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Research Article
Plant Growth

Ameliorative Effect of Plant Growth Promoting Rhizobacteria Enhancing micronutrient uptake of Groundnut (Arachis hypogaea. L).

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Groundnut (Arachis hypogaea L.) is a premier oil seed crop in India occupying 45 per cent of total oil seed production. Though India leads both in area and production of groundnut, the country ranks eight in productivity due to fertilizer management and erratic response of the crop in terms of yield. Innovative agriculture is an immediate need of today's world that has increased tremendously in its population. Hence, we need to increase the agricultural produce substantially with the help of plant growth promoting rhizobacteria. A pot experiment was undertaken under botanical garden in Annamalai university, with three bacterial strains (Rhizobium sp.), (Pseudomonas sp.) and (Bacillus sp.), applied in our experiment. The treatment involving consortium inoculation with Rhizobium + Pseudomonas + Bacillus recorded highest values of Mg, Zn and Cu can improve nutrient uptake with a shoot portion of groundnut when compared to the root portion.

Keywords: biofertilizer, micronutrients, zinc, copper, iron, pgpr, groundnut

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1. Introduction

Agricultural crop productivity and food security are currently the challenges in the center of global attention for resilience and sustainable food system towards eradication of all versions of malnutrition and world hunger. It was predicted that in 2020 between 720 and 811 million people in the world faced hunger. Furthermore, food insecurity and malnutrition have been now exacerbated beneath the shadow of the covid-19 pandemic that downturns all the progress made in agriculture (FAO, IFAD, UNICEF, WFP AND WHO, 2021). To tackle this critical demand, FAO focuses on the implementation offour betters(better production, better nutrition, better environment, and better life) to ensure food security, persistent livelihoods and double the food production to attain the zero hunger target by 2030 (FAO, 2021).

Groundnut or peanut (Arachis hypogaeaL.) Is an important and fourth major economic oil yielding crop worldwide with the production of 42.4 million tons. It is an invaluable source of protein, essential fatty acids and other biologically active compounds (Deshmukh *et al.,* 2020). The concerted efforts must be undertaken to face the ever increasing demand for high groundnut production due to its incredible therapeutic values to treat malnutrition (Sayyed *et al*., 2019). Conventional approaches such as chemical fertilizers and pesticides have been adopted to ensure maximum food production. However, the indispensable input of these chemical products creates a global concern due to their harmful impacts on environments (Rachidi *et al*., 2021). Thus, the incorporation of plant bio stimulant as a novel and environment-friendly strategy in agriculture hold a promise to secure crop performance, yield stability and soil quality (Rouphael *et al*., 2020;Basu *et al*., 2021;Hamid *et al*., 2021).

The modern system of farming, it is progressively more felt, is becoming indefensible as evidenced by moribund crop productivities, damage to environment, chemical contaminations, etc. The essential of having an alternative agriculture method which can function in a friendly eco-system while sustaining and increasing the crop productivity is realized now. Organic farming and biofertilizers is recognized as the best-known alternative to the conventional agriculture (Yadav *et al*., 2013).

Groundnut (*Arachis hypogaea* L.) belongs to family Leguminosae is the “King of oilseed” in our country is an important crop both for oil and food. About 2/3 of the crop produce in the world is crushed to extract oil and 1/3 is used to make other edible products. Groundnut naturally enriches the soil through symbiosis. Organic sources which are good for improvement of soil properties, besides supplying nutrients for longer period of time without leaving ill effects on soil has been realized. Biofertilizers are the most useful technology necessary to support developing organic, sustainable, green and non-polluted agriculture. Although plant growth-promoting rhizobacteria (PGPR) have been reported to influence plant growth, yield and nutrient uptake by an array of mechanisms, the specific traits by which PGPR promote plant nutrient uptake were limited to the expression of one or more of the traits expressed at a given environment of plant–microbe interaction. Mineral elements are classified into two groups (macro elements and micro elements) depending on their importance in the plant. Macro elements (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and Sulphur (S)) are those which are required by plants in large amount while microelements (iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), chlorine (Cl), boron (B), nickel (Ni) and molybdenum (Mo)) are those which are required by plants in small amount (White *et al* 2012). Shortage in any one of these elements restricts plant growth and reduces crop yields (Arunachalam *et al*., 2013). Bioavailability of mineral elements such as zinc (Zn), iron (Fe), copper (Cu) and manganese (Mn) and their uptake by plants is essential for crop production (Makoi *et al*., 2013). The aim of current experiment was conducted to investigate the response of groundnut to PGPR on enhancing micronutrients uptakes such as (Mg, Cu and Zn).

