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Effect of Cyanobacterial Combinations on Peanut Yield

Gehan Mohamed Salem [a], Alaa Abd Elghany Shaheen [b] and Mona Fekry Ghazal [a*]

[a] *Department of Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.*
[b] *Department of Sandy and Calcareous Research, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The characteristics of two cyanobacterial strains, *Anabaena oryzae* and *Nostoc muscorum*, were studied in order to use them as biofertilizers in a field experiment conducted in the two winter seasons of 2021 and 2022 at the Ismailia Agricultural Research Center Station to study the effect of both strains on peanut plant in sandy soil. Cyanobacterial strains were used individually by coating seed, soil drench, and foliar applications, as well as mixed applications of two strains in various ways. Both cyanobacterial strains morphological examination revealed that they both have heterocysts, nitrogen, phosphorus, and potassium in their culture filtrate, and they were able to produce chlorophyll a and phosphatase enzymes. The results of an agricultural experiment showed that using *Nostoc muscorum* and *Anabaena oryzae* separately had a positive effect on peanut plants in a variety of applications, but combining both of these applications with 75% nitrogen increased the growth traits, nutrient contents, and soil biological activities in both peanut plants and their rhizosphere soil. The soil drench treatment with *A. oryzae* and *Nostoc muscorum* plus 75%

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*Corresponding author: E-mail: drmonaghazal@gmail.com;

nitrogen produced the highest growth results and peanut yields in a single application. The *A. oryzae* Soil Drench Application (S) + *N. muscorum* Foliar Application (F) with 75% N reported the best outcomes in mixed treatments. However, compared to single applications, all blended applications displayed better growth and yield characteristics. The results of the study suggest that employing cyanobacteria in a mixed application will enhance its advantages over a single use.

Keywords: Anabaena oryzae; Nostoc muscorum; phosphate dissolving; nitrogen fixers; biofertilizers; single application; mixed application.

1. INTRODUCTION

In tropical and subtropical areas, peanut is one of the most significant and economically viable oilseeds and it is primarily farmed for its oil, protein and carbs [1]. For peasants in underdeveloped tropical nations, it is a significant cash crop [2]. Growth and yield response of groundnut (*Arachis hypogaea* L.) to plant densities and phosphorus on an Ultisol in Southeastern Nigeria. The majority of Egypt's newly reclaimed land has sandy soils, which are often low in organic matter and lacking in plant nutrients. Bio-fertilizers are environmentally friendly and have been shown to be an effective and cost-effective substitute for chemical fertilizers with lower capital and energy input [3]. *Anabaena oryzae* and *Nostoc mascorum* are cyanobacteria organs in the family Nostocaceae, order Nostocales. Cyanobacteria and green algae contribute to nutrient enrichment through nitrogen fixation and mineralization. Microalgae's photosynthetic capacity and application in soil result in carbon enrichment, which improves soil organic matter, promotes other mineralization processes and improves macro and micronutrient utilization in soil and rhizosphere [4]. Furthermore, microalgae can produce metabolites such as phytohormones, polysaccharides, and so on, which can benefit agricultural production. By stimulating soil microbial activity, this fascinating group of organisms can help to improve nutrient availability, maintain soil organic carbon and fertility, and enhance plant growth and crop yield [5]. Cyanobacteria, as a biofertilizer, are known to form associations with vascular/nonvascular plants and produce growth-promoting substances [6]. They are also known to increase soil fertility by increasing available N and P levels and have demonstrated an economic view that it can compensate for approximately 50% of the recommended doses of N, P, and K. [7]. Therefore, the objective of this study is to investigate the effect of using two cyanobacterial strains, *Anabaena oryzae* and *Nostoc mascorum*, as an alternative to nitrogen fertilization by various ratios by different types of applications as an individual addition and mixed addition by various ways of treatments on peanut plant growth features, with the goal of reducing chemical nitrogen fertilization.

2. MATERIALS AND METHODS

2.1 Cyanobacterial Strains

The strains of cyanobacteria that fix nitrogen dioxide, *A. oryzae* and *N. muscorum*, were provided by the Agric. Microbiol. Dept. of the ARC's Soils, Water, and Environmental Research Institute in Giza, Egypt. According to Rippka et al. [8] and Zarrouk [9] independent cyanobacterial strains were grown on BG_{11} media in growth chambers with constant lighting of 2000 lux and a temperature of 25°C. Regular visual examinations were performed by Olympus mod. IX71 microscope with a coupled digital camera.

