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Zinc Solubilizing and Sulphur Oxidizing Bacteria as Plant Probiotic for Summer Groundnut

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ABSTRACT: Looking to importance of Zn and S as an essential micro element, their availability to the crops from the soil needs to be ensured. We can add them in the soil in form of chemical amendment but, the entire applied amount is not available for the plant. To ensure their availability to plant, microbial inoculants are proved best. A research study focused on the use of Zinc Solubilizing Bacteria (ZSB) and Sulphur Oxidizing Bacteria (SOB) as plant probiotics for summer groundnut involved native strains of ZSB (*Beijerinckia fluminensis* AAU ZSB F2) and SOB (*Pseudomonas aeruginosa* AAU PF 3 and *Bacillus tropicus* AAU SOB 1). A micro-plot experiment was conducted using the bacterial consortium, individual bacteria, and control treatments to test their effects on groundnut growth, yield, and quality parameters. The treatment that received 100% RDF with $ZnSO_4$ and Gypsum, along with ZSB and SOB, showed significant highest in plant height at 30 and 60 days (19.72 cm and 35.61 cm respectively), pods per plant (12.33), pod yield (8.86 g per plant), haulm yield (19.77 g per plant) and kernel yield (8.8 g per plant). In terms of quality parameters, the treatment that received 100% RDF with Gypsum and SOB exhibited the highest oil percentage (54.12%) in the kernel, while the treatment with $ZnSO_4$ and Gypsum along with ZSB and SOB had the highest protein content (31.82%). Regarding soil chemical parameters, the treatment with the native SOB strain resulted in lower pH, whereas the treatment with the ZSB strain showed higher pH. No significant difference was observed in organic carbon content and available phosphorus among the different treatments. The treatment with 100% RDF, $ZnSO_4$, Gypsum, ZSB, and SOB had the highest available zinc and sulphur content in the soil, as well as higher zinc and sulphur content and uptake by the kernel and haulm. Overall, the study demonstrated the potential of the bacterial consortium in promoting groundnut growth, yield, and quality parameters, as well as enhancing soil nutrient availability. The microorganisms based nutrient availability proved very cost effective and eco-friendly for sustainable agriculture as well as to improve quality of the groundnut.

Keywords: Sulphur Oxidizing Bacteria, Zinc Solubilizing Bacteria, Plant Probiotics, Groundnut, Sulphur, Zinc.

INTRODUCTION

When chemical fertilizers were first used in agriculture in the 20th century, most of the issues that farmers encountered in order to boost crop yield were resolved. However, chemical fertilizers gradually began to reveal their adverse effects on the environment and agro-ecosystems, eventually affecting people and other creatures. In light of this, it was advised to utilize Biofertilizers and organic practices in an integrated manner to optimize plant nutrient availability and thus promote sustainable crop production (Han *et al*., 2006; Manva *et al*., 2019). Biofertilizers are basically living microorganisms that colonize the rhizosphere, or the area around plant roots. Through biological processes, these bacteria are able to change the unavailable form of macronutrients like nitrogen, phosphorus, and potassium (NPK), which are crucial for human health, into the available form (Ahmed, 2012; Patel *et al*, 2022). The term "plant growth-promoting rhizobacteria" (PGPR) or 'Plant Probiotic' is another name for this group of bacteria. Important genera within this category include *Pseudomonas, Azospirillum, Burkholderia, Bacillus, Enterobacter, Rhizobium, Erwinia, Serratia, Alcaligenes, Arthrobacter, Acinetobacter*, and *Flavobacterium* (Rodriguez and Fraga 1999; Krishnaiah *et al*., 2005). In the root-soil interface, soil microorganisms like fungi and bacteria interact with plant roots and soil components to make certain nutrients more readily available to plants. They also produce plant hormones to encourage plant growth and antimicrobial compounds to shield plants from biotic stresses. Soil bacteria have been used in crop production for decades. The main functions of these bacteria are (1) to supply nutrients to crops; (2) to stimulate plant growth, e.g., through the production of plant growth hormones; (3) to control or inhibit the activity of plant pathogens; (4) to improve soil structure; and (5) bioaccumulation or microbial leaching of inorganics (Hayat *et al*., 2010).

Zinc as an essential nutrient. Zinc (Zn) is an essential micronutrient required for plants, animals and human beings for their normal healthy growth and reproduction. In plants, zinc plays a key role as a structural constituent or regulatory co-factor of a wide range of different enzymes and proteins in many important biochemical pathways which are mainly concerned with carbohydrate, protein metabolism and resistance to infection by certain pathogens (Di *et al*., 1998; Martino *et al*., 2003). Zinc needed by plant in micro quantity but in critical concentration and if the amount available is not adequate, plants will suffer from physiological stress (Alloway, 2008). Zinc deficiency is the highest priority among micronutrients for agriculture to address. According to a survey made on the available status of micronutrients in India, Zn recorded as the micronutrient deficient in all the 20 states assessed and also deficient in all the agro-ecological regions of India. (Anonymous, 2002).