2. Materials and Methods

2.1 Seed Materials

The groundnut seed (*Arachis hypogaea* L.) var. VRI 2 was obtained from Regional Research Station, Tamil Nadu Agricultural University, Virudhachalam, Cuddalore District, Tamil Nadu, India.

2.2 Plant Growth Promoting Rhizobacteria (PGPR)

Plant growth promoting rhizobacteria (*Rhizobium, Pseudomonas,* and *Bacillus*) were obtained from the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu.

2.3 Seed Treatment

The seeds of groundnut were surface sterilized with 80 per cent ethanol and 0.1 per cent mercuric chloride and washed with distilled water for 3 to 4 times. The seeds were mixed with carrier-based plant growth promoting rhizobacteria either as individual organisms or consortium of organisms and shade dried for 30 min. After shade drying, the seeds were sown.

Treatments	Plant growth promoting rhizobacteria
T0	Seed without PGPR
T1	Rhizobium
T2	Pseudomonas
T3	Bacillus
T4	Rhizobium + Pseudomonas
T5	Rhizobium + Bacillus
T6	Pseudomonas + Bacillus
T7	Rhizobium + Pseudomonas + Bacillus

2.4 Pot Culture Experiment

Pot experiments were conducted in Botanical Garden, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

2.5 Sowing Method

Ten seeds of groundnut were sown in cement pots filled with soil, with treatments of various plant growth promoting rhizobacteria.

2.6 Irrigation Schedule

Pre-sowing irrigation was given to ensure uniform germination. Irrigation was given at 3 DAS with due care to avoid excess flooding of water. Uniform irrigation was done for two times per week.

2.7 Pot Culture Experiment Details

Crop : Groundnut (*Arachis hypogaea* L.)

Variety : VRI 2

Design : Complete Randomized Block Design

Sampling Days : 25, 50, 75 and 100 DAS

Parameters Studied : Micronutrient content plant sample.

Plant materials were estimated by the following methods.

2.8 Magnesium (Yoshida *et al*., 1972)

2 mL of the filtrate was mixed with 2 mL of 5 per cent lanthanum chloride solution and diluted with 10 mL of 1N hydrochloric acid. The solution was fed into an atomic absorption spectrophotometer at 211.9 nm for calcium and 285.4 nm for magnesium. Standard curve was prepared by using calcium chloride/magnesium chloride.

2.9 Zinc, Copper (De Vries and Tiller, 1980)

1 mL of sulphuric acid and 15 mL of double distilled water were added to a kjeldahl flask containing 0.5 g of dried powdered material and incubated at 80°C for overnight. After that, 5 mL of acid mixture (nitric acid and perchloric acid in the ratio of 3:1) was added and then digested. The digested material was cooled, made up to 50 mL and filtered through Whatmann No. 42 filter paper. The sample was aspired into an atomic absorption spectrophotometer with air acetylene flame for the estimation of zinc (214 nm), copper (324.6 nm), iron (568 nm) and manganese (530 nm). The readings were taken and recorded.

Statistical Analysis

Statistical significance was assessed at the $P<0.05$ level using one way ANOVA and means were separated by Duncan’s multiple range test ($P<0.05$) with the help of SPSS 16 software. Means and ± standard deviations were calculated from three replicates.

