

# Influence of cyanobacterial inoculum on the growth features and yield of peanut plants in sandy soil

**Comment [E1]:** Influence of Cyanobacterial Inoculum on the Growth Features and Yield of Peanut Plants in Sandy Soil

## Abstract

During the growing seasons in summer of 2021 and 2022, two field experiments were carried out at the Ismailia Agricultural Research Center Station, Egypt, to investigate the effect of cyanobacterial inoculation (*Anabaena oryzae* and *Nostoc muscorum*) on peanut plant growth, yield, and certain soil biological activities under different nitrogen fertilization ratios and There are three different applications: soil drenching with cyanobacteria filtrate, seed coating with cyanobacterial powder, and foliar spray with a dose of 60 ml per liter. The findings demonstrated that cyanobacteria inoculation of peanut plants generally improved peanut plant growth, resulting in significantly higher peanut and grain yields than uninoculated treatments. When compared to other tested treatments and types of applications, *N. muscorum*+ 75% N produced the highest peanut yield and plant characteristics, followed by *A. oryzae* + 75% N in soil drench application. Overall, cyanobacteria increased the amount of N, P, K, and Ca in peanut plants. Cyanobacteria inoculation improved soil fertility by increasing total bacterial and cyanobacterial count CO<sub>2</sub> evolution, and indole acetic acid contents in the peanut rhizosphere. In general, peanut growth in sandy soil conditions can benefit from cyanobacteria inoculation with 75% nitrogen amounts.

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**Comment [E4]:** The findings demonstrated that cyanobacteria inoculation of peanut plants generally improved plant growth, resulting in significantly higher grain yields than uninoculated treatments.

**Comment [E5]:** ...+ 75% nitrogen (N) produced ...

**Comment [E6]:** ...the amount of N, phosphorus (P), potassium (K), and calcium (Ca) in peanut plants.

**Comment [E7]:** ...count, carbon dioxide (CO<sub>2</sub>) evolution...

**Key words:** peanut, cyanobacteria, *Anabaena oryzae*, *Nostoc muscorum*, indole acetic acid (IAA).

## 1. INTRODUCTION

Peanut (*Arachis hypogea* L.) is one of the most important and cost-effective oleaginous crops farmed in the world's tropical and subtropical climates, owing to its oil, protein, and carbs

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From [Abdelghany et al. \(2022\)](#)

The peanut (*Arachis hypogea* L.) is one of the most essential and economical oleaginous crops grown in tropical and subtropical regions of the world, mainly because of its oil, protein, and carbohydrates [1]. Peanut seeds contain oil (45%), protein (26–28%), carbohydrates (20%), and fiber content (5%) [2], indicating their high nutritional value for both humans and animals. Peanuts have recently gained significant attention in Egypt, as they are able to grow under newly reclaimed sandy soil conditions [3].

[1]. Peanut seeds have a significant nutritional value for both people and animals since they contain oil (45%), protein (26-28%), carbs (20%), and fiber (5%) [2]. Groundnut oil, derived from the seed of the groundnut plant (*Arachis hypogea*), contains more potassium(K) than sodium(Na) and is a rich source of calcium (Ca), phosphorus(P), and magnesium (Mg), thiamin, vitamin E, selenium (Se), zinc (Zn), and arginine are also present. Researches demonstrated that diets high in groundnut oil are as effective as olive oil in preventing heart disease and are better for your heart than very low-fat diets [3].Peanuts have lately acquired popularity in Egypt due to their ability to grow on newly restored sandy soil conditions [4].

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...the groundnut plant contain more....

Cyanobacteria/blue-green algae have been around for over three billion years and are the most diverse and widespread group of photosynthetic Gram negative, structurally complex prokaryotes [5]. From obligate phototrophs to heterotrophs, these organisms have a diverse set of nutritional and environmental abilities [6]. Cyanobacteria are new microorganisms with potential for long-term agricultural development [7]. Cyanobacteria are Diazotrophes that can be used to produce inexpensive and environmentally acceptable biofertilizers. They can control the nitrogen (N) deficiency in plants and improve soil aeration, water retention, and vitamin B12 availability [8]. These are utilized as inoculants to increase crop yields, specifically for rice, as well as the fertility and structure of the soil [9]. Cyanobacteria can create vitamins, amino acids, and hormones like auxins(IAA), gibberellins, cytokinins, or abscisic acid to aid in growth. Molecules exhibiting pharmacological, immunosuppressive, or enzyme-inhibiting characteristics, in addition to antibiotics and poisonous substances, have been reported by [10]. More and more attention has been paid in recent years to the use of cyanobacteria, which occur in almost every environment on the planet, and several species are known to form symbiotic associations with bacteria as well as eukaryotes [11]. Many cyanobacteria are capable of N<sub>2</sub> fixation also organic