Role of zinc solubilizing bacteria (ZSB). The chemical forms of zinc applied as a zinc supplement gets easily transferred into hydroxide form and other complex forms that cannot be assimilated by plants and thus in turn leads to zinc deficiency in crops. Some soil microorganisms possess ability to solubilize insoluble metals by mean of chelation, acidification, exchange reaction and release of organic acid in soil (Hafeez *et al.*, 2013; Patil *et al*., 2022). Various researchers have overcome this problem exploring metal solubilizing bacteria belonging to genera *Bacillus, Pseudomonas* and *Serratia* for improving the micronutrient deficiency in plant. The major advantage of applying bacteria as a metal solubilizer is elimination of pollution created due to repeated application of zinc salts and improvement in growth and development of plant by plant growth promoting bacteria (Hansda *et al.*, 2014). Recent study describes effect of Zn mobilizers which significantly conquer the deficiency symptoms of Zn and by increasing the total biomass and grain yield (White *et al.*, 2001).

Sulphur as nutrient. Sulphur/Sulfur (S) is most important nutrient in addition to NPK which limits the growth of plants and it is one of the essential plant nutrients for the synthesis of proteins, oils, vitamins and flavoured compounds. The amino acids methionine, cysteine and cystine contain 21, 26 and 27% S, respectively (Tandon and Messick, 2002). Sulphur contributes to an increase in crop yields in three different ways like, it provides a direct nutritive value or indirect nutritive value as soil amendment, especially for calcareous and saline alkali soils and it also improves the use efficiency of other essential plant nutrients, particularly nitrogen and phosphorus. The main forms of S present in nature are sulphide and sulphate in water or soil and sulphur dioxide in the aerial environment as a sulphur source; whilst sulfoxides, elemental sulphur, thiosulphate and polythionate plays a smaller but significant role. Sulphur can be distributed into two forms **I)** organic and **II)** inorganic sulphur compounds. About 90% of the sulphur in soils is in bounded to the organic molecules in S is found either in oxidized or reduced form (Chaudhary, 2018).

Sulphur deficiency in soil. Asparagine, glutamine, and arginine accumulate in plant tissues as a result of sulphur deficit in soils and plants, which also limits protein synthesis. In areas where high-S utilizing crops like oilseeds and pulses are regularly produced, sulphur insufficiency is more prevalent. Sulfur shortage affects about 41% of Indian soils, and it is expanding quickly in regions where intensive cropping practices, high yielding cultivars, and high analysis chemical fertilizers, particularly sulphur free fertilizers like diammonium phosphate and urea, are utilized (Singh, 2001). Sulfur is crucial for plant nutrition, and its deficiency can significantly lower output and lower quality. The growth, yield, and quality of crops and other products are ultimately impacted by sulphur-deficient plants' greater decrease in chlorophyll content in leaves, inhibition of protein synthesis and carbohydrate metabolism, protein composition, supply of mineral nutrients, nitrogenase activity in root nodules, etc.

Role of sulphur oxidizing bacteria (SOB). Physiologically different types of microorganisms, which comprise of both heterotrophic and autotrophic mode of nutrition, are involved in S cycle for transformations of inorganic sulphur compounds in nature. Sulphate is captured as a nutrient and reduced to sulphide, which is then included into sulphur containing amino acids and enzymes (Friedrich *et al*., 2001). Bacteria, which are having 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 (SOB). The sulphur oxidizers also play a vital role in removal of poisonous hydrogen sulphide (H_2S) from the atmosphere. Sulphur oxidizing bacteria generally belongs to various genus like *Thiobacillus, Beggiatoa, Thiothrix, Thiomicrospira, Desulphuromonas, Chromatium* etc. but the oxidation process is unlimited to the true sulphur bacteria as it is found in the bacteria having heterotrophic mode in nature (Das *et al*., 1996; Hao *et al*., 2019). Belongs to the genera *Pseudomonas, Escherichia, Alcaligens, Xanthobacter* etc. sulphur oxidizing heterotrophic bacteria are isolated from different environments like root rhizosphere, biogas digester, lagoons, swamps, mangroves, composting heap, sulphur containing hot water spring/pond, etc. (Starkey, 1935; Kuenen and Beudeker 1982).

Zn and S deficiency in groundnut. In Zn deficiency symptoms in groundnut are light yellow stripes along with veins of leaf blade under acute condition-veinally chlorosis and cessation of growth of terminal bud. Older leaves may show slight chlorosis. S deficient plants are look light green. Stunted growth, uniformly chlorotic plants, thin stemmed and spindle appearance.

Looking to the above facts and essentiality of Zn and S for quality groundnut production, the research was carried out to study plant probiotics potential of AAU native ZSB and SOB isolates on summer groundnut.

