

Research Article

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Field trial to correlate mineral solubilization activity of *Pseudomonas aeruginosa* and biochemical content of groundnut plants

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Abstract: The excessive use of phosphorus (P) fertilizers increases crop production but can lead to P-induced zinc (Zn) deficiencies, making both nutrients unavailable to plants. Plant–microbe interactions, such as with *Pseudomonas aeruginosa*, can alleviate these constraints by solubilizing Zn and P in soil. A soil incubation study revealed that applying *P. aeruginosa* with farmyard manure (FYM) significantly increased Zn and P solubilization (6.86 mg/l; 14.83 mg/l)

compared to control (3.15 mg/l; 13.67 mg/l). A field experiment evaluated the effects of *P. aeruginosa* on the biochemical composition of groundnut plants under five treatments. The T2, T3, and T4 treatments had the highest protein, carbohydrate, and chlorophyll levels, likely due to the heterogeneous activity of FYM and the mineral solubilizing ability of *P. aeruginosa*. Groundnut seeds from T3 (combined liquid inoculant and FYM) had the highest iodine (88.47 mg KOH/g), saponification value (195.56 mg KOH/g), and free fatty acid content (2.23 g oleic acid). The pH of the T3 soil decreased from 8.3 to 7.5, and significant increases were observed in electrical conductivity (from 2.88 to 0.30 dS/m), calcium carbonate (2.53–1.7%), organic carbon (0.39–1.91%), nitrogen (273.75–788.25 kg/ha), P (20.1–59.65 kg/ha), potassium (182.25–346.5 kg/ha), and Zn (1.53–7.24 mg/kg). The study suggests that the combined application of liquid formulants of *P. aeruginosa* with FYM is advantageous, as FYM supports microbial growth by providing essential nutrients for mineralization. Moreover, liquid inoculants formulated with polyvinylpyrrolidone as an osmo protectant demonstrated enhanced shelf-life and mineral solubilization, contributing to improved biochemical properties in groundnut plants.

Keywords: *Pseudomonas aeruginosa*, zinc, phosphorus, solubilization, field study, carbohydrates, proteins, chlorophyll, oil, soil, fertility

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1 Introduction

The presence of fourteen mineral elements in the soil is essential for plant growth as they regulate plant metabolic processes and produce crops with excellent quality and yield. They can be categorized into macronutrients like nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) and micronutrients like chlorine (Cl), boron (B), iron (Fe), manganese (Mn), copper

(Cu), zinc (Zn), nickel (Ni), and molybdenum (Mo) [1]. Farmers focus more on macronutrients since they are critical for plant development and growth, ranging from structural elements to redox-sensing agents [2]. Despite being crucial for crop quality and productivity, providing inherent protection against disease and adverse conditions, micronutrients have received less attention. They also act as co-factors for several enzymes involved in metabolizing various organic compounds, including proteins, lipids, carbohydrates, and nucleic acids [3–5]. Micronutrient deficiencies in the soil are a universal phenomenon these days due to the crop's inability to absorb micronutrients, as well as positive or negative interactions with macronutrients accumulating in the soil as a result of excessive application of chemical fertilizers by the agrarian communities [6–9]. It is estimated that 49, 31, 15, 14, 10, and 3% of agricultural soils worldwide are deficient in Zn, B, Mo, Cu, Mn, and Fe, respectively [10,11]. Of the micronutrients, only Zn is directly linked in the food chain such that deficiency is extensive in humans, food crops, and soil. The Zn deficiency is therefore the highest priority among micronutrients for agriculture to address [12].

Plants require Zn as a micronutrient because it is vital to many of their bio-physicochemical processes, including protein synthesis, gene regulation and activation, carbohydrate metabolisms, morphological and anatomical participation in bio-membranes, and numerous other critical cellular processes like ion homeostasis, metabolic and physiological processes, and enzyme activation [13–15]. Zn must therefore be properly absorbed, transported, and distributed throughout plant tissues, cells, and intracellular spaces to sustain optimal growth and development [16]. Stunted growth and development, chlorosis, necrosis, membrane breakdown, poor metabolism, nutritional imbalance, and increased vulnerability to biotic and abiotic stresses are the symptoms of Zn deficiency in plants [17]. Some of the factors that affect the quantity of Zn available to plants are soil type, soil pH, and the presence of other minerals that compete with Zn absorption, one such interaction is between Zn and P [18]. The Zn-induced P deficiencies are uncommon because farmers usually use more P fertilizer than Zn, which could prevent Zn from being absorbed [19]. According to Sharma *et al.* [20], precipitation on Fe, Al, and Ca complexes causes 75–90% of the applied P fertilizer to accumulate in the soil. The formation of H^+ ions by phosphate salts causes the soil's phosphate content to rise, which in turn prevents Zn from being absorbed by plants due to their co-precipitation as Zn phosphate ($Zn_3(PO_4)_2$) in both alkaline soils (submerged conditions) and acidic soils. This causes Zn an inaccessible soil element to be absorbed by plants [21,22].

The Zn and P are essential for the metabolism of carbohydrates in plants, to catalyze the enzymes such as carbonic anhydrase, fructose-1,6-bisphosphate, and aldolase. The rapid drop-in activity of these three enzymes slows the metabolism of carbohydrates in plants [21–25]. Nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (NADP), the crucial nutrients involved in plant glucose metabolism, are made up exclusively of P [26]. The ribosome, a location for protein synthesis, disintegrates without Zn because it is part of its structural makeup. Protein synthesis and protein levels are severely reduced in Zn-deficient plants, which results in the accumulation of free amino acids, as Zn is essential for synthesizing numerous groups of amino acids into enzymes and proteins [25,27–29]. Consequently, a deficiency in Zn prevents the plant from producing specific enzymes and proteins. P, which is a crucial component of adenosine triphosphate (ATP) and aids in the activation of amino acids for protein synthesis, operates similarly [26].

The availability of mineral nutrients to plants also affects the production of chlorophyll. The dynamics of leaf surface development and area are greatly influenced by mineral nutrition, which is reflected in leaf surface area, photosynthetic capability, and net photosynthetic productivity. Among the minerals necessary for photosynthesis, Zn and P are essential for the production of chlorophyll in plants. Zn is a key enzyme co-factor necessary for the regular functioning of the photosynthetic apparatus and plays a significant role in the control of chlorophyll production in plants. In addition, P contributes to the stability of the chlorophyll molecule as a crucial component [30].

For millions of people around the world, groundnuts (*Arachis hypogaea* L.) are a significant source of cooking oil. As integrated food components [31] and oleo chemicals [32], they give food products a distinct flavor and texture. In seeds, Zn plays a significant role in the production of proteins and lipids [33]. Groundnut-growing soils in India have significant yield loss owing to a 50% Zn shortfall [34,35]. Zn deficiency in groundnuts results in interveinal yellow-vein chlorosis and uneven mottle on upper leaves. The entire leaflet turned chlorotic in conditions of severe deficit [36].