compound uptake and assimilation, which may play an important role in secondary metabolism [12]. Furthermore, some cyanobacteria may produce plant growth promoting substances and contribute significantly to soil fertility [10]. In a sustainable agriculture regime, cyanobacteria strains from the genera *Nostoc* and/or *Anabaena* can be used as an alternative to increase soil productivity and plant growth. There is currently a lot of interest in forming new relationships with various plants. Aref and Al-Kassas [11] investigate the effect of cyanobacterial inoculation as a nitrogen source that can partially substitute on maize plants. They discovered that using cyanobacteria increased maize yield significantly above the control.

Because of the previous benefits of cyanobacteria, the current study was carried out to investigate the effect of two cyanobacterial strains *A. oryzae* and *N. muscorum* as single strains on growth and productivity of peanut plant with different nitrogen ratios and compare between using full nitrogen fertilization and replacing it with cyanobacteria by 50,75% nitrogen ratios, in order to exchange chemical N fertilizers with biofertilizers and improve the biological performance.

Comment [E10]: ..by 50 and 75% nitrogen ratios

## 2. MATERIAL AND METHODS

### 2.1. Field experiment

The current investigation was conducted during two successive summers in 20201 and 2022 at the Ismailia Agricultural Research Station (ARC), Ismailia Governorate, Egypt (30° 35' 41.901" N and 32° 16' 45. 843" E), in a field experiment on sandy soil. Distinct cyanobacterial strain treatments and nitrogen fertilization ratios were added using three different techniques. *A. oryzae* and *N. muscorum* cyanobacterial strains that fix N<sub>2</sub> were supplied by the Agric. Microbiol. Dept. of the ARC's Soils, Water and Environmental Research Institute in Giza, Egypt. Cyanobacterial strains were cultivated independently on BG11 media [13], [14] and were

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...summers in 2021 and 2022 ...

cultured in growth chambers at a temperature of 25°C with constant lighting of 2000 lux. The developed cyanobacterium cultures were centrifuged at a speed of 3000 rpm min<sup>-1</sup>, and the supernatants from each strain were mixed to produce the cyanobacterial culture filtrate. The filtrate was used as soil drench treatment for peanut seeds after planting directly and a foliar spray at a rate of 60 L fed<sup>-1</sup> and the third treatment was seed coating by the powder of each cyanobacterial strain individually. During soil preparation, recommended doses of potassium and phosphorous fertilization were added as potassium sulphate (48% K<sub>2</sub>SO<sub>4</sub>) and monocalcium super phosphate (15.5% P<sub>2</sub>O<sub>5</sub>), and nitrogen as ammonium nitrate (33.5% kg N fed<sup>-1</sup>), doses were tested by 50 and 75% of recommended dose, with both individual cyanobacterial strains due to decrease the addition of full recommended dose of nitrogen while complete recommended ratios were applied as a control treatment. According to Page et al. [15] this study was carried out in sandy soil, which has the soil characteristics shown in Table (1). Samples were collected after 70 days of planting and at harvest time in both seasons.

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**Comment [E16]:** ... monocalcium super phosphate (15.5% P<sub>2</sub>O<sub>5</sub>), and N as...

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## 2.2. Plant analysis

### 2.2.1. Growth and seed yield characters

- 1- Plant height (cm)
- 2- No. of pods plant<sup>-1</sup>.
- 3- Pod weight plant<sup>-1</sup> (g)
- 4- Seeds yield fed.<sup>-1</sup> (kg)
- 5- 100- seed weight (g).

### 2.2.2. Nitrogen (N), potassium (K), phosphorus (P) and calcium(Ca) contents in plant

- 1- N, P, and K contents in peanut leaves, straw and seed after harvest were measured using the procedures outlined by Jackson [16].

2- Soil exchangeable  $\text{Ca}^{2+}$  was extracted using 1 mol/L ammonium acetate (pH 7.0)

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according to Rasmussen et al. [17]

## 2.3. Soil analysis

### 2.3.1. Some chemical and physical properties of soil

To determine the particle size distribution, soil samples were collected, air dried, softly crushed, and passed through a 2mm sieve using the international pipette method with hexametaphosphate as the dispersion agent. The modified Walkelt and Blak technique was used to calculate the organic matter (OM) content of the soil [18], pH of soil measured by pH metre, accessible N, P, and K as well as water-soluble cations and anions were measured using a Collins Clcimeter[16] The extraction of soil exchangeable  $\text{Ca}^{2+}$  used 1 mol/L ammonium acetate (pH 7.0)[17].