MATERIALS AND METHODS

Compatible test. *In vitro* plate bioassay was carried out to determine compatibility of selected isolates on nutrient agar plate. The respective bacterial cultures *viz*., AAU ZSB F2 (*Beijerinkia* spp), *Pseudomonas aeruginosa* AAUPF 3 and AAU SOB 1 (*Bacillus tropicus*) were cross streaked on nutrient agar plates and incubated at 30±2 ºC for 5 days and observed every 24 hrs for interaction or inhibition of any of the test cultures at intercept on plate (Manva *et al*., 2019).

Development of bacterial formulation. Liquid formulation of AAU native zinc solubilizing bacteria, sulphur oxidizing bacteria and combination of ZSB-SOB were prepared as per standard methodology (As per FCO gazette notification for introduction of NPK consortia biofertilizers, Dept. of Agriculture & co-operative, Ministry of Agriculture, GOI vide S. O. 1181(E) dated 30.04.2014). For preparation of liquid formulations, bacterial isolates were grown in their respective media/nutrient broth. After the sufficient growth, formulations were prepared using suitable additives/stabilizing agents and bacterial population was maintain at/above 10^8 cells/ml throughout the experimental period. The pH of the formulations was adjusted using suitable buffer and stored at room temperature for six months (Goljanian-Tabrizi *et al*., 2016).

Shelf-life study. Microbial population of individual as well as combined formulation was recorded at initial stage. Shelf life of the formulation was monitored at regular intervals at 1 month (up to 6 months) by serial dilution of 1.0 ml of formulation in sterile distilled water and results were recorded as cfu/ml (Acharya, 2008).

Micro-Plot study. The experiment was conducted in a micro-plot at the Bidi Tobacco Research Station, Anand Agricultural University, Anand, Gujarat, India. A complete randomized block design with the 11 treatment and 3 repetitions was used. The unit plot size was 1.10 m × 1.10 m. Seeds of groundnut (cv. GG 34) were sown at 45 cm between rows. Recommended dose of fertilizers (25-50-00 N-P_2O_5-K_2O) and FYM (10 t/ha) applied at the time of sowing. Weeding and plant protection measures were undertaken as and when required.

This research study is based on the solubilization of zinc and the oxidizing of sulphur bacterial isolates. ZSB F2 (*Beijerinckia* spp.) & *Pseudomonas aeruginosa* AAUPF 3 used as zinc solubilizing bacteria and AAU SOB 1 (*Bacillus tropicus*) used as a sulphur oxidizing bacteria. For this nutrient $ZnSO_4$ and Gypsum used as a zinc and sulphur source. Following treatments are used in experiment. **T1**: Control; **T2**: 100% RDF; **T3**: 100% RDF + $ZnSO_4$ (25 kg/ha); **T4**: 100% RDF + Gypsum (111 kg/ha); **T5**: 100% RDF + $ZnSO_4$ (25 kg/ha) + ZSB; **T6**: 100% RDF + $ZnSO_4$ (25 kg/ha) +SOB; **T7**: 100% RDF + $ZnSO_4$ (25 kg/ha) + ZSB & SOB; **T8**: 100% RDF + Gypsum (111 kg/ha) +ZSB; **T9**: 100% RDF + Gypsum (111 kg/ha) +SOB; **T10**: 100% RDF + Gypsum (111 kg/ha) + ZSB & SOB.

Initial Analysis of Soil. The soil type was sandy loam. Chemical properties of soil were, pH- 7.63; EC- 0.27dS/m; OC- 0.30%; Avail. Phosphorus-35.70 kg/ha; Avail. Sulphur-8.10 ppm; Avail. Zinc-0.42 ppm; Total microbial count-8.1×10^4 (cfu/g); ZSB count-3.1×10^3 (cfu/g) and SOB count-4.9×10^3 (cfu/g).

Data acquisition. Plant height observation recorded at 30 and 60 days after sowing (DAS). The crop was harvested at physiological maturity and after harvest following data pods per plant, pod yield haulm yield and kernel yield recorded.

Oil and protein content determined in kernel recorded for quality parameters. After harvest the soil physio-chemical properties analyzed with different methods like, pH (1:2.5 (Soil: Water) Potentiometer (Jackson, 1973), electrical conductivity (EC)(Jackson, 1973), organic carbon (OC) (modified Walkley and Black method) (Walkley and Black, 1934), available P_2O_5 (Olsen's (0.5 M $NaHCO_3$) Method (Olsen *et al*., 1954)), Zinc (DTPA (0.01 M $CaCl_2.2H_2O$) Extraction (AAS) Method (Lindsay and Norvell, 1978)) and Sulphur (Turbidometric (0.15% $CaCl_2$ extractable) Method (Chesnin and Yien 1951)) and also recorded the total microbial count, zinc solubilizing count and sulphur oxidizing bacterial count by serial plate count method (Solanki, 2021). Zinc and Sulphur content and uptake (sulphur by Turbidimetric method (Chaudhary and Cornfield, 1966) and zinc by Atomic Absorption spectrophotometry (AAS) (Lindsay & Norvell 1978)) by kernel and haulm data collected from research.