The Zn fertilization boosts nodulation, chlorophyll content, and pod yield in soils with low levels of Zn. According to ILZRO [37], a deficit impaired pegging but had no overt leaf symptoms. Numerous enzymes are activated by Zn [38,39], and this can directly affect developmental processes that result in noticeable variations in seed oil quality. The P is also necessary for the generation of high-quality seeds because it is a coenzyme in reactions

that transfer energy; in photosynthesis, energy is used in the form of ATP and NADP. Following that, this energy is utilized in the photosynthetic fixation of CO_2 and the production of lipids and other vital organic molecules [26].

Thus, employing Zn and P becomes crucial for plant development as they are necessary for controlling the biochemical activities in plants. Even though they accumulate in the soil, they are often found in insoluble forms as a result of their antagonistic interactions. The relationship between the plant and microbes is self-sustaining; the former uses the latter to extract soluble nutrients from the soil, which increases soil fertility. In the rhizosphere, microorganisms that can convert a variety of unavailable metal forms into available ones can be isolated and used to supply plants with Zn and P in their stable forms.

Pseudomonas aeruginosa is a rhizobacterium that stimulates plant development by secreting phytohormones, siderophores, hydrogen cyanide, and lytic enzymes, solubilizing P, K, and Zn, and so gaining great attention in the field of Biofertilization [40]. Therefore, in the current investigation, the impact of *P. aeruginosa*'s mineral solubilizing activity on the biochemical content of the groundnut plants cultivated under various treatments at regular 30-day intervals was examined.

2 Materials and methods

Laboratory assessment of the Zn-solubilizing ability of *P. aeruginosa* was carried out qualitatively and quantitatively under different cultural conditions [41,42]. Field experiments were also conducted to test its influence on the growth and yield of *A. hypogaea* [43].

The primary goal of the present investigation was to evaluate the impact of *P. aeruginosa*'s mineral (Zn and P)-solubilizing activity on the biochemical characterization of *A. hypogaea* L. A field study was performed on an agricultural farm at Kangeyam, Tirupur District, Tamil Nadu. The *P. aeruginosa* was formulated in a variety of ways, including solid forms employing lignite at a rate of 100 ml (10^9 CFU/ml) per 200 g and liquid forms using 3% poly vinyl pyrrolidone (PVP), which has a cell load of 10^9 CFU/ml. Before sowing, 100 seeds were treated with 5 g of solid-based inoculant with a cell load of 10^9 CFU/ml and also 5 ml of liquid inoculum with a cell load of 10^9 CFU/ml per 100 seeds and incubated for 6 h at room temperature.

The experimental field was set up using a randomized complete block design with five treatments (T1: control [uninoculated seeds]; T2: seeds treated with liquid inoculant;

T3: seeds treated with liquid inoculant and the soil amended with farmyard manure [FYM]; T4: soil amended with FYM alone; T5: seeds treated with lignite [solid] based bioinoculant) replicated four times on a 3 m by 3 m plot size. At regular intervals of 30 days, *P. aeruginosa*'s effects on the biochemical components of the randomly chosen plants were investigated.

2.1 The efficiency of *P. aeruginosa* in solubilizing minerals (Zn and P) present in the soil reserve (soil incubation study) [44]

The *P. aeruginosa*'s effects on the release of Zn and P from the soil reserve were investigated through a soil incubation study using field soil that was taken from an agricultural farm in Kangeyam, Tirupur District, Tamil Nadu. A 1 kg of field soil was taken in a series of pots. FYM was applied to one set of pots at a rate of 5%, while another set was left untreated. The test bacterial culture was centrifuged, and the supernatant solution was discarded after the bacteria were cultured in nutrient broth for 48 h. The bacterial pellets were rinsed with sterile distilled water, and 10 ml (10^9 CFU/ml) of cell suspension per 1 kg of soil was used to inoculate the pots. Additionally, an uninoculated control was also maintained. Throughout the incubation phase, the soil's moisture content was maintained. Minerals were extracted and their Zn and P availabilities were evaluated after 10 days of incubation.

2.1.1 Estimation of soil's available Zn [45]

Diethylene triamine penta acetic acid (DTPA) solution (0.005 M) in a 1:2 ratio was poured into 20 ml of a 100 ml Erlenmeyer flask containing 10 g of soil, and the mixture was then agitated for 2 h. The extract was obtained after the contents were filtered via the Whatman No. 42 filter paper. The amount of available Zn in the soil was measured using an atomic absorption spectrometer.

2.1.1.1 Preparation of DTPA extraction solution

To make the DTPA extraction solution, 200 ml of distilled water was used to dissolve 149.2 g of 0.1 M triethanolamine, 19.67 g of 0.005 M DTPA, and 14.7 g of 1 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. This solution was then concentrated up to 10 l. 1 N HCl was used to bring the pH down to 7.3 ± 0.05 .

2.1.2 Estimation of available P in soil

The available P in soil was estimated by Olsen's method [46].

2.1.2.1 Reagents preparation

- i. Sodium bicarbonate (0.5 M),
- ii. Activated carbon, and
- iii. 5 N sulfuric acid.

Conc. H_2SO_4 , 137 ml was added to 1 l of distilled water

2.1.2.2 Reagent A

- (a) Ammonium molybdate (12 g) was dissolved in distilled water (250 ml).
- (b) Antimony potassium tartrate (0.291 g) was dissolved in distilled water (100 ml).
- (c) 100 ml of 5 N H_2SO_4 was prepared by dissolving 137 ml of conc. H_2SO_4 in 1 l of distilled water.
- (d) The three reagents were mixed as described above and the volume was increased up to 2 l with distilled water.

2.1.2.3 Reagent B

Ascorbic acid (1.056 g) was dissolved in reagent A (200 ml).

2.1.2.4 Procedure

One teaspoon of activated carbon and 50 ml of 0.5 M sodium bicarbonate was added to a 100 ml Erlenmeyer flask containing 5 g of soil. The mixture was filtered through Whatman No. 40 filter paper after being agitated for 30 min in an orbital shaker. To get a clear filtrate, additional activated carbon may have to be added. Using 5 N H_2SO_4 , 5 ml of filtrate was pipetted into a 25 ml volumetric flask and acidified to pH 5.0. After the mixture was diluted to 20 ml, 4 ml of freshly prepared reagent B was added, and the volume was then increased to 25 ml with distilled water. The flask was then thoroughly shaken and let to stand for 10 min. In a Vis spectrophotometer, the resulting blue color's absorbance was detected at 660 nm. Distilled water was used as blank. The available P is represented in mg/l, and the unknowns were determined using the standard graph.

2.2 Biochemical characterization of the groundnut plants

The biochemical contents of the fresh leaves and seeds of groundnut plants such as total carbohydrate, soluble

protein, total chlorophyll content of the leaves, oil percentage, iodine value, saponification value, and free fatty acids content of the oil extracted from the groundnut seeds were assessed at regular 30-day intervals in triplicates.

2.2.1 Total carbohydrate

The total carbohydrate content of groundnut plant leaves and seeds was evaluated by the Hedge and Hofreiter method [47]. A 1 g of the fresh sample (leaves as well as seeds of groundnut plants) was hydrolyzed individually in a water bath for 3 h with 2.5 N HCl (5 ml), and after cooling to room temperature, it was neutralized with Na_2CO_3 . 4 ml of Anthrone was added to 0.1 ml of individual extracts in test tubes after they had been prepared to a volume of 1 ml with distilled water. Color intensity at 630 nm was measured after the tubes had been kept boiling for 5 min. The glucose standard graph was used to compute the total carbohydrate concentration of the samples.