Comment [E18]: ..the international pipette method with hexametaphosphate as the dispersion agent.

Table (1). Physical and chemical properties of experimental soil

pH (1:2.5) soil suspension	EC dSm <sup>-1</sup> (Soil paste)	Soluble cations (mmol c l <sup>-1</sup> )				Soluble anions ( mmol c l <sup>-1</sup> )			
		Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
8.14	1.04	2.92	2.18	1.30	2.34	--	2.47	3.53	2.74
Coarse sand (%)		Fine sand (%)		Silt (%)	Clay (%)	CaCO <sub>3</sub> (%)	Organic Matter (%)	Texture class	
62.35	30.08	2.35	2.30	0.18	0.21	Sandy			
Available N (mg Kg <sup>-1</sup> )				Available P (mg Kg <sup>-1</sup> )		Available K (mg Kg <sup>-1</sup> )			
34.67				2.1		147			

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Comment [E20]: (mmol c L<sup>-1</sup>)

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### 2.2.2. Some soil Biological activities

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Soil biological activity of rhizosphere peanut plants was determined in soil samples collected from peanut rhizosphere soil at 70 days after planting and at harvest time in terms of

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**total bacterial** count was performed on nutrient agar using the spread plate method [19], **total cyanobacterial** count was conducted by plating ten-fold serial soil suspension-dilutions in triplicate onto agarized BG11 medium [20], **CO<sub>2</sub>evaluation** was determined according to Gaur et al.[21] and **indole acetic acid (IAA)** in soil determined according to Gordon and Weber [22].

## 2.4. Statistical Analysis

The experimental data obtained were subjected to analysis of variance (ANOVA), according to the procedures outlined by Snedecor and Cochran [23].

## 3.RESULTS AND DISCUSSION

Both selected cyanobacterial strains significantly improved peanut plant development parameters and nutrient content in soil and plant analysis.

### 3.1. Plant Analysis

#### 3.1.1. Plant Growth and yield parameters

Data in Table 2 indicated the effect of individual cyanobacterial treatment, with different nitrogen rates and different application methods on some plant growth parameters after 70 days from planting and seeds after harvest in two seasons 2021 and 2022. In all treatments inoculation of both individual cyanobacterial strains with 75% nitrogen ratio recorded the greatest significant plant height in both seasons, *N. muscorum* + 75% nitrogen treatment recorded the highest plant height (31.22 cm), then treatment of *A.oryzae* + 75% nitrogen (30.48 cm) while the lowest plant height was in inoculation with only *N. muscorum* (25.23cm) followed by treatment with only *A. oryzae* (26.15 cm), in seed coating application ,also in soil drench application, *N. muscorum* +

**Comment [E27]:** I suggest: .... **Total bacterial** count was performed on nutrient agar using the spread plate method [19]. **Total cyanobacterial** count was conducted by plating ten-fold serial soil suspension-dilutions in triplicate onto agarized BG11 medium [20]. **CO<sub>2</sub>evaluation** was determined according to Gaur et al.[21] and **indole acetic acid (IAA)** in soil determined according to Gordon and Weber [22]

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In all treatments inoculation of both individual cyanobacterial strains with 75% nitrogen ratio recorded the greatest significant .....

75% nitrogen treatment showed the most significant plant height (31.86 cm), then *A.oryzae* +75% nitrogen (31.17cm) and the same in foliar treatment, *N. muscorum* + 75% nitrogen showed the largest significant plant height at all (32.06 cm), then *A. oryzae* + 75% nitrogen treatment (31.51cm), and lowest plant height was in soil drench treatment in inoculation with *A.oryzae* without nitrogen (25 cm) and the lowest plant height at all was control treatment (23.76 cm). Furthermore number and weight of nodules increased by using both individual cyanobacterial strains + 75% nitrogen, the greatest number of nodules was in foliar application, *N. muscorum* with 75% nitrogen (32 nodules) and its weight was (48.51gm) followed by *A.oryzae* with 75% nitrogen in the same application and *N. muscorum* + 75% nitrogen in soil drench application (31.33 and 31.15 nodules) and their weight were (44.93 and 43.1 gm) respectively, the lowest number and weight of nodules was in seed coating application with treatments of individual *N. muscorum* and *A.oryzae* (23 and 21.3 nodules) respectively. These values were not significantly differed from those given using control (100% N).