Statistical analysis. The experimental data of different observation parameter obtained to statistical analysis using Completely Randomized Design. Data were subjected to analysis of variance and means were compared by Duncan's New Multiple Range Test (DNMRT) (Steel and Torrie, 1980). The statistical analysis was complete with the assistance of Department of Agricultural Statistics, B. A. College of Agriculture, Anand Agricultural University, Anand.

RESULTS AND DISCUSSION

Revival and Colony Characterization of Native Zinc Solubilizing Bacteria and Sulphur Oxidizing Bacteria. In this experiment three native ZSB and SOB strains were used. These native micronutrients solubilizing bacterial strain were isolated earlier and preserved at culture collection center at Department of Ag Microbiology, BACA, AAU, Anand.

Native bacterial isolates were screened for their solubilization efficiency of sulphur and zinc in agar plates. Native SOB strain *Bacillus tropicus* AAU SOB 1showed sulphur solubilizing zone of 14.41 mm. While in case of zinc solubilization, strain *Pseudomonas aeruginosa* AAU PF 3showed 16.25mm and *Beijerinckia fluminensis* AAU ZSB F2 shown 10.61 mm of zone.

Compatibility study of AAU native isolates. ZSB F2 (*Beijerinckia* spp.), *Pseudomonas aeruginosa* AAUPF & AAU SOB 1 (*Bacillus tropicus*) isolates were cross streaked on nutrient agar plate to check out their compatibility and observed that these three isolates did not inhibit each other showed great compatibility among each other.

Analysis microbial population (cfu/ml) of Formulation. The bacterial formulation was subjected to shelf-life assessment up to 120 days (December, 2021 to February, 2022) at initial, 60, 90, 120, 150 and 180 days in Fig. 1. They were observed count by serial dilution and plate count method and during shelf-life assessment the data pertaining to microbial population revealed that, the bacterial population was maintained above 10^8 cfu/ml up to 180 days, indicated sufficient numbers of viable microorganisms in the formulation. The same trend is also reflected in graph

An experiment was conducted in micro-plot condition during summer 2022 to test the plant growth promotion effect of zinc solubilizing and sulphur oxidizing bacterial formulation on Groundnut. A total of three native SOB (*Bacillus tropicus* AAU SOB 1) and ZSB (*Pseudomonas aeruginosa* AAU PF 3and *Beijerinckia fluminensis* AAU ZSB F2) strains and their consortium were tested.

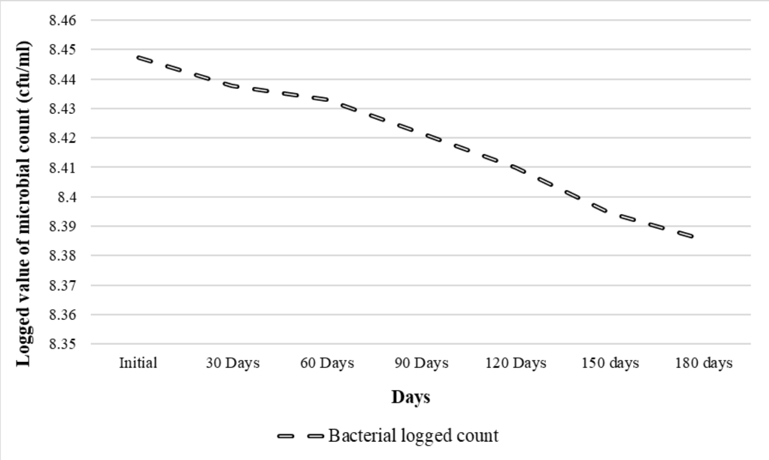

Fig. 1. Shelf-life study of microbial population in consortium formulation at different time intervals.

Growth parameter of groundnut. The results pertaining to plant growth promotion effect of ZSB and SOB with/without $ZnSO_4$ and gypsum on ground growth and yield are narrated hereafter.

Plant height. The observations pertaining to plant height measured at 30 and 60 DAS and presented result in Fig. 2 and results revealed that plant height showed significant differences. Data revealed that T_{11} recorded with significantly higher plant height at 30 DAS (19.72 cm) and 60 DAS (35.61 cm) which was found at par with treatment receiving application of T_{10} (19.70 cm, 34.80 cm) and 100% RDF + $ZnSO_4$ (25 kg/ha) + Zinc Solubilizing Bacteria & Sulphur Oxidizing Bacteria (19.14 cm, 34.91 cm) respectively.

Table 1: Effect of various treatments on growth and yield parameters in summer groundnut.