2.2.2 Soluble protein

By using Bradford's method [48], the total soluble protein in fresh leaves and seeds was determined. The samples were weighed at 0.1 g, homogenized in 2 ml of 0.1 M phosphate buffer pH 6.8 in a mortar and pestle that had been pre-chilled while homogenization was taking place, maintained on ice, then centrifuged at 5,000 rpm for 10 min at 4°C. Equal parts of 20% trichloroacetic acid that had already been cooled were added to the supernatant before being centrifuged once more at 5,000 rpm. The particle was rinsed with acetone and then dissolved in 1 ml of 0.1 N NaOH after the supernatant was discarded. For optimal color development, tubes were covered with aluminum foil and kept in the dark, and 5 ml of Bradford reagent was added to a 1 ml aliquot. The amount of soluble protein was estimated and represented as mg/g using the standard bovine serum albumin (Sigma-Aldrich) as a reference.

2.2.3 Chlorophyll content of the leaves [49]

2.2.3.1 Extraction of chlorophyll

A 20 ml of 80% acetone was used to grind one gram of fresh leaves. It was then centrifuged for 5 min at 5,000 rpm. The process was repeated until the residue was colorless after the supernatant was transferred. At 645 and 663 nm, the solution's absorbance was read in comparison to a solvent (acetone) blank.

2.2.3.2 Estimation of chlorophyll content

The following equation was used to determine the levels of chlorophyll *a* (1), chlorophyll *b* (2), and total chlorophyll (3):

$$\text{Chlorophyll } a: 12.7(A663) - 2.69(A645), \quad (1)$$

$$\text{Chlorophyll } b: 22.9(A645) - 4.68(A663), \quad (2)$$

$$\text{Total Chlorophyll: } 20.2(A645) + 8.02(A663). \quad (3)$$

2.2.4 Extraction of groundnut oil

Groundnut seeds were powdered using a laboratory Ball Mill. Using petroleum ether (60°C) for 6 h, oil was extracted using the Soxhlet technique [50].

2.2.4.1 Determination of percentage yield

The percentage (%) yield was calculated using [51]

$$\text{Percentage yield of oil} = \frac{\text{Weight of oil}}{\text{Weight of sample(g/nut)}} \times 100.$$

2.2.4.2 Iodine value

According to the AOAC method [51], the iodine value of oils was determined. A conical flask containing 0.25 g of oil was filled with a 10 ml solution of CCl_4 . Similarly, 30 ml of the Hanus solution was added, and the mixture was left to stand for 45 min while being occasionally shaken. Any free iodine on the stopper was rinsed down with 10 ml of 10% KI solution and 100 ml of distilled water. The iodine was titrated using a previously standardized $\text{Na}_2\text{S}_2\text{O}_3$ solution, which was added gradually while being constantly shaken until the yellow solution became nearly colorless. The addition of a few drops of the starch indicator was followed by a series of titrations until the blue color completely vanished. Iodine that was still present in the CCl_4 solution was vigorously shaken out of the bottle so that it could be absorbed by the KI solution. The amount of $\text{Na}_2\text{S}_2\text{O}_3$ solution needed for the experiment was recorded. Along with the sample, a control experiment was conducted.

The following calculation was used to determine the percent weight of iodine that the oil sample had absorbed:

$$I = \frac{(B - A) \times N \times 0.127 \times 100}{W},$$

1 ml 1 N $\text{Na}_2\text{S}_2\text{O}_3$ = 0.127 g; *B* = ml of 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ required by blank; *A* = ml of 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$ required by oil sample; *N* = normality of $\text{Na}_2\text{S}_2\text{O}_3$; and *W* = weight of oil in g.

2.2.4.3 Saponification value

To calculate the saponification value, 1.0 g of oil was placed in a conical flask together with 25 ml of 0.5 N alcoholic KOH, which was then heated under a reserved condenser for 30–40 min to ensure that the sample was completely dissolved. Phenolphthalein was added and titrated with 0.2 N HCl after chilling the sample until a pink endpoint was obtained [52].

The saponification value was calculated by the following formula:

$$\text{Saponification value } (S) = \frac{(B - T) \times N \times 56.1}{W}$$

S = saponification value; *B* = ml of HCl required by blank; *T* = ml of HCl required by oil sample; *N* = normality of HCl; *W* = weight of oil in g; and 56.1 = equivalent weight of KOH.

2.2.4.4 Free fatty acids content of the oil

Around 2 ml of 1% phenolphthalein solution was added to 20 ml of ethanol–diethyl ether (1:1 v/v) mixture, and the mixture was neutralized with 0.10 M NaOH solution. The neutralized mixture was then combined with 5 g of each oil sample, and the combination was titrated against a 0.10 M NaOH solution while being constantly shaken until a pink color appeared and lasted for 15 min. The free fatty acid value was calculated using the titer values [53].

2.3 Analysis of the characteristics of soil samples subjected to different treatments

The different parameters of the soil samples subjected to different treatments such as pH, electrical conductivity (EC), CaCO_3 , organic carbon, N, P, K, and Zn were analyzed before and after the cultivation of the groundnut

2.3.1 pH

In a conical flask, 10 g of soil and 100 ml of distilled water were combined, and the mixture was shaken vigorously for 30 min. It was filtered through muslin cloth after 30 min, and the filtrate was used to calculate the pH using an Elico pH meter.

2.3.2 EC

The soil water ratio of 1:10 was used to determine the EC of the soil samples, and the results are expressed in dS/m, according to the method of Jackson [54].

2.3.3 CaCO₃

The soil samples were crushed and sieved through a 2 mm sieve after being air-dried. As stated by Dreleimanis [55], the equivalent amount of calcium carbonate (E-CaCO₃) was measured using 0.5 M HCl for dissolving CaCO₃ and determined between the two titrations of the surplus acid by using 0.2 M NaOH.

2.3.4 Organic carbon

The titration method, recommended by Walkley and Black [56], was used to determine the amount of organic carbon in the soil samples.

2.3.5 N content

The Kjeldahl method was used to determine the soil sample's total N content, and the calculation was made per Vogel's [57] recommendations.

2.3.6 K content

By using the titration method recommended by Jackson [54], the K content of the soil sample was calculated.

2.3.7 Zn

By using the method outlined in Section 2.1.1, the available Zn present in the soil samples was determined.

2.3.8 P

By using the method outlined in Section 2.1.2, the available P present in the soil samples was determined.

2.4 Statistical analysis

A randomized complete block design with four replicates of each treatment was used to set up plots with various treatments in field trials. Analysis of variance was used to analyze the data, and Duncan's multiple range test was used to compare means at $p \leq 0.05$. The SPSS software (2019 version) was used for all analyses.