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**Table (2). Effect of treatment with individual cyanobacterial strains, and with different nitrogen (N) rates by different types of applications on some plant growth parameters of peanut plants at 70 days and seeds at harvest time during two seasons 2021 and 2022.**

Treatments		Plant height (cm)	Number of Pods plant <sup>-1</sup>	Weight of pods (g)	Seed yield (kg /fed.)	100- Seeds weight (g)
Control		23.67 (c)	28.00 (c)	40.33(c)	1335.48(d)	72.86 (bc)
Seed coating	<i>Anabaena-oryzae</i>	26.15(f)	21.30(f)	32.50 (f)	750.00 (g)	60.73(d)
	<i>A.oryzae</i> +50% N	27.25 (d)	26.95(d)	36.88 (c)	1328.00 (c)	66.33(c)
	<i>A.oryzae</i> +75% N	30.48(b)	29.25(d)	40.93 (a)	1608.88 (a)	73.75(b)
	<i>Nostoc- muscorum</i>	25.23(e)	23.00 (e)	33.35 (e)	1001.28 (f)	63.50 (d)
	<i>N. muscorum</i> +50% N	29.50(c)	28.5 (c)	39.43 (c)	1486.18(b)	71.18(b)
	<i>N. muscorum</i> + 75% N	31.22(a)	30.00(a)	42.10 (b)	1715.25(e)	74.00(a)
ch	<i>A.oryzae</i>	25.00(f)	21.75 (f)	35.36(f)	791.2(g)	63.35(d)
	<i>A.oryzae</i> +50% N	29.20(d)	27.00 (d)	39.88 (c)	1487.13(c)	73.30(c)

	<i>A.oryzae</i> +75% N	31.17(b)	30.86 (d)	47.42 (a)	1644.65(a)	78.45 (b)
	<i>N.muscarum</i>	25.65(e)	26.00(e)	39.88 (e)	1032.50 (f)	65.27(d)
	<i>N.muscarum</i> +50% N	30.79 (c)	29.25(c)	45.95(c)	1550.30 (b)	73.28(b)
	<i>N.muscarum</i> + 75% N	31.86 (a)	31.15(a)	43 .10 (b)	1752.00(e)	79.10(a)
Foliar application	<i>A.oryzae</i>	25.20(f)	22.05 (f)	34.50(f)	876.50(g)	64.80 (d)
	<i>A.oryzae</i> +50% N	29.20 (d)	27.00 (d)	39.88(c)	1487.13(c)	73.30(c)
	<i>Anabeana</i> +75% N	31.51(b)	31.33 (d)	44.93 (a)	1800.2 (a)	81.25(b)
	<i>N.muscarum</i>	26.50(e)	26.27(e)	37.35(e)	1298.25 (a)	67.50(d)
	<i>N.muscarum</i> +50% N	30.90 (c)	30.75(c)	44.43(c)	1663.5 (f)	78.51(b)
	<i>N.muscarum</i> + 75% N	32.06 (a)	32.00(a)	48.51(a)	1835.55 (e)	81.98(a)
L.S.D. (0.05)		1.02	1.53	1.41	15.38	5.22

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### 3.1.2. Nitrogen (N), potassium(K), phosphorus (P) and calcium(Ca) contents in Pplants

In respect to the interaction effect between cyanobacteria treatments and nitrogen rates, total N, P, K and Ca contents of peanut crop under different nitrogen rates and different types of applications conditions shown in Table (3), results exhibited the same trend achieved for leaves, straw and grain yield of peanut in response of cyanobacteria treatments combined with different nitrogen levels. In foliar application the corresponding highest values were 5.34 % (N), 0.56 % (P) and 2.89% (K) for peanut leaves and 2.26% (N), 0.31% (P) and 0.42% (K) for peanut straw, while seeds recorded 4.54% (N), 0.53% (P) and 1.56% (K) in treatment of *N.muscorum*+75 % N, followed by *A.oryzae* +75 % N treatment 5.16% (N), 0.55% (P) and 2.83 % (K) for peanut leaves and 2.21% (N), 0.28% (P) and 0.35% (K) for peanut straw, while seeds recorded 4.66% (N), 0.50% (P) and 1.5% (K), while soil drench application was slightly less than foliar application in N, P, K content 5.15% (N), 0.41% (P) and 2.77% (K) for peanut leaves and

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2.22% (N), 0.30% (P) and 0.36 % (K) for peanut straw, while seeds recorded 4.51% (N), 0.48% (P) and 1.26% (K) in the same treatments respectively. Seed coating application recorded the lowest N, P and K percentages of peanut crop in the same treatments. Total calcium contents of peanut leaves, straw and seeds, the behavior was in contrast for total since inoculation with both individual cyanobacterial strains + 75% N led to slightly increase the total calcium content in peanut leaves, straw and seeds compared to the other examined treatments. Also, the treatment of *N. muscorum*+ 75 % N then, *A. oryzae* +75 % N in foliar applications were the least significant values of total calcium contents of peanut leaves, straw and seeds compared to the other tested treatments and applications. These relative least Ca values were 2.56, 0.86, 1.76 % for leaves, straw and seeds, respectively.