Tr. No.	Treatment	No. of pods per Plant	Pod Yield (g/plant)	Haulm Yield (g/plot)	Kernel Yield (g/plot)	Oil Content (%)	Protein Content (%)
T_1	Control	5.17^{g}	6.86^{g}	738.72	276.00	46.05^{f}	21.94^{h}
T_2	100% RDF	8.17^{f}	7.78^{e}	745.38	322.05	46.55^{ef}	24.53^{g}
T_3	T_2+ $ZnSO_4$ (25 kg/ha)	8.50^{ef}	7.94^{de}	749.52	327.15	47.91^{e}	25.79^{f}
T_4	T_2+ Gypsum (111 kg/ha)	9.00^{def}	7.42^{f}	746.82	322.35	52.08^{bc}	27.17^{e}
T_5	T_3 + ZSB	9.50^{de}	7.40^{f}	849.03	329.40	50.53^{d}	28.37^{de}
T_6	T_3+ SOB	9.83^{cd}	8.16^{cd}	761.73	363.60	51.51^{cd}	30.18^{bc}
T_7	T_3+ ZSB & SOB	11.50^{ab}	8.62^{ab}	888.33	384.90	52.03^{bc}	30.91^{ab}
T_8	T_4+ ZSB	10.67^{bc}	8.36^{bc}	854.73	373.50	50.40^{d}	27.91^{de}
T_9	T_4+ SOB	10.83^{bc}	8.49^{bc}	863.73	377.10	54.12^{a}	28.93^{cd}
T_{10}	T_4+ ZSB & SOB	11.50^{ab}	8.65^{ab}	885.00	387.15	52.90^{abc}	31.51^{a}
T_{11}	T_2+ $ZnSO_4$ (25 kg/ha) + Gypsum (111 kg/ha) + ZSB & SOB	12.33^{a}	8.86^{a}	895.08	396.00	53.06^{ab}	31.82^{a}
S.Em. ±		0.326	0.11	14.86	3.18	0.446	0.375
C.D. (P=0.05)		0.955	0.32	43.57	9.32	1.310	1.101
CV %		5.78	2.37	3.15	1.57	1.53	2.31

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

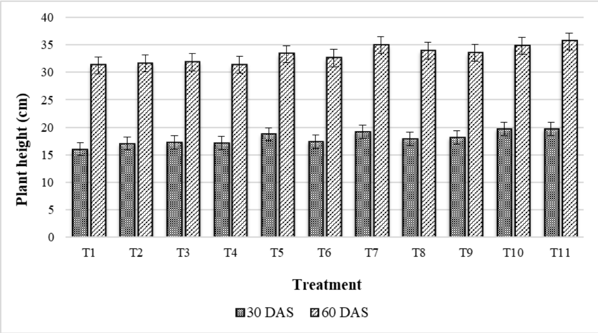

Fig. 2. Plant height of groundnut GG 34 at 30 and 60 days after sowing.

Pod Yield. Observations on pods per plant are narrated in Table 1. Results revealed that pods per plant showed significant differences. Data revealed that T_{11} showed significantly higher pods 12.33 per plant which was found at par with T_{10} (11.50 pods per plant) and T_7 (11.50 pods per plant). Pod yield (g/plant) are represented in Fig. 3. Results revealed that pod yield showed significant differences. Data revealed that T_{11} showed significantly higher pod yield 8.86 g plant^{-1} which was found at par with T_{10} (8.65 g plant^{-1}) and T_7 (8.62 g plant^{-1}). The use of SOB in legume crops has been found to increase yield and symbiotic nitrogen fixation, according to Chaudhary *et al*. (2022). In addition to having an impact on the growth and productivity of legumes, sulphur is a crucial nutrient. Chaudhary *et al*. (2019) reported a favorable response to SOB inoculation on mustard growth in terms of height, weight and viable rhizospheric bacterial count.

Haulm yield and kernel yield. The data pertaining to total haulm yield/plot presented in Table 1. The data showed significant differences among different treatments and revealed that treatment T_{11} receiving soil application of 100% RDF and 25 kg/ha $ZnSO_4$ and 111 kg/ha Gypsum with treatment of Zinc Solubilizing Bacteria & Sulphur Oxidizing Bacteria showed significantly higher Haulm yield 895.08 g per micro-plot which was found at par with T_{10} (885 g/plot), T_9 (863.73), T_8 (854.73) and T_7 (888.33 g/plot). Data related to haulm yield and kernel yield were presented in fig 3. From the data it was concluded that the both showed significant differences and data revealed that T_{11} showed significantly higher haulm yield 19.77 g plant^{-1} and kernel yield 8.8 g plant^{-1} which was found at par with T_{10} (19.52 g plant^{-1}; 8.6 g plant^{-1} respectively) and T_7: (19.71 g plant^{-1}; 8.55 g plant^{-1} respectively). Similar trend was recorded for kernel yields also (Table 2). Statistically significant differences were recorded among different treatments and data revealed that T_{11} receiving soil application of 100% RDF and 25 kg/ha $ZnSO_4$ and 111 kg/ha Gypsum with treatment of Zinc Solubilizing Bacteria & Sulphur Oxidizing Bacteria showed significantly higher kernel yield 396 g/plot which was found at par with T_{10} (387.15) and T_7 (384.9). According to Joshi *et al*., (2018) study and investigation, liquid biofertilizers are an essential component of integrated nutrient management and have a considerable impact on groundnut production, both in term of the pod and Haulm yield. His research concluded that the increase in values could be attributed to the synergistic effect of inorganic and liquid biofertilizers, which enhanced the soil environment and improved water absorption, nutrient uptake, and overall plant development, leading to higher photosynthetic activity, which in consequently increased pod and Haulm yield. Kusale *et al*. (2021) discovered that SOB *Klebsiella variicola* inoculation reduced salt stress and enhanced wheat and maize growth and nutrition. Under salinity stress at 45 DAS, *klebsiella variicola* inoculation enhanced the physicochemical characteristics of the soil and enhanced seed germination, root length, shoot height, and chlorophyll content in wheat and maize seedlings. The PGP features and enhanced mineral uptake of nitrogen, phosphorus, potassium, and magnesium are associated to the improvement in plant growth. According to Chaudhary *et al*. (2019) favorable response to SOB inoculation on mustard growth in terms of number of siliquae, weight of 100 seeds and leaf chlorophyll content.