3 Results and discussion

3.1 Efficiency of *P. Aeruginosa* in solubilizing minerals (Zn and P) present in the soil reserve (soil incubation study)

This investigation was carried out to ascertain the impact of *P. aeruginosa* inoculation to solubilize Zn and P present in the soil. The results showed no significant difference in the mineral solubilizing ability of bacteria that is applied alone versus applied along with FYM. But the efficacy of the organism to solubilize Zn was little enhanced when it was applied along with FYM (6.86 mg/l) compared to mineral solubilizing bacteria alone (6.02 mg/l) and control (3.15 mg/l) (Table 1). By providing a nutrient-rich environment that supports the growth and activity of this beneficial microbe (*P. aeruginosa*), and by enhancing their effectiveness in producing organic acid, which may have increased Zn availability in the soil, FYM application has the potential to increase available Zn concentrations beyond the deficiency threshold limits. Several forms of Zn, including ZnO, ZnCO₃, and ZnS, that are present in the soil can be dissolved by the Zn-solubilizing bacterial strains producing organic acids mainly 2-ketogluconic acid and gluconic acid [58,59]. Furthermore, FYM has a significant role in regulating temperature, retaining moisture, increasing soil organic carbon (SOC) availability, and improving nutrient availability and soil pH [60–62]. These actions and conditions generate an environment that is conducive to microbial growth and activity [63,64]. Since certain Zn-solubilizing microorganism (ZSM) species are copiotrophic which favor habitats that are high in carbon and nutrients, the addition of FYM may increase the availability of carbon and nutrients in the soil, which will in turn encourage the activity of copiotrophic ZSM species in soil [65,66]. Applying FYM and Zn-solubilizing bacteria raised the soil's DTPA-Zn content, as reported by Senthil Kumar et al. [67]. ZSMs are therefore

Table 1: Release of minerals in the soil inoculated with *P. aeruginosa*

Treatments	Amount of minerals (mg/l)	
	Zn	P
Control	3.15 ± 0.50 ^b	13.67 ± 0.58 ^b
MSB	6.02 ± 0.18 ^{ab}	14.33 ± 0.29 ^{ab}
MSB + FYM	6.86 ± 0.03 ^a	14.83 ± 0.29 ^a
SD CD ($p < 0.05$)	0.2511	0.3333
	0.6145	0.8157

Values are mean ± SD of three samples; MSB: mineral-solubilizing bacteria; FYM: farmyard manure.

viable substitutes for increasing Zn bioavailability and supplementing plants in an economical, environmentally responsible, and sustainable way [68,69].

Regarding the amount of P that was readily available, inoculating *P. aeruginosa* with FYM led to the highest concentration (14.83 mg/l), surpassing both the control (13.67 mg/l) and the organism alone (14.33 mg/l) (Table 1). By encouraging microbial growth in soils, FYM enables organic matter to be mineralized by microorganisms, which releases nutrients into the crop gradually [44]. Primary P minerals, such as apatite, and secondary clay minerals, such as calcium, iron, and aluminum phosphates, are sources of P in soil and play a crucial role in preserving P availability and building via desorption and dissolution processes [70]. Applying FYM and biofertilizer causes the manure to break down and release macro and micronutrients, including P. It also dissolves the type of P that is not available by releasing organic acids throughout the breakdown process [70]. Because of the mineralization, solubilization, and translocation action of P-solubilizing bacteria through the generation of organic acid and proton extrusion, the use of biofertilizers has also increased the availability of P to plants [71,72]. Nahas [73] attributed the increase in solubilization of insoluble phosphate due to organic acids produced by microbes to the drop in soil pH, soil cations chelating, and competition with phosphate for adsorption sites in the soil solution. Kaur and Reddy [74] and Shahzad et al. [75] also observed improved soil P status with inoculation with phosphate-solubilizing bacteria resulting from higher alkaline phosphatase activity. Thus, the addition of FYM not only adds up to nutrient pools but also creates a favorable root zone environment for better root activity and nutrient uptake.

3.2 Effect of different treatments on the total carbohydrate and soluble protein contents of the leaves and seeds of groundnut plants

Mineral-solubilizing bacteria positively influence the biochemical content of plants by enhancing Zn and P uptake, which in turn improves chlorophyll synthesis, protein content, carbohydrate metabolism, antioxidant activity, phytohormone levels, nutrient use efficiency, and secondary metabolite production. This results in healthier plants with better growth, higher stress resistance, and improved overall productivity [76]. Therefore, the ability of the mineral-solubilizing bacterium *P. aeruginosa* to raise the carbohydrate and soluble protein content of the leaves of groundnut plants grown under diverse circumstances was evaluated at regular intervals of 30 days. The findings showed that the soluble protein and carbohydrate content of the leaves of T2, T3, and T4 plants recorded maximum values in comparison to the control (Table 2). On the 120th day of harvest, estimates of the groundnut plants' seeds' carbohydrate and soluble protein contents were also made. Additionally, the results revealed that T2, T3, and T4 plants' groundnut seeds had the highest value when compared to the control (Table 3). This could be because of the common factor of all the three treatments "*P. aeruginosa* and FYM" provided vital nutrients for controlling biochemical activity in plants along with as nearly all of the enzymes and coenzymes involved in biochemical activity are activated by Zn [23] and P [77,78], respectively, through its mineral solubilizing ability. Studies have shown that MSB can increase the carbohydrate content in plants. For instance, a study on wheat, rice, and maize found

Table 2: Effect of different treatments on the total carbohydrate and protein contents of the leaves of groundnut plants

Treatments	Total carbohydrate content of the plants (mg/g)				Protein Content of the Plants (mg/g)			
	30th	60th	90th	120th	30th	60th	90th	120th
T1	0.20 ± 0.03 ^c	0.21 ± 0.02 ^c	1.64 ± 0.11 ^c	2.52 ± 0.19 ^b	2.17 ± 0.11 ^b	3.44 ± 0.22 ^a	3.68 ± 0.17 ^c	3.93 ± 0.28 ^b
T2	0.32 ± 0.02 ^{ab}	0.42 ± 0.04 ^a	2.24 ± 0.16 ^b	2.96 ± 0.21 ^{ab}	2.25 ± 0.16 ^b	3.85 ± 0.19 ^a	3.94 ± 0.60 ^c	4.09 ± 0.21 ^b
T3	0.41 ± 0.09 ^a	0.49 ± 0.05 ^a	2.68 ± 0.24 ^a	3.53 ± 0.31 ^a	3.25 ± 0.34 ^a	4.05 ± 0.71 ^a	4.86 ± 0.21 ^a	5.06 ± 0.30 ^a
T4	0.32 ± 0.04 ^{ab}	0.46 ± 0.07 ^a	2.34 ± 0.17 ^{ab}	3.08 ± 0.74 ^{ab}	2.5 ± 0.38 ^b	3.93 ± 0.64 ^a	4.05 ± 0.77 ^{ab}	4.88 ± 0.29 ^a
T5	0.3 ± 0.06 ^b	0.31 ± 0.08 ^b	1.73 ± 0.36 ^c	2.56 ± 0.39 ^b	2.16 ± 0.18 ^b	3.68 ± 0.37 ^a	3.81 ± 0.74 ^c	4.15 ± 0.44 ^b
Treatments: 17.393***Days: 568.473***					Treatments: 14.139***Days: 81.132***			
Treatments × days: 2.652***					Treatments × days: 0.829***			

Values are mean ± SD of four replication samples in each group; *** indicates $p < 0.001$; ** indicates $p < 0.01$; and * indicates $p < 0.05$ versus control. T1: control; T2: seeds treated with liquid inoculant; T3: seeds treated with liquid inoculant and the soil amended with FYM; T4: soil amended with FYM alone; T5: seeds treated with lignite (solid) based bioinoculant.