The need for contamination-free, healthful food has grown in direct and indirect proportion to the increase in population. By year 2029, the World Health Organization estimates that 50 percent of world food production will have increased. "Green Revolution" approaches are also helping to boost agriculture productivity while lowering the risk of chemical-based fertilizers harming human health and the environment. Researchers have therefore exploited 'green technology' to create an eco-friendly atmosphere by utilizing bacteria. The use of cyanobacteria to boost crop productivity and soil fertility is discussed in detail in Green Technology. TheCyanobacteria may breakdown a wide range of contaminants and play a variety of roles in the soil ecosystem, all of which help to maintain soil fertility [24]. Inorganic nitrogen fertilizers have a very high production cost [25]. Biofertilizer is used to address nitrogen deficiency in crops in a sustainable manner. Biofertilizers such as bacteria can fix less than 10 kg of nitrogen per hectare while cyanobacteria can fix about 10–30 kg/ha of nitrogen each year. Based on their purposes

and modes of operation, biofertilizers are divided into many categories. The most often used biofertilizers are plant growth-promoting rhizobacteria (PGPR), potassium solubilizer, phosphorus solubilizer, and nitrogen fixer (N-fixer). Ahmed et al. [26] investigated the effects of organic and mineral fertilization on plants growing in sandy soil. Biofertilizers increased peanut pod yields, cereals yield, and wheat straw yields. Cyanobacteria offer promising models for investigating the metabolic cost hypothesis. This may be attributed to increased nitrogen fixation, which enabled the plants absorb and use all of their nutrients more effectively. This led to increased concentrations of N, P, and K in the kernel and haulm, which led to increased uptake of N, P, and K [27].

**Table (3.)** Effect of treatment with cyanobacterial strains with different nitrogen (N) rates by different types of applications on nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) uptake by peanut plants at 70 days, plant straw and seeds at harvest time during two seasons 2021 and 2022.