3. Results and Discussion

3.1 Magnesium

The results on the effect of plant growth promoting rhizobacteria on magnesium content in root and leaf portion of groundnut at various stage of its growth. The highest magnesium content (63.92, 71.76, 84.59 and 97.89 ppm) of dry weight at 25, 50, 75 and 100 DAS were recorded in leaf portion of groundnut grown in consortium treatment (*Rhizobium* + *Pseudomonas* + *Bacillus*) of PGPR. The lowest magnesium content (30.53, 36.42, 48.93 and 63.42 ppm) of dry weight at 25, 50, 75 and 100 DAS were recorded in root portion of groundnut crop grown without PGPR treatment.

Magnesium is a part of the chlorophyll in all green plants with a chief role in essential photosynthesis. It is also helps to activate many plant enzymes needed for growth. The magnesium content of the crop is higher in leaf portion in 75day crop grown in the combined treatment of PGPR. The lowest magnesium content was recorded in root portion of the crop in control treatment. The maximum level of Magnesium content was obtained in *Pseudomonas* + *Azotobacter* and *Pseudomonas* + *Azospirillum* treatments, (Sharaf Zadeh, 2012). Erturk *et al*. (2011) reported that, the concentration of macro and micronutrient such as magnesium content of plant tissue increased by bacterial treatment in hazelnut plant. Bacterial applications significantly affected concentrations of magnesium, when compared to the control in apple (Karakurt and Aslantas, 2010). Plant growth benefits due to the addition of PGPR include the increase in magnesium content in faba bean (Abd El-Azeem *et al.,* 2007). This observed enhancement in biomass might be due to increased macro- and micro-nutrient (e.g. N, P, K, S, Mg, and Fe) uptake by chickpea seedlings in those treatments as reported by Hartz *et al*. (1996).

3.2 Zinc

The results on the effect of plant growth promoting rhizobacteria on zinc content in root and leaf portion of groundnut at various stage of its growth. The highest zinc content (52.28, 56.58, 62.99 and 69.70 ppm) of dry weight at 25, 50, 75 and 100 DAS were recorded in leaf portion of groundnut grown in consortium treatment (*Rhizobium* + *Pseudomonas* + *Bacillus*) of PGPR. The lowest zinc content (20.87, 25.42, 29.83 and 36.42 ppm) of dry weight at 25, 50, 75 and 100 DAS were recorded in root portion of groundnut crop grown without PGPR treatment.

Zinc is directly or indirectly involved in several enzyme system, auxin production, RNA synthesis, protein synthesis, seed production and rate of maturity. The zinc content of the crop is higher in leaf portion in 75-day crop grown in the combined treatment of PGPR. The lowest zinc content was recorded in root portion of the crop in control treatment.

Both direct and indirect PGPR pathways may have an impact on plant performance. Producing substances that stimulate plant growth (phytohormones), increasing the availability and uptake of nutrients in the soil through biological nitrogen fixation, releasing fixed forms of nutrients into plant-useful systems (P, K, and Zn), chelating nutrients (Fe) through the production of siderophores, and other similar processes are examples of direct mechanisms (Kour*etal*., 2023;Upadhyay*etal*., 2022). Zinc is an indispensable micronutrient, required in a small amount for crops to play numerous important functions in their life cycle. It is involved in various physiological and biochemical functions of plants (Kumar *et al*., 2019). Plant growth promoting and nitrogen fixing microorganisms improved the plant dry biomass, N, P, K, Zn, and Fe contents when compared to the uninoculated control of Phaseolus vulgaris L. (Mishra et al., 2014). PGPR can increase Zn-availability to plants by solubilizing complex Zn compounds and thus alleviate Zn deficiency in plants (Saravanan *et al.,* 2011). Elkoca *et al*. (2008) found significant increases of zinc concentration in the common bean with PGPR treatments. Shirmardi *et al*. (2010) reported that rhizobacteria inoculants produce higher copper, Fe and Mn concentration than that of the control. Bacterial inoculations especially mixed inoculation, significantly helps in increased uptake of micronutrients (Fe, Mn, Zn, and Cu) of leaf, and straw part of the plant (Turan *et al*., 2010). Esitken *et al*. (2006) observed in co-inoculation of *Pseudomonas* + *Bacillus* increase of zinc contents in leaves. The bacterial applications affected the zinc contents in rabbit eye blueberry (De Silva *et al.,* 2000).