Table 3: Effect of different treatments on the carbohydrate and protein contents of the seeds

Treatments	Total carbohydrate (mg/g)	Soluble protein (mg/g)
T1	1.68 ± 0.27 ^c	7.13 ± 0.77 ^c
T2	2.22 ± 0.30 ^{ab}	8.82 ± 0.16 ^b
T3	2.56 ± 0.45 ^a	10.1 ± 0.68 ^a
T4	2.41 ± 0.19 ^{ab}	8.88 ± 0.13 ^b
T5	1.96 ± 0.44 ^{bc}	8.08 ± 0.53 ^b

Values are mean ± SD of four replication samples.

T1: control; T2: seeds treated with liquid inoculant; T3: seeds treated with liquid inoculant and the soil amended with FYM; T4: soil amended with FYM alone; and T5: seeds treated with lignite (solid)-based bioinoculant.

that MSB increased the carbohydrate content in these crops [79,80]. This is likely since Zn and P play a crucial role in carbohydrate metabolism, and increased Zn and P availability can enhance photosynthesis and carbohydrate production. MSB has also been found to increase the protein content in plants. A study on groundnut plants found that zinc solubilizing bacteria (ZSB) increased the protein content in the plants, likely due to the increased availability of Zn, which is essential for protein synthesis [43,81]. Additionally, Zn-mobilizing plant growth-promoting rhizobacteria (PGPR) have been found to increase protein content in rice [82]. Additionally, threonine, an amino acid, has been shown to promote bacterial solubilization and plant uptake of nutrients, including P, which can lead to increased protein production [83]. According to Yousefi and Sadeghi [84], the application of FYM and vermicompost significantly increased the carbohydrate and protein content of wheat. This is likely due to the improved soil fertility and nutrient availability provided by FYM, which promotes healthy plant growth and development [85]. A similar finding was made by Prathiba and Siddalingeshwara [86], who investigated the impact of plant growth-promoting activity of *Bacillus subtilis* and *Pseudomonas fluorescens* as *Rhizobacteria* on seed quality of sorghum and observed that both strains were effective in enhancing seed quality, including seed germination, vigor index, and nutritional quality, including protein and carbohydrate contents. The favorable impact of *P. fluorescens* strain Psd on wheat production and nutritional quality was also evaluated by Sirohi *et al.* [87], who reported that seeds from plants collected from the strain Psd-infected soil had higher carbohydrate and protein contents than seeds from controls. Furthermore, Mathivanan *et al.* [88] evaluated the impact of PGPR (*Rhizobium* + *Pseudomonas* + *Bacillus*) on the nutritional value of groundnut (*A. hypogaea* L.) seedlings. Their findings showed that high biochemical content was observed in the groundnut seedlings grown using PGPR

compared to uninoculated control. The current study thus shows the ability of *P. aeruginosa* to promote plant growth with effected the biochemical composition of the plants in conjunction with organic manure, which acted as a nutrient reservoir for its microbial activity.

3.3 Effect of different treatments on the total chlorophyll content of the leaves of groundnut plants

The ability of *P. aeruginosa* to promote plant growth by increasing the chlorophyll content (chlorophyll “a,” chlorophyll “b,” and total chlorophyll) of the leaves of groundnut plants raised under different treatments was assessed in the current experimental study at regular intervals of 30 days. The results demonstrated that, in comparison to the control, the groundnut leaves of T3 plants showed a significant ($p < 0.05$) increase in the levels of chlorophyll (chlorophyll “a,” chlorophyll “b,” and total chlorophyll) (Figure 1). Plants with low levels of Zn have less efficient photosynthesis [89]. It causes a 50–70% reduction in net photosynthesis in many plant species [90,91]. This decline in net photosynthesis in plants is ascribed to a reduction in Hill reaction activity, which may be brought on by a drop in chlorophyll concentration [92] and a disruption in the structure of the chloroplasts [25] in Zn-deficient plants. Under Zn-deficient conditions, Sharma *et al.* [92] reported that cauliflower leaves had significantly lower stomatal conductance, which resulted in a drop in CO₂ supply, which in turn caused a considerable decrease in photosynthesis. In addition, Zn controls the synthesis of carotenoids and chlorophyll in plants, which is crucial for the efficient operation of the photosynthetic apparatus [93]. The application of MSB along with FYM can have a positive impact on the chlorophyll contents of plants. The chlorophyll content of plants has also been found to be influenced by the application of FYM and bioinoculants. Mean chlorophyll content values were higher with the application of 9.6 ton/ha of FYM [94]. Additionally, the use of bioinoculant has been found to promote the growth of beneficial microorganisms in the soil, which can help to increase the availability of nutrients, including sulfur, which is essential for chlorophyll formation [95]. Zn plays a crucial role in the synthesis of chlorophyll, and its deficiency can lead to reduced chlorophyll content and impaired photosynthesis [43]. Studies have demonstrated that the application of ZSB along with FYM can result in increased chlorophyll content, leading to improved photosynthesis and plant productivity [96,97]. The exact mechanisms behind this interaction are not fully