Treatments	Leaves (at 70 days)				Straw (Harvest time)				Seeds			
	N	P	K	Ca	N	P	K	Ca	N	P	K	Ca
<b>Control</b>	4.32 (e)	0.27(f)	2.38 (e)	2.16(e)	1.96(cd)	0.24(d)	0.15(a)	0.76(cd)	3.72(e)	0.38 (f)	1.49(d)	1.58(cd)
<i>Anabaena oryzae</i>	3.92(g)	0.28(e)	1.99(g)	1.65(e)	1.87(d)	0.21(e)	0.07(e)	0.69 (f)	3.41(g)	0.32(e)	1.43(f)	1.49(f)
<i>A.oryzae</i> +50% N	4.22 (d)	0.30(d)	2.22 (d)	1.75 (d)	1.95(d)	0.23(cd)	0.16(d)	0.74 (d)	3.62(d)	0.36(d)	1.46(d)	1.56(d)
<i>A.oryzae</i> +75% N	4.34(C)	0.35(c)	2.39 (c)	2.20(C)	2.02 (b)	0.25 (b)	0.24(c)	0.79(b)	3.94(b)	0.42(b)	1.53(b)	1.65(b)
<i>Nostoc muscorum</i>	3.98 (f)	0.28 (f)	2.07 (e)	1.68 (e)	1.93 (cd)	0.23 (d)	0.07(e)	0.71(e)	3.47(f)	0.32(e)	1.44 (e)	1.51(e)
<i>N. muscorum</i> +50% N	4.31(b)	0.33 (b)	2.37(b)	1.86(b)	1.99 (c)	0.25(bc)	0.19(d)	0.78 (c)	3.80(c)	0.39(c)	1.49(c)	1.62(c)
<i>N. muscorum</i> + 75% N	4.34(a)	0.35(a)	2.39(a)	2.20(a)	2.16 (a)	0.29 (a)	0.28(b)	0.83 (a)	4.20(a)	0.45(a)	1.58(a)	1.69(a)
<i>A.oryzae</i>	4.06(g)	0.31(e)	2.25(g)	1.74(e)	1.89(d)	0.21 (e)	0.14(e)	0.71(f)	3.46 (g)	0.32(e)	1.46(f)	1.54(f)
<i>A.oryzae</i> +50% N	4.49 (d)	0.35 (d)	2.48(d)	2.12 (d)	1.99 (d)	0.24(cd)	0.21(d)	0.75(d)	3.76(d)	0.37(d)	1.53(d)	1.62(d)
<i>A.oryzae</i> +75% N	4.98(C)	0.38 (c)	2.69(C)	2.37 (C)	2.17 (b)	0.28 (b)	0.29(c)	0.81(b)	4.42(b)	0.43(b)	1.59(b)	1.70(b)
<i>N.muscarum</i>	4.10 (f)	0.32(f)	2.33 (e)	1.76(e)	1.96 (cd)	0.24 (d)	0.17(e)	0.73(e)	3.5(f)	0.33(e)	1.47(e)	1.57(e)
<i>N.muscarum</i> +50% N	4.61(b)	0.36(b)	2.54(b)	2.26(b)	2.15 (c)	0.27(bc)	0.28 (d)	0.81(c)	3.89(c)	0.40(c)	1.58(c)	1.67(c)
<i>N.muscarum</i> + 75% N	5.15(a)	0.41(a)	2.77(a)	2.44(a)	2.22 (a)	0.30 (a)	0.36(b)	0.85 (a)	4.51(a)	0.48(a)	1.62(a)	1.74(a)
<i>A.oryzae</i>	4.14 (g)	0.34 (e)	2.37 (g)	1.95(e)	2.00 (g)	0.21 (e)	0.10(e)	0.71 (f)	3.81 (g)	0.35(e)	1.46(f)	1.55(f)
<i>A.oryzae</i> +50% N	4.58 (d)	0.45(d)	2.68(d)	2.24(d)	2.10 (d)	0.25 (e)	0.22(d)	0.76 (d)	4.19(d)	0.44(d)	1.55(d)	1.64(d)
<i>Anabaena</i> +75% N	5.16(C)	0.55(C)	2.83(c)	2.47 (C)	2.21 (b)	0.28(cd)	0.35(c)	0.83 (b)	4.66(b)	0.50(b)	1.60(b)	1.72(b)
<i>N.muscarum</i>	4.21 (f)	0.37(f)	2.40(e)	1.98 (e)	2.06 (cd)	0.24 (d)	0.14(e)	0.73 (e)	3.88(f)	0.37(e)	1.48(e)	1.58(e)

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<i>N.muscarum</i> +50% N	4.65 (b)	0.52 (b)	2.72(b)	2.32 (b)	2.18 (c)	0.28(bc)	0.30(d)	0.79 (c)	4.42(c)	0.48(c)	1.58(c)	1.68(c)
<i>N.muscarum</i> + 75% N	5.34(a)	0.56(a)	2.89(a)	2.56(a)	2.26 (a)	0.31(a)	0.42(b)	0.86 (a)	4.74 (a)	0.53(a)	1.65(a)	1.76(a)
<b>L.S.D. (0.05)</b>	0.05	0.02	0.05	0.07	0.59	0.03	0.14	0.06	0.38	0.04	0.26	0.24

After nitrogen, phosphorus, and potassium, calcium is a vital secondary macronutrient for the healthy growth of the plant. It frequently plays an important role in the development of cell walls, cell components, and accumulates in plant. When plants in peanuts absorb Calcium from the soil, it remains in the plant tissues rather than being transferred to developing pods, where Calcium is more needed [28]. In addition to Calcium's beneficial effects on yield enhancement, Calcium has very important roles in peanuts include an increase in germination rate, oil content, protein content, seed Calcium concentration, increase in the number of nodules, and a decrease in aflatoxin contamination. Because Calcium is vital for plumule formation, it has been directly connected to seed quality [29]. Treatments with cyanobacterial strains increase Calcium contents in soil and plant subsequently. For the plant to develop and flourish properly, seventeen vital ingredients are needed. Among these, relatively high amounts of nitrogen (N), phosphorus (P), and potassium (K) are required [30]. Numerous microorganisms, such as cyanobacteria, which dissolve phosphate and fix nitrogen in soil, as well as molds and fungus are frequently utilized as biofertilizers [31]. They give the plant nutrients that promote development, such as indole acetic acid (IAA), amino acids, and vitamins, and they also increase the soil's fertility and productivity while preserving crop output. Similar to this, microorganisms that produce phytohormones are also utilized in the creation of biofertilizers [32]. Biofertilizers may play an important role in long-term crop productivity and soil fertility while minimizing the environmental load associated with fertilizer production and nutrient leaching into groundwater deposits [33].

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After N, P, and K, Ca is a vital secondary....