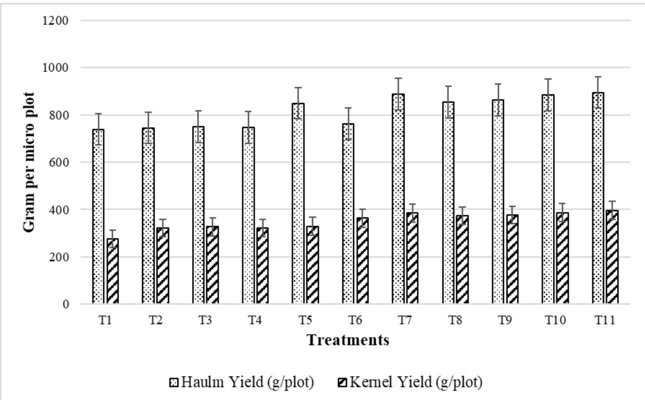

Fig. 3. Effect on haulm kernel yield per plot of groundnut.

Quality Parameter. The data pertaining to oil content (%) are narrated in table 1and results revealed that oil content showed significant differences among different treatments. As shown in the results treatment T_9 showed significantly higher oil content 54.12 % which was found at par with T_{11} (53.06 %). The observations of protein content (%) are presented in Table 1 and significant differences were recorded among protein content of different treatments. As shown in the table treatment T_{11} showed significantly higher protein content 31.82 % which was found at par with T_{10} (31.51 %) and T_7 (30.91%). According to Joshi *et al*. (2018) higher oil and protein content as well as yield may be attributed to the use of biofertilizers combined with inorganic sources, which increased nutrient availability at timely and accelerated up crop growth, improving groundnut quality parameters. The availability of all the necessary nutrients as a result of its continuous mineralization can be attributed to this increase in oil content while bioformulation application. Joshi *et al*. (2021) studied SOB, a crucial component of soil sulphur metabolism. High levels of sulphur are needed for oil seed crops, which are crucial for the synthesis of proteins, vitamins, and enzymes. In oil seed crops, it enhances yield, oil content, and protein content. *Thiobacillus* are crucial because they help with mineralization and the absorption of additional essential nutrients as well as pH lowering during sulphur oxidation. You can use these SOB inoculants to improve soil fertility and sulphate transformation.

Soil Parameter. The results pertaining to soil chemical parameters *viz*., pH, EC (dS/m), OC (%) and available P are presented in Table 2. Results revealed that pH showed significant differences. Soil application of T_5having higher pH (8.23) which is at par with soil application of T_8 (8.13). Whenever soil application T_9 have 7.67 pH. In case of EC (dS/m) significant differences were observed on soil EC receiving different treatments. Among them, soil application of T_5 having higher EC (0.33 dS/m) and soil application with T_9 having lower EC (0.23 dS/m). An inspection of data represented in table 2 showed that different treatment had non-significant effect on organic carbon content (%) of the soil after harvest of the crop, the indicated that the organic carbon content of soil was found in range of 0.302 to 0.326 % in the soil. Soil available phosphorus contain was not affected significantly by different treatments and varied in range of 40.25 to 42.39 kg/ha in the soil.

The results of available zinc (ppm)are represented in Table 2. Results revealed that available zinc showed significant differences and concluded that T_5 showed significantly higher available zinc 0.45 ppm which was found at par with soil application of T_{11} (0.44 ppm) and T_7 (0.44 ppm). The data related to available sulphur (ppm) are narrated in table 2. Results revealed that available sulphur content showed significant differences and from above data it can be concluded that T_{11} receiving soil showed significantly higher available sulphur 9.38 ppm which was found at par with T_{10} (9.08 ppm).

Table 2: Effect of various treatments on different soil chemical parameters.