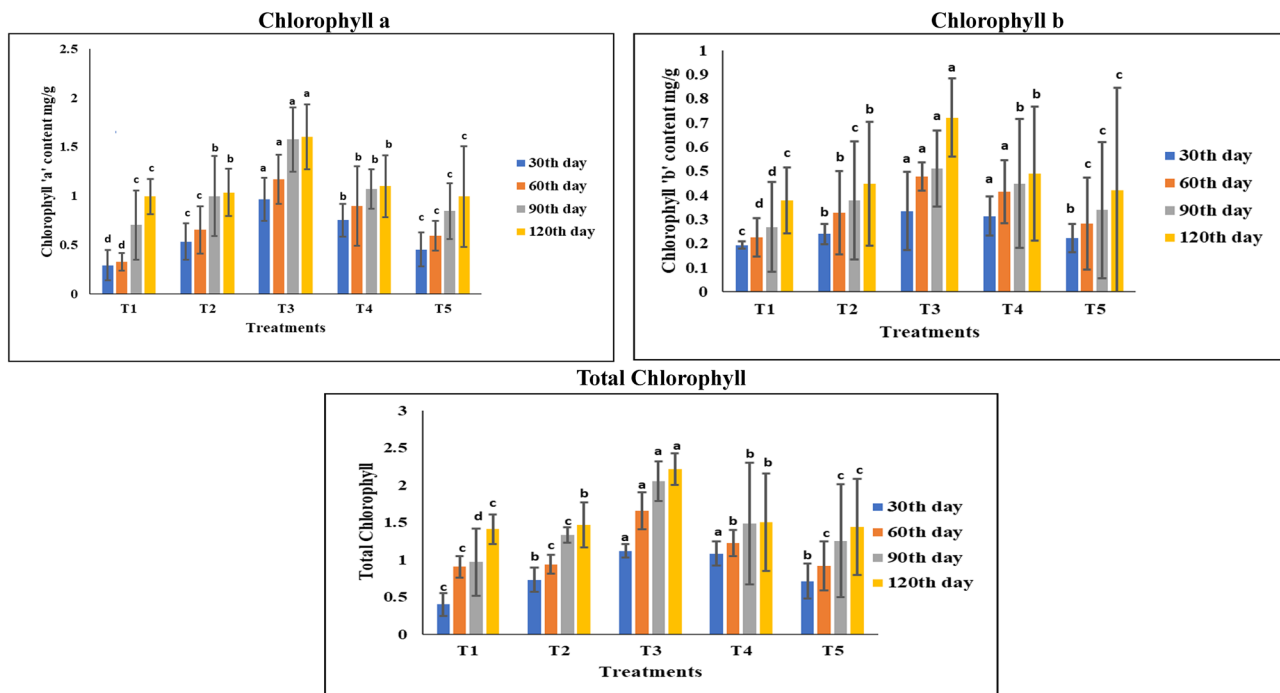


Figure 1: Effect of different treatments on the chlorophyll content of the leaves of groundnut plant. Values are mean $\pm$ SD of four replication samples. T1: control; T2: seeds treated with liquid inoculant; T3: seeds treated with liquid inoculant and the soil amended with FYM; T4: soil amended with FYM alone; and T5: seeds treated with lignite (solid)-based bioinoculant.

understood, but it is believed that the ZSB's ability to solubilize Zn and make it available to plants, combined with the nutrient-rich properties of FYM, contributes to the observed effects [79]. Research has shown that ZSB can increase the availability of Zn in the soil, which is an essential micronutrient for plant growth and development [98]. Studies have demonstrated that the application of phosphorus solubilizing bacteria (PSB) along with FYM can lead to a significant increase in chlorophyll content in plants. For instance, a study on okra plants found that the integrated use of PSB and FYM resulted in a 62% increase in chlorophyll content [99]. Similarly, another study on tomato plants showed that the inoculation of PSB strains, either singly or in combination, significantly increased the chlorophyll content of leaves [100]. The increased chlorophyll content in plants can be attributed to the ability

of PSB to solubilize P in the soil, making it available to plants. The P is an essential nutrient for plant growth and development, and its availability can limit plant productivity. By solubilizing P, PSB can enhance plant growth and development, leading to increased chlorophyll content [101].

3.4 Analysis of percentage of oil content and the chemical characteristics of the oil extracted from the groundnut seeds harvested on the 120th day

Groundnut oil is an organic substance made from groundnut seeds that is known to taste and smell like its parent legume.

Table 4: Effect of different treatments on the oil percentage and the chemical characteristics of the oil extracted from the groundnut seeds

Treatments	Oil (%)	Iodine (mg KOH/g oil)	Saponification (mg KOH/g oil)	Free fatty acid (mg KOH/g oil)
T1	44.03 $\pm$ 1.34 ^c	84.88 $\pm$ 0.77 ^c	191.01 $\pm$ 0.91 ^d	3.62 $\pm$ 0.51 ^a
T2	46.05 $\pm$ 1.14 ^c	86.92 $\pm$ 0.09 ^b	194.36 $\pm$ 0.99 ^{ab}	2.73 $\pm$ 0.38 ^{bc}
T3	54.13 $\pm$ 1.54 ^a	88.47 $\pm$ 0.44 ^a	195.56 $\pm$ 1.05 ^a	2.23 $\pm$ 0.53 ^c
T4	50.08 $\pm$ 1.39 ^b	87.17 $\pm$ 0.51 ^b	192.85 $\pm$ 0.75 ^c	3.12 $\pm$ 0.48 ^{ab}
T5	45.1 $\pm$ 1.29 ^c	86.24 $\pm$ 0.85 ^b	193.06 $\pm$ 0.77 ^{bc}	2.97 $\pm$ 0.30 ^{ab}

Values are mean $\pm$ SD of four replication samples in each group.

Oil from the seeds of the following treatments: T1: control; T2: seeds treated with liquid formulation; T3: seeds treated with liquid formulation and the soil amended with FYM; T4: soil amended with FYM alone; and T5: seeds treated with lignite (solid)-based bioinoculant.

In Table 4, the oil content and chemical properties of the oil extracted from the peanut seeds of the plants through various treatments are shown. It was a light-yellow tint and liquid at ambient temperature (Figure 2). The amount of crude oil in the peanut seeds of five different treatments ranged from

54.13 to 44.03% of the dry weight of the seeds (Table 4). The crude oil content varied significantly ($p \leq 0.05$) among the seeds of the five different treatments. This may be attributable to the additive effects of liquid inoculant (*P. aeruginosa*) and organic manure, which would have enhanced seed oil content.