## 3.2. Soil Analysis

### 3.2.1. Response of soil biological activities and indole acetic acid (IAA) contents in rhizosphere of peanut plants

Due to soil biological activities, data in ~~Table (4)~~ showed that total bacterial and cyanobacterial counts, CO<sub>2</sub> and ~~IAA indole acetic acid~~ content in rhizosphere of peanut plants in both seasons, all recordings were higher in all treated plants than in the control treatment, with the highest bacterial count was 89 and 85 x 10<sup>6</sup> CFU g<sup>-1</sup>rhizosphere soil<sup>-1</sup> recorded by *N. muscarum* + 75% N and *N. muscarum* + 50% N treatment respectively in soil drench application, followed by 84 x 10<sup>6</sup> CFU g<sup>-1</sup>rhizosphere soil<sup>-1</sup> which recorded by *A. oryzae* +75 % N treatment in the same type of application, then 76 and 74 x 10<sup>6</sup> CFU g<sup>-1</sup>rhizosphere soil<sup>-1</sup> recorded by *N. muscarum* + 75 % N and *N. muscarum* + 50 % N treatment respectively in foliar application. While cyanobacterial total count increased in most treatments due to presence of cyanobacterial inoculant, but the highest cyanobacterial count was 38, 37 x 10<sup>6</sup> CFU g<sup>-1</sup>rhizosphere soil<sup>-1</sup> documented by *A. oryzae* +75% N and *N. muscarum* + 75% N treatments respectively in soil drench application, followed by 32, 29 x 10<sup>6</sup> CFU g<sup>-1</sup>rhizosphere soil<sup>-1</sup> documented by *N. muscarum* + 75% N and *A. oryzae* +75% N in foliar application. CO<sub>2</sub> evolution indicates the respiration activities of soil microbial community therefore the highest CO<sub>2</sub> evolution was 462 mg CO<sub>2</sub> 100 g dry soil recorded by *N. muscarum* + 75% N treatment in soil drench application and 374 mg CO<sub>2</sub> 100 g dry soil recorded by *A. oryzae* +75% N treatment in foliar application type, then 363, 352 which recorded by *N. muscarum* + 50% N and *A. oryzae* +75% N treatments in soil drench application while seed coating application was the lowest microbial activities and CO<sub>2</sub> evolution in all treatments. IAA was able to be released into the soil by both cyanobacterial strains and rhizobacteria that support plant growth, which raised the

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And so on, in other cases.... 100 g<sup>-1</sup>

amount of IAA in the soil. The highest IAA in soil were 1.863 and 1.844 mg g<sup>-1</sup> soil which recorded by *N. muscarum* + 75% N treatment in soil drench application and foliar application respectively, followed by 1.842 and 1.743 which recorded by *A. oryzae* +75% N and *N. muscarum* + 75% N treatments in foliar application type. The IAA in soil for all other treatments ranged from 0.8 to 1.477 mg g<sup>-1</sup> soil in all application types, with the control treatment having the lowest IAA at 0.722 mg g<sup>-1</sup> soil.

**Table (4.)** Effect of treatment with individual cyanobacterial strains and with different nitrogen (N) rates by different types of applications on total bacterial count, total cyanobacterial count, CO<sub>2</sub> and indole acetic acid (IAA) in rhizosphere of peanut plants at 70 days and seeds at harvest time during two seasons 2021 and 2022.

**Comment [E40]:** Please pay attention to the correct position inside the text, for Table 4.

	Treatments	Total Bacterial count (x 10 <sup>6</sup> cfu g dry rhizosphere soil <sup>1</sup> )	Total cyanobacterial count x 10 <sup>6</sup> cfu g dry rhizosphere soil <sup>1</sup>	CO <sub>2</sub> evolution (mg CO <sub>2</sub> 100 g dry rhizosphere soil <sup>1</sup> day <sup>-1</sup> )	IAA mg g <sup>-1</sup> soil
	Control	71	18	200	0.772
Seed coating	<i>Anabaena</i> - <i>oryzae</i>	73	19	252	1.27
	<i>A. oryzae</i> +50% N	33	15	274	0.894
	<i>A. oryzae</i> +75% N	30	25	341	1.842
	<i>Nostoc</i> - <i>muscarum</i>	54	10	286	1.102
	<i>N. muscarum</i> +50% N	55	14	220	1.333
	<i>N. muscarum</i> + 75% N	68	23	330	1.743
Soil drench	<i>A. oryzae</i>	19	18	264	1.456
	<i>A. oryzae</i> +50% N	62	14	220	1.301
	<i>A. oryzae</i> +75% N	84	38	352	0.895
	<i>N. muscarum</i>	68	12	286	0.919
	<i>N. muscarum</i> +50% N	85	24	363	1.407
	<i>N. muscarum</i> + 75% N	89	37	462	1.863
application	<i>A. oryzae</i>	30	17	231	0.935
	<i>A. oryzae</i> +50% N	37	16	374	1.296