Tr. No.	Treatment	pH (1:2.5)	EC 1:2.5 (dS/m)	OC (%)	Avail. P_2O_5 (kg/ha)	Avail. S (ppm)	Avail. Zn (ppm)
T_1	Control	7.79^{c}	0.31^{bc}	0.302	42.22	8.36^{d}	0.39^{e}
T_2	100% RDF	7.87^{bc}	0.31^{bc}	0.303	42.39	8.40^{cd}	0.40^{de}
T_3	T_2 + $ZnSO_4$ (25 kg/ha)	7.88^{bc}	0.32^{ab}	0.306	41.65	8.43^{cd}	0.42^{bcd}
T_4	T_2 + Gypsum (111 kg/ha)	7.73^{c}	0.29^{de}	0.304	41.52	8.47^{bcd}	0.41^{cde}
T_5	T_3 + ZSB	8.23^{a}	0.33^{a}	0.319	40.28	8.62^{bcd}	0.45^{a}
T_6	T_3 + SOB	7.70^{c}	0.29^{de}	0.314	40.88	8.79^{abcd}	0.43^{abc}
T_7	T_3 + ZSB & SOB	7.76^{c}	0.3^{cd}	0.321	40.40	8.81^{abcd}	0.44^{ab}
T_8	T_4 + ZSB	8.13^{ab}	0.32^{ab}	0.315	40.51	8.73^{bcd}	0.43^{abc}
T_9	T_4 + SOB	7.67^{c}	0.28^{e}	0.310	42.25	9.03^{abc}	0.41^{cde}
T_{10}	T_4 + ZSB & SOB	7.7^{c}	0.28^{e}	0.320	40.34	9.08^{ab}	0.42^{bcd}
T_{11}	T_2 + $ZnSO_4$ (25 kg/ha) + Gypsum (111 kg/ha) + ZSB & SOB	7.78^{c}	0.30^{cd}	0.326	40.69	9.38^{a}	0.44^{ab}
S.Em. ±		0.10	0.01	0.01	0.56	0.19	0.01
C.D. (P=0.05)		0.30	0.02	NS	NS	0.57	0.03
CV %		2.30	3.67	2.99	2.34	3.82	3.51

Pourbabaee *et al*. (2020) examined the combined effects of native SOB *Thiobacillus* sp. and various sources of sulphate, such as elemental sulphate, granular sulphate, and without sulphate, on pH, electrical conductivity, sulphate content in soil, plant growth indices, and uptake of sulphate by maize under various moisture circumstances. Stem diameter, plant height, shoot weight, and sulphate uptake by maize plants were all considerably increased by the application of 570 mg of sulphur per kg in the form of elemental sulphur and bacterial inoculum. When elemental sulphur and Thiobacillus are combined, elemental sulphur oxidation improves, increasing the availability of nutrients in the soil and, ultimately, the growth of plants.

The data pertaining to total microbial, ZSB and SOB count represented in Table 3. Results reveled that the T_{11} application found highly significant in total count (1.77×10^7 cfu/ g of soil), ZSB count (9.3×10^6 cfu/ g of soil) and SOB count (8.1×10^6 cfu/ g of soil) compared to control and other treatment. Chaudhary *et al*. (2019) reported a favourable response to SOB inoculation on mustard growth in terms of height, weight, number of siliquae, weight of 100 seeds, oil content, leaf protein content, leaf chlorophyll content, and viable rhizospheric bacterial count.

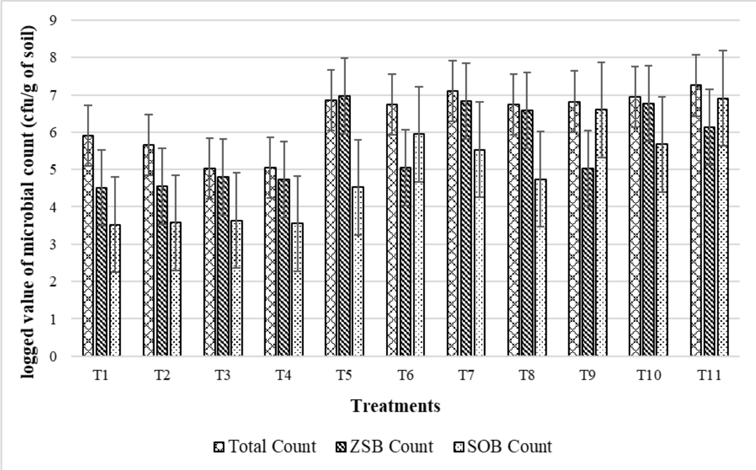

Fig. 4. Effect on totalmicrobial, ZSB and SOB countof soil after harvest.