3.3 Copper

The results on the effect of plant growth promoting rhizobacteria on copper content in root and leaf portion of groundnut at various stage of its growth. The highest copper content (24.10, 26.58, 29.39 and 31.88 ppm) of dry weight at 25, 50, 75 and 100 DAS were recorded in leaf portion of groundnut grown in consortium treatment (Rhizobium + Pseudomonas + Bacillus) of PGPR. The lowest copper content (8.10, 10.36, 13.27 and 15.42 ppm) of dry weight at 25, 50, 75 and 100 DAS was recorded in root portion of groundnut crop grown without PGPR treatment.

Copper plays an important role in reproductive growth.The copper content of the crop is higher in leaf portion in 75-day crop grown in the combined treatment of PGPR. The lowest copper content was recorded in root portion of the crop in control treatment.

PGPR inoculations (*Bacillus subtilis*, *Bacillus atrophaeus*, *Bacillus spharicus*, sub group *Staphylococcus kloosii*, and *Kocuria erythromyxa*) significantly increased the nutrient uptake of copper in strawberry plants when compared to control (Karlidag *et al*., 2013). Yolcu *et al*. (2011) reported that PGPR treatments increased the concentrations of copper than that of the control in Italian ryegrass.

Canbolat *et al*. (2006) reported that phosphate solubilizing and N_2-fixing PGPR increased the uptake of copper content in barley, spinach and wheat.

Table 1: Effect of plant growth promoting rhizobacteria on magnesium content (ppm/dr. wt.) in leaf of groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T_0)	38.16 ± 1.14g	46.00 ± 1.38g	58.83 ± 1.76g	72.13 ± 2.16h
Rhizobium (T1)	41.84 ± 1.25fg	49.68 ± 1.49fg	62.51 ± 1.87fg	75.81 ± 2.27g
Pseudomonas (T2)	45.52 ± 1.37ef	53.36 ± 1.60ef	66.19 ± 1.99ef	79.49 ± 2.38f
Bacillus (T3)	49.20 ± 1.48de	57.06 ± 1.71de	69.89 ± 2.10de	83.19 ± 2.50e
Rhizobium + Pseudomonas (T4)	52.88 ± 1.59cd	60.72 ± 1.82cd	73.55 ± 2.21cd	86.85 ± 2.61d
Rhizobium + Bacillus (T5)	56.56 ± 1.70bc	64.40 ± 1.93bc	77.23 ± 2.32bc	90.53 ± 2.72c
Pseudomonas + Bacillus (T6)	60.24 ± 1.81ab	68.08 ± 2.04ab	80.91 ± 2.42ab	94.21 ± 2.83b
Rhizobium + Pseudomonas + Bacillus (T7)	63.92 ± 1.92a	71.76 ± 2.15a	84.59 ± 2.54a	97.89 ± 2.94a
S.Ed.	2.20	1.85	2.00	1.69
CD (P = 0.05)	4.66	3.91	4.24	3.59

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P £ 0.05 (L.S.D.)