<i>A. oryzae</i> +75% N	63	29	275	1.477
<i>N. muscarum</i>	65	22	209	1.259
<i>N. muscarum</i> +50% N	74	24	352	1.368
<i>N. muscarum</i> + 75% N	76	32	374	1.844

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The findings of this study revealed an increase in total bacterial and cyanobacterial count, which corresponded with the results of Zulpa et al. [34], who investigated the effect of cyanobacteria on soil microbiological activity and nutrient content. The biomass and extracellular products of both strains increased soil microbial activity. Abbas et al [35] discovered that cyanobacteria inoculation increased soil biological activity in general, CO<sub>2</sub> evolution increased in different treatments when compared to the control treatment that did not

receive inoculation, which was due to an increase in microbial count and biological activities. Indole-3-acetic acid (IAA) is a well-studied auxin found in a variety of bacteria as well as some freshwater cyanobacteria. Cyanobacteria are primarily involved in biological nitrogen fixation and have been used as agricultural biofertilizers [36], plant hormones are essential for plant growth and development [37]. Mazhar et al. [38] demonstrated that cyanobacteria can produce IAA, the results of this study revealed that cyanobacterial-treated plant rhizospheres had higher levels of IAA than control treatments. When compared to the control treatment without inoculation, cyanobacterial inoculation dramatically increased biological soil activities like total bacterial count, cyanobacteria count, CO<sub>2</sub> evolution, and indole acetic acid generation [39].

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From these outcomes may be explained by the fact that cyanobacteria release a variety of stimulating compounds, but primarily ~~IAA indole acetic acid~~, gibberellins, and cytokines. These beneficial benefits of cyanobacteria could encourage plant growth, nutrient uptake and efficiency, as well as photosynthetic metabolism. These findings are consistent with those of Maqubela et al. [40] cyanobacteria have shown promise as a biofertilizer. They can transform solar energy into biomass by utilizing CO<sub>2</sub>, water, and nutrients. The use of cyanobacteria in agricultural techniques has been reported as a cost-effective way to decrease global warming by lowering CO<sub>2</sub> emissions. According to the findings, cyanobacteria biomass can be used to improve food quality, soil physicochemical qualities, prevent soil-borne diseases, add organic matter, release growth-promoting chemicals, solubilize insoluble phosphates, use as nutraceuticals, and even be used in pharmaceuticals. As a result, cyanobacteria-based biofertilizers are both cost-effective and environmentally beneficial [41]. Bio-fertilization with cyanobacteria, resulted in increases in the soil microbial population, including soil fungus, actinomycetes, and bacteria according to Alvarez et al. [42], increased microbial activity in the

Comment [E41]: These outcomes may be explained.....

soil resulted in higher organic matter content. In addition to nitrogen, cyanobacteria benefit crop plants by producing gibberellins, auxins such as indole-3-acetic acid, indole-3-propionic acid, vitamin B<sub>12</sub>, free amino acids such as serine, arginine, glycine, aspartic acid, threonine, glutamic acid, etc., extra- and intracellular polysaccharides such as xylose, galactose, fructose. Such compounds have a number of beneficial benefits, including enhanced soil structure, promotion of agricultural plant growth as well as beneficial microorganisms, and heavy metal chelation [43].

## CONCLUSION

Based on the previous findings, the current study found that cyanobacteria play a beneficial role in improving the status of soil in terms of physical, chemical, and biological properties. As a result, cyanobacteria biofertilizers are recommended for use in agriculture as renewable natural nitrogen fixing biofertilizer for various crop plants. They are non-polluting, inexpensive, and use renewable resources in addition to free available solar energy, atmospheric nitrogen, and water. The results of this study clearly demonstrated that combining cyanobacteria with 75% recommended dose of mineral N with soil drench application resulted in the highest plant growth, yield, and yield attributes than the other types of applications. This combination may also reduce by the amount and cost of N mineral fertilizer applied to peanuts in sandy soil conditions by 25%, thereby preserving soil health. To confirm and truly support this study, it must be repeated with peanuts and other crops.

### Comment [E42]: CONCLUSION

The current study found that cyanobacteria play a beneficial role in improving the status of soil in terms of physical, chemical, and biological properties.

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### Comment [E43]: REFERENCES

Please carefully verify the section.

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