Nutrient content and uptake. The data of zinc content (ppm)in kernel and haulmare represented in Fig. 5 and results revealed significant difference in zinc content among different treatments. As shown in the table treatment T_{11} receiving showed significantly higher zinc content 43.167 ppm in kernel and 68.03 ppm in haulm. This treatment was found at par with soil application of T_7 42.233 ppm in kernel and 65.58 ppm in haulm. The data regarding sulphur content (%) in kernel and haulm are noted in fig. 5 and results revealed that sulphur content showed significant differences among different treatments. According to experimental results treatment T_{11} receiving showed significantly higher sulphur content 0.36 % in kernel and 0.33 % in haulm. Sulphur for kernel content his treatment was found at par with soil application of T_{10} (0.35%) and T_9 (0.35%). Where in case of haulm soil application of T_{10} (0.32 %) significantly at par with T_{11}. The data of zinc uptake (g/ha) by kernel and haulm revealed that haulm and kernel uptake zinc content showed significant differences. According to results, significantly higher uptake zinc content18.54 mg/plot in kernel and 71.49 mg/plot in haulm. Were in case of haulm, soil application of T_5 (67.86 mg/plot) significantly at par with T_{11}. Similarly, the results for sulphur uptake (g/ha) by kernel and haulm revealed that haulm and kernel sulphur uptake showed significant differences between different treatments. As represented in the fig, data revealed that T_{11} showed significantly higher uptake sulphur content1.54 g/plot in kernel and 3.50 g/plot in haulm. Where in case of haulm soil application of T_{10} (3.32 g/plot) significantly at par with T_{11}. Abhijit *et al*. (2014) conducted a field experiment to examine how sulphur and sulphur oxidizing strains of *T. thioxidans* affected mustard variety B-85 yield metrics. Three *Thiobacillus* strains RS002, RS004, and RS005 and sulphur was administered at a rate of 20 kg/ha *via* elemental S, gypsum, and pyrite. The average seed production had 14.5% more elemental S than the control, and with the addition of S oxidizers, this increase reached 30.6%. With sulphur oxidizing inoculant RS004, the oil output, which was 25.7% higher in elemental S or gypsum over control after the second year up to 42%. Gypsum or elemental S had the highest sulphur level in seeds, and this content increased in the second year of the study. Yasmin *et al*. (2021) carried out experiment on the isolation, characterization, and role of zinc-solubilizing bacteria in chickpea growth revealed that the improvement in plant growth parameters may be connected to the bacterial ability to solubilize nutrients and make them available to plants. By reducing the quantity of ethylene, IAA and ACC deaminase activity by bacterial isolates enhances root growth and increases root surface area more suited for nutrient uptake. Some members of the *Pseudomonas* genus have been identified as phytohormone, IAA, and zinc solubilization-producing plant growth promoters.

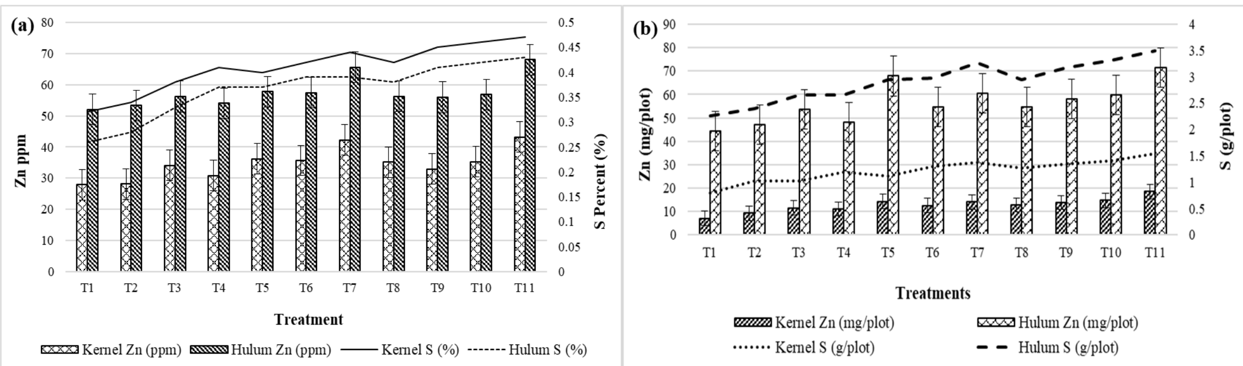

Fig. 5. (a) Effect on Zinc and Sulphur content in kernel and haulm of groundnut; **(b)** Zinc and Sulphur uptake by groundnut kernel and haulm.

CONCLUSIONS

From the above results it can be concluded that, the native zinc solubilizing and sulphur oxidizing bacterial strains were proved effective for nutrient solubilization and found compatible with each other for preparation of consortium formulation with sufficient self-life. Moreover, these native ZSB, SOB and its combined formulation found to increase groundnut growth, yield and kernel quality along with increase in sulphur and zinc nutrient content and uptake.

FUTURE SCOPE

To meet the current demand for healthy and nutrient rich food along with restoration of soil fertility, improving productivity and lowering the use of expensive chemical fertilizer inputs, it is essential to use microbial resources, which are valuable and effective for restoring soil nutrients and promoting sustainable crop production while preserving agroecosystems.

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Conflict of Interest. None.

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