Table 2: Effect of plant growth promoting rhizobacteria on magnesium content (ppm/dr. wt.) in root of groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T0)	30.53 ± 0.92h	36.42 ± 1.09g	48.93 ± 1.48f	63.42 ± 1.90g
Rhizobium (T1)	34.28 ± 1.03g	40.17 ± 1.20fg	52.68 ± 1.58ef	67.17 ± 2.01fg
Pseudomonas (T2)	38.03 ± 1.14f	43.92 ± 1.32ef	55.43 ± 1.66e	69.92 ± 2.10ef
Bacillus (T3)	41.78 ± 1.25e	47.67 ± 1.43de	60.18 ± 1.80d	74.67 ± 2.24de
Rhizobium + Pseudomonas (T4)	45.53 ± 1.37d	51.42 ± 1.54cd	63.93 ± 1.92cd	78.42 ± 2.35cd
Rhizobium + Bacillus (T5)	49.28 ± 1.48c	55.17 ± 1.65bc	67.68 ± 2.03bc	82.17 ± 2.46bc
Pseudomonas + Bacillus (T6)	53.03 ± 1.59b	58.92 ± 1.77ab	71.43 ± 2.14ab	85.92 ± 2.58ab
Rhizobium + Pseudomonas + Bacillus (T7)	56.78 ± 1.70a	62.67 ± 1.88a	75.18 ± 2.25a	89.67 ± 2.69a
S.Ed.	1.73	2.02	2.14	2.30
CD (P = 0.05)	3.67	4.28	4.53	4.89

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P £ 0.05 (L.S.D.)

Table 3: Effect of plant growth promoting rhizobacteria on zinc content (ppm/dr. wt.) in leaf of groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T0)	25.12 ± 0.75g	29.42 ± 0.88h	35.83 ± 1.07g	42.54 ± 1.28h
Rhizobium (T1)	29.00 ± 0.87fg	33.30 ± 1.00g	39.71 ± 1.19fg	46.42 ± 1.39g
Pseudomonas (T2)	32.88 ± 0.99ef	37.18 ± 1.11f	43.59 ± 1.31ef	50.30 ± 1.51f
Bacillus (T3)	36.76 ± 1.10de	41.06 ± 1.23e	47.47 ± 1.42de	54.18 ± 1.62e
Rhizobium + Pseudomonas (T4)	40.64 ± 1.22cd	44.94 ± 1.35d	51.35 ± 1.54cd	58.06 ± 1.74d
Rhizobium + Bacillus (T5)	44.52 ± 1.34bc	48.82 ± 1.46c	55.23 ± 1.66bc	61.94 ± 1.86c
Pseudomonas + Bacillus (T6)	48.40 ± 1.45ab	52.70 ± 1.58b	59.11 ± 1.77ab	65.82 ± 1.97b
Rhizobium + Pseudomonas + Bacillus (T7)	52.28 ± 1.57a	56.58 ± 1.70a	62.99 ± 1.89a	69.70 ± 2.09a
S.Ed.	1.86	1.70	1.88	1.55
CD (P = 0.05)	3.95	3.61	3.98	3.29

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P £ 0.05 (L.S.D.)

Table 4: Effect of plant growth promoting rhizobacteria on zinc content (ppm/dr. wt.) in root of groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T0)	20.87 ± 0.62g	25.42 ± 0.76g	29.83 ± 0.89g	36.42 ± 1.09g
Rhizobium (T1)	24.08 ± 0.72fg	28.63 ± 0.86fg	33.04 ± 0.99fg	39.63 ± 1.19fg
Pseudomonas (T2)	27.29 ± 0.82ef	31.84 ± 0.95ef	36.25 ± 1.09ef	42.84 ± 1.28ef
Bacillus (T3)	30.50 ± 0.91de	35.05 ± 1.05de	39.46 ± 1.18de	46.05 ± 1.38de
Rhizobium + Pseudomonas (T4)	33.71 ± 1.01cd	38.26 ± 1.15cd	42.67 ± 1.28cd	49.26 ± 1.48cd
Rhizobium + Bacillus (T5)	36.92 ± 1.11bc	41.47 ± 1.24bc	45.88 ± 1.38bc	52.47 ± 1.57bc
Pseudomonas + Bacillus (T6)	40.13 ± 1.20ab	44.68 ± 1.34ab	49.09 ± 1.47ab	55.68 ± 1.67ab
Rhizobium + Pseudomonas + Bacillus (T7)	43.34 ± 1.30a	47.89 ± 1.44a	52.30 ± 1.57a	58.89 ± 1.77a
S.Ed.	1.69	1.64	1.88	1.92
CD (P = 0.05)	3.59	3.48	3.99	4.06

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P £ 0.05 (L.S.D.)