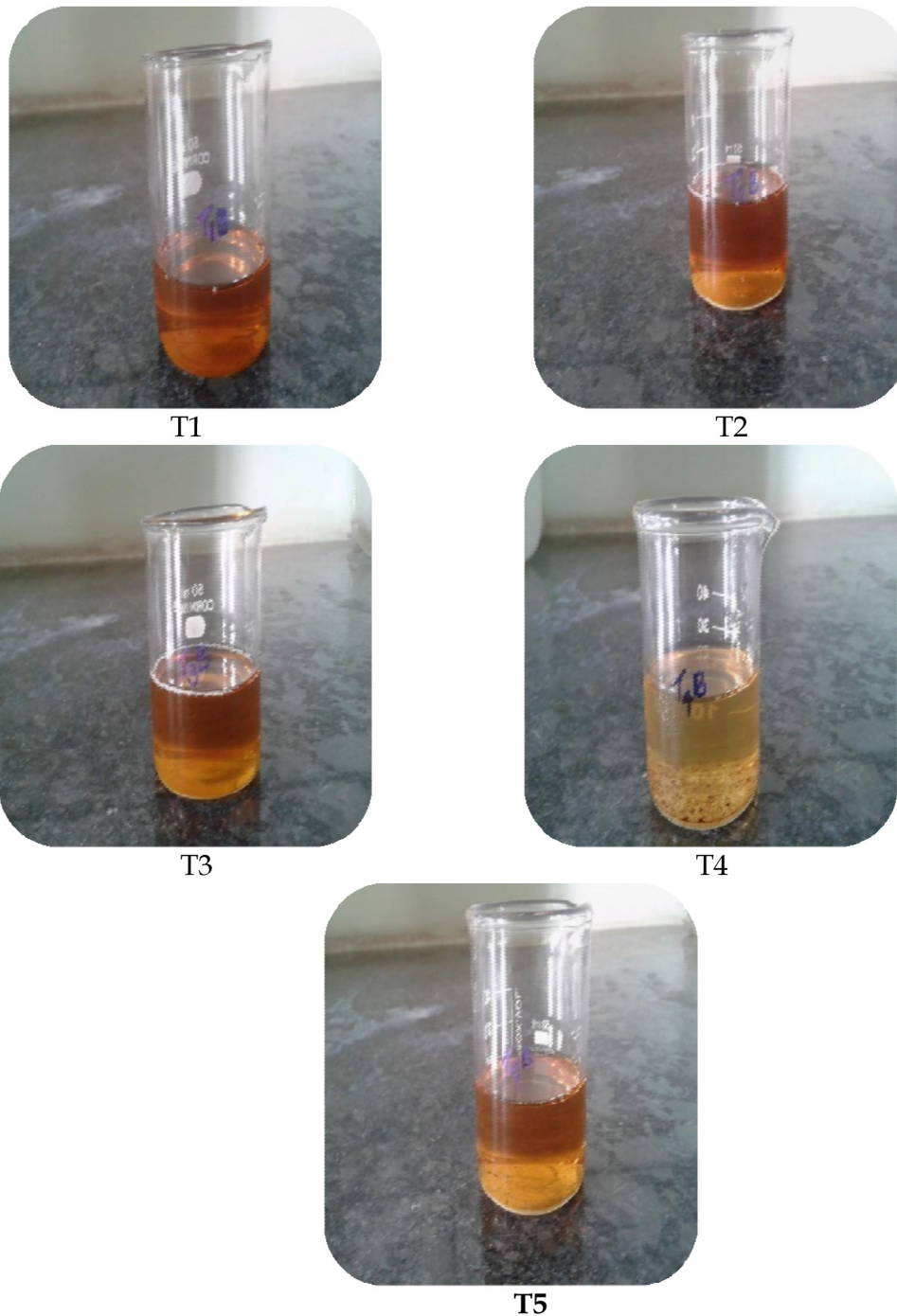


Figure 2: Oil extracted from the seeds of groundnut plants harvested on the 120th day. Oil from the seeds of the following treatments: T1: control; T2: seeds treated with liquid inoculant; T3: seeds treated with liquid inoculant and the soil amended with FYM; T4: soil amended with FYM alone; and T5: seeds treated with lignite (solid)-based bioinoculant.

The degree of oil unsaturation can be gauged by the iodine value. For certain kinds of oil or fat, it remains constant. The iodine value is a valuable metric for analyzing the oxidative rancidity of oils since the higher the unsaturation, the higher the probability of the oils becoming rancid [102]. The findings of the iodine value of oil that was extracted from groundnut seeds under various treatments are shown in Table 4. The control had the least iodine content, at 84.88 mg KOH/g oil. A decrease in iodine value is a sign of lipid oxidation, and this could be because metallic ions were present and accelerated or allowed oxidation after hydroperoxide formed [103–107]. Oil from plant seeds grown using a combination of organic manure and liquid inoculant (T3) had the highest iodine content (88.47 mg KOH/g oil) when compared to organic manure alone (87.17 mg KOH/g oil). The iodine content of the oil extracted from the seeds of T2 and T5 plants yielded identical results.

The total quantity of milligrams of potassium hydroxide that reacts with one gram of sample is the saponification value, which is used to assess the amount of alkali reactive groups in fats and oils [101]. In comparison to the control (191.01 mg KOH/g), the oil from the seeds of T3 plants had a greater saponification value (195.56 mg KOH/g). Similar outcomes were seen in the seeds of the T2 and T5 plants. Higher saponification values indicate a higher percentage of lower fatty acids since they are inversely related to the average molecular weight or chain length of the fatty acids [108]. Due to the oil's high saponification value, its potential use in the saponification sector may be suggested.

The acid number or acid value refers to the quantity of free fatty acids. It specifies the oil's functional characteristics, shelf life, nutritional value, and flavors [109]. So, the amount of free fatty acids determines the oil's quality. The results for the oil's free fatty acid content are displayed in Table 4. Compared to control plants (3.62 g oleic acid), the oil recovered from the seeds of T3 plants contained the least quantity of free fatty acid (2.23 g). In comparison to other treatments, the oil extracted from the seeds of the T2 and T5 plants produced comparable results. The stability of the products is shown by the low free fatty acid content of the oil [110]. If oil is stored for a long time, its unpleasant flavor and odor are caused by the existence of free fatty acids and other fatty components [111]. Because the sample of oil obtained from various treatments had a pleasant smell. This can be caused by the low levels of free fatty acids present. The oil's suitability for human ingestion increases with a decrease in free fatty acid content [112].

As shown by the results, the application of liquid inoculant along with organic manure (T3) had a significant impact on the oil yield and its chemical characteristics,

which may be attributable to the availability of vital nutrients like Zn and P due to *P. aeruginosa*'s activity in mineral solubilizing. Similar results were observed by Radwan and Awad [113] who discovered that applying biofertilizer (*Azospirillum* sp. and *Pseudomonas* sp.) together with organic amendments significantly increased the oil yield and quality of peanut seeds in comparison to chemical fertilizer treatment. Safwat and Badran [114] additionally observed that the cumin plants' essential oil production was increased when organic amendments mixed with *Azotobacter* and *Bacillus megaterium* were applied. Mekki et al. [115] also stated that soybean plants fertilized with biofertilizers in addition to organic manure had higher soybean seed oil levels. The largest amount of essential oil was produced when fennel plants were fertilized with organic manure and biofertilizers (*Azotobacter* and *Azospirillum*), according to Azzaz et al. [116]. As a result, in the current study, liquid inoculants and organic manure improved the oil content of the seeds of groundnut plants by making vital nutrients available to the plants through their action to promote plant growth.

3.5 Analysis of characteristics of soil before and after treatments

The addition of mineral-solubilizing bacteria to field soil in the form of a liquid bioinoculant would alter the soil's characteristics because of the microorganisms' ability to promote plant growth. Therefore, both before and after treatments, the parameters of the soil, such as pH, EC, CaCO_3 , organic carbon, and mineral content, including NPK and Zn, were investigated in this study. The results showed that all treatments significantly improved the soil's properties in comparison to the control (Table 5).

The pH of the soil, which represents the degree of acidity in the soil, has a big impact on microbial activity, plant nutrition availability, and even soil aggregate durability. The pH of the soil in the T3 plot was 7.5, which is lower than in the other treatments. This suggests that the addition of FYM and liquid bioinoculant affected the physical, chemical, and biological processes as well as the characteristics of the soil [117,118]. The pH of all the treated soils slightly decreased after the last groundnut plant was harvested. This could be because the bioinoculant *P. aeruginosa* produced organic acids, which led to a drop in pH, which is thought to be the main mechanism of metal solubilization. Because of the soil's natural ability to act as a buffer in this experiment, it was discovered that soil pH decline was far lower than in the culture medium. Similar

