiafluminensis*	6.79^{ab} (2.7×10^{6})	5.78^{ab} (8.5×10^{5})	72.69^{bc}
T_{10}	T_3 + *Klebsiellavariicola*	6.33^{b} (1.3×10^{7})	4.70^{d} (6.3×10^{4})	69.15^{cd}
T_{11}	T_3 + *Bacillus tropicus* + *Beijerinckiafluminensis* + *Klebsiellavariicola* Consortium	7.17^{a} (2.4×10^{5})	5.97^{a} (1.0×10^{6})	75.66^{ab}
	S.Em. ±	0.21	0.16	1.62
	C.D. at 5%	Sig.	Sig.	Sig.
	C.V. %	5.77	5.45	4.56

Results suggested that in presence of elemental / bentonite sulphur, seed treatment of *Bacillus tropicus, Beijerinckia fluminensis* and *Klebsiella variicola* sulphur oxidizing bacterial (SOB) consortium or individual SOB has wide scope as agriculturally beneficial bio-input in increasing growth and biomass yield of an important oil seed crop mustard (*Brassica juncea* L.)

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