Table 5: Effect of plant growth promoting rhizobacteria on copper content (ppm/dr. wt.) in leaf of groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T0)	11.64 ± 0.35g	14.12 ± 0.42g	16.93 ± 0.51f	19.42 ± 0.58g
Rhizobium (T1)	13.42 ± 0.40fg	15.90 ± 0.48fg	18.71 ± 0.56ef	21.20 ± 0.64fg
Pseudomonas (T2)	15.20 ± 0.46ef	17.68 ± 0.53ef	20.49 ± 0.61def	22.98 ± 0.69ef
Bacillus (T3)	16.98 ± 0.51de	19.46 ± 0.58de	22.27 ± 0.67cde	24.76 ± 0.74de
Rhizobium + Pseudomonas (T4)	18.76 ± 0.56cd	21.24 ± 0.64cd	24.05 ± 0.72bcd	26.54 ± 0.80cd
Rhizobium + Bacillus (T5)	20.54 ± 0.62bc	23.02 ± 0.69bc	25.83 ± 0.77abc	28.32 ± 0.85bc
Pseudomonas + Bacillus (T6)	22.32 ± 0.67ab	24.80 ± 0.74ab	27.61 ± 0.83ab	30.10 ± 0.90ab
Rhizobium + Pseudomonas + Bacillus (T7)	24.10 ± 0.72a	26.58 ± 0.80a	29.39 ± 0.88a	31.88 ± 0.96a
S.Ed.	1.63	1.56	1.79	1.56
CD (P = 0.05)	3.45	3.30	3.80	3.31

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P £ 0.05 (L.S.D.)

Table 6: Effect of plant growth promoting rhizobacteria on copper content (ppm/dr. wt.) in root of groundnut (*Arachis hypogaea* L.)

Treatments	Age of the plant in days			
	25	50	75	100
Control (T0)	8.10 ± 0.24f	10.36 ± 0.31f	13.27 ± 0.40e	15.42 ± 0.46e
Rhizobium (T1)	9.46 ± 0.28ef	11.72 ± 0.35ef	14.63 ± 0.44de	16.78 ± 0.50de
Pseudomonas (T2)	10.82 ± 0.32def	13.08 ± 0.39def	15.99 ± 0.48cde	18.14 ± 0.54cde
Bacillus (T3)	12.18 ± 0.36cde	14.44 ± 0.43cde	17.35 ± 0.52bcde	19.50 ± 0.58bcde
Rhizobium + Pseudomonas (T4)	13.54 ± 0.41bcd	15.80 ± 0.47bcd	18.71 ± 0.56abcd	20.86 ± 0.63abcd
Rhizobium + Bacillus (T5)	14.90 ± 0.45abc	17.16 ± 0.51abc	20.07 ± 0.60abc	22.22 ± 0.66abc
Pseudomonas + Bacillus (T6)	16.26 ± 0.49ab	18.52 ± 0.56ab	21.43 ± 0.64ab	23.58 ± 0.71ab
Rhizobium + Pseudomonas + Bacillus (T7)	17.62 ± 0.53a	19.88 ± 0.60a	22.79 ± 0.68a	24.94 ± 0.75a
S.Ed.	1.54	1.55	2.01	2.04
CD (P = 0.05)	3.27	3.29	4.27	4.32

Data are average values of three replicates ± SD. Mean with different letters in the same column differ significant P £ 0.05 (L.S.D.)

4. Conclusion

The micronutrients were analyzed in dried plant material of leaf and root portion. The high amounts of minerals were recorded in leaf portion when compared to root. The highest minerals were recorded in consortium treatment of PGPR (*Rhizobium* + *Pseudomonas* + *Bacillus*). Efficient plant nutrition management should ensure both enhanced and sustainable agricultural production and safeguard the environment. Hence, there is an urgent need for integrated nutrient management that targets agricultural inputs and lowers the adverse environmental impacts of agricultural fertilizers and practices.

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