Table 5: Characteristics of the soil before and after treatments

Treatments	pH	EC (dS/m)	N (kg/ha)	P (kg/ha)	K (kg/ha)	Zn (mg/kg)	CaCO ₃ (%)	SOC (%)
Before treatment	8.3 ± 0.17 ^a	2.88 ± 0.32 ^a	273.75 ± 2.06 ^f	20.1 ± 2.12 ^e	182.25 ± 4.5 ^e	1.53 ± 0.10 ^d	2.53 ± 0.20 ^a	0.39 ± 0.03 ^d
T1	8.08 ± 0.15 ^{ab}	1.35 ± 0.15 ^b	417.00 ± 2.94 ^e	29.6 ± 0.81 ^d	204.75 ± 4.27 ^d	2.09 ± 0.78 ^{cd}	1.13 ± 0.28 ^c	0.75 ± 0.02 ^c
T2	7.8 ± 0.25 ^{bc}	0.37 ± 0.09 ^d	478.75 ± 1.70 ^c	37.33 ± 1.58 ^c	218.00 ± 4.89 ^c	5.38 ± 0.97 ^b	1.3 ± 0.24 ^c	0.84 ± 0.04 ^{ab}
T3	7.5 ± 0.31 ^c	0.30 ± 0.05 ^d	788.25 ± 1.25 ^a	59.65 ± 1.71 ^a	346.5 ± 4.72 ^a	7.24 ± 0.83 ^a	1.7 ± 0.14 ^b	1.91 ± 0.05 ^a
T4	7.9 ± 0.22 ^b	0.35 ± 0.10 ^d	483.75 ± 2.62 ^b	47.83 ± 1.22 ^b	231.5 ± 2.51 ^b	6.79 ± 1.26 ^a	1.3 ± 0.40 ^c	0.89 ± 0.06 ^a
T5	8 ± 0.18 ^{ab}	0.93 ± 0.14 ^c	432.00 ± 2.82 ^d	33.15 ± 1.49 ^d	207.25 ± 4.11 ^d	3.22 ± 0.99 ^c	1.23 ± 0.09 ^c	0.81 ± 0.08 ^{bc}

Values are mean ± SD of four replication samples.

T1: control; T2: seeds treated with liquid formulation; T3: seeds treated with liquid formulation and the soil amended with FYM; T4: soil amended with FYM alone; and T5: seeds treated with lignite (solid)-based bioinoculant.

to this, Son et al. [119] showed that the main source of mineral (such as Zn and P) solubilization was the acidification of the culture by bacteria; however, a high level of metal solubilization compared to *in vitro* may not be possible in soil because most soils have a great pH buffering capacity. Lakshmi et al. [120] also observed similar results, i.e., the highest reduction in soil pH with the combined application of biofertilizers.

Soil EC has a direct impact on plants growing in the soil, it is a valuable indication for controlling agricultural systems [121] as the quantity of moisture that soil particles can hold varies with soil EC. Good soil health is often indicated by an EC range of 0–1 dS/m [122]. According to the current study, all of the inoculant-treated soil had an EC range of 0.3–1.3, which is acceptable and demonstrates the soil's fertility [123].

The T3 plot showed a drop in EC contents, which is consistent with findings from Sushila et al. [124], who proposed that applying biofertilizers to saline soil would reduce soil salinity because the biofertilizers activate microorganisms in soil and increase the production of the enzyme dehydrogenase led to decrease the soil salinity compared with control. Additionally, Shaban and Attia [125] demonstrated that the values of EC dropped with an increase in mineral fertilizer in combination with biofertilizer, as compared to mineral fertilizers alone.

SOC plays a significant role in improving soil quality by improving ion exchange capacity, supporting biological component function, reducing bulk density, increasing water holding capacity, and increasing macro- and micronutrient availability [126,127]. SOC concentration greater than 1.7% indicates fertile soil [128]. In the current study, a T3 plot with a SOC concentration of 1.9% demonstrated that biofertilizers both directly and indirectly promote SOC accrual through microbial biomass turnover, the secretion of organic extracellular polymeric substances, or promoting plant growth through nutrient solubilization, associations, hormonal changes, etc. [129].

The physical and chemical properties of soil are impacted by the presence of CaCO₃. Excessive lime concentration might hinder root penetration and water circulation. Within the soil's fertile range, all the plots had CaCO₃ concentrations ranging between 1 and 2.5% [123]. As a result, all of the inoculant-treated soil has optimal pH, EC, SOC, and CaCO₃ content. Additionally, plant nutrients including N, P, K, and Zn were found to be within the fertile range of >480, >56, >280, and >0.6 kg/ha [123].

In terms of soil characteristics, the incorporation of mineral solubilizing bacteria with organic manure (T3) performed better than the other treatments, although there is no significant difference in the properties of the soil treated with different treatments when compared to liquid inoculant (T2), organic manure (T4), and control (T1) (Table 5). This could be a result of the advantageous effects of organic manures on nutrient availability. They accomplish this by directly or indirectly influencing the chemical processes that alter soil microbial activity and nutrients. These findings also correspond with the study by Mengel et al. [130], who stated that adding organic manure and biofertilizer promoted the synthesis of chemicals that control growth, enhancing the physico-chemical characteristics, microbial activity, and soil's ability to retain water. Mahapatra et al. [131] also observed that bacterial biofertilizers are crucial for boosting root accessibility to soil components including N, P, K, and Zn, which promotes rhizosphere biocontrol. Thus, the current study observed that the liquid inoculant (*P. aeruginosa*) and organic manure had a positive impact on the availability of N, P, K, and Zn as well as other soil parameters, demonstrating the essential role of this organism in the transformation reaction of vital nutrients in the soil through its numerous plant growth-promoting activities, such as mineral solubilization and phytohormone synthesis. Regenerative agriculture practices involving the incorporation of organic matter (FYM application and residue retention) among others [132] can strongly influence the soil physicochemical parameters and

biological properties and enhance soil fertility by stimulating microbial abundance and nutrient cycling and regulating SOC availability [99]. The application of organic matter (i.e., FYM) has been shown to improve soil physiochemical conditions [60] alongside stimulating a positive microbial link between nutrient availability [133], the regulation of SOC, and moisture.

4 Conclusion

The current study reveals that *P. aeruginosa* can effectively improve *A. hypogaea* L.'s biochemical profile by providing essential minerals (Zn and P) through the solubilization process. The incorporation of PVP proved to be a potential ingredient in *P. aeruginosa* liquid formulations as it protects bacteria by reducing their mortality and prolonging their shelf life. Liquid formulants supplementation in conjunction with FYM is also very beneficial because it provides the bioinoculant with a carbon and energy source, which improves nutrient availability, prolongs nutrient retention in the soil, and increases soil carbon sequestration, which reduces climate change by absorbing and storing atmospheric carbon dioxide. Thus, the study implies that crops cultivated using liquid bioinoculants will be more nutritious, leading to healthier products for consumers, as they boost nutrient availability and uptake. This kind of bioinoculant has the potential to replace chemical fertilizers, thereby reducing their adverse ecological impacts and leading to more sustainable agricultural practices. This is consistent with customers' growing demands for environmentally friendly and sustainable food production methods. Overall, bioinoculants generally aid in the advancement of a more sustainable agricultural system that is advantageous to both food producers and consumers.

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Ranjithkumar: performed the experiments and analyzed and interpreted the data and writing manuscript; Sinouvassane Djearamane, Lai-Hock Tey, Ling Shing Wong, and Saminathan Kayarohanam: methodology, writing and editing the manuscript; Natarajan Arumugam, Abdulrahman I. Almansour: investigation of the whole project; and Sakkarapalayam M. Mahalingam: writing and editing the manuscript.

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