2.2 Some Characteristics of Cyanobacterial Strains

2.2.1 Nutrients contents in cyanobacterial cultures

The pH of cultures was determined using a pH metre and the available N, P, and K, were determined using a Collins Cloimeter [10,11] used the modified Walkelt and Blak technique to calculate the organic matter (OM) content of the culture filtrate.

2.2.2 Pigment content

Chlorophyll a (Chl a) was estimated by the method of Meeks and Castenholz [12] Triplicate subsamples (10 ml of cell suspension) were harvested after their centrifugation at 10,000 rpm for 10 min and washed twice with sterile water. Cell pellets were resuspended in 5 ml 90% (v/v) methanol and incubated at 25^{o} C for 15 min in the dark. The methanol extract was determined at 663 nm. The Chl a content was estimated using the equation:

Chl a (µg/ ml) = OD663 nm x 12.7

2.2.3 Phosphate solubilization

Phosphate solubilization (P-sol) activity was demonstrated by growing the strains in culture medium containing 2.5% tricalcium phosphate. A zone of clearance of the medium was detected in the case of phosphate solubilizing colonies after 14 days of incubation at 3^0C [13].

2.3 Field Experimental Design

The current study was carried out in a field experiment on sandy soil at the Ismailia Agricultural Research Station (ARC), Ismailia Governorate, Egypt (30^o 35′ 41.901″ N and 32^o 16′ 45.843″ E), over the course of two successive summers in 2021 and 2022. Three distinct methods were used to add different cyanobacterial strain treatments and nitrogen fertilization ratios. The supernatants from each strain of cyanobacterium were blended after the established cultures were centrifuged at a speed of 3000 rpm min^{-1} to create the cyanobacterial culture filtrate. The filtrate was utilised as a foliar spray at a rate of 60 L fed^{-1}, a soil drench treatment for peanut seeds after direct planting, and a third treatment was seed coating by the powder of each cyanobacterial strain separately. During soil preparation, the recommended amounts of potassium, phosphorus, and nitrogen fertilization were added as ammonium nitrate. Both individual cyanobacterial strains were tested at doses of 50 and 75 percent of the recommended amount of nitrogen. The addition of the full recommended dose of nitrogen served as a control. Cyanobacterial strains were added in three ways: seed coating (c) with cyanobacterial powder before planting, soil drench (S) and foliar application (F) with culture filtrate after planting, mixed applications were added as following,

1) *A oryzae* (S) + *N. mascorum* (C*)*
2) *A oryzae* (S) + *N. mascorum* (F)
3) *A oryzae* (C) + *N. mascorum* (S)
4) *A oryzae* (C) + *N. mascorum* (F)
5) *A oryzae* (F) + *N. mascorum* (S)
6) *A oryzae* (F) + *N. mascorum* (C)

2.3.1 Plant analysis

Growth and seed yield characters:

(1) Plant height (cm),
(2) No. of pods $plant^{-1}$,
(3) Pod weight $plant^{-1}$ (g),
(4) Seeds yield $fed.^{-1}$ (kg)
(5) 100- seed weight (g).

Nitrogen, potassium and phosphorus contents in Plant: N, P, and K contents in peanut leaves, straw and seed after harvest were measured using the Standard method by Jackson [10] .

2.3.2 Soil analysis

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 pipte method with hexameta phosphate as the dispersion agent. The modified Walkelt and Blak technique was used to calculate the organic matter (OM) content of the soil Walkley [11], 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 Colorimeter Jackson [10].

Total bacterial and cyanobacterial count: The biological activity of the soil in the rhizosphere of peanut plants was measured 70 days after planting and at harvest time. The spread plate method was used to count the total number of bacteria on nutrient agar APHA [14]. The total cyanobacterial count was determined by plating ten-fold serial soil suspension dilutions in triplicate onto agarized BG_{11} medium [15].

Table 1. Soil particle size and some chemical properties for soil utilized in agriculture experiment

Soil particle size		Some chemical properties	
Coarse sand %	69.58	pH (1:2.5 suspension)	7.8
Fine sand %	23.51	EC dSm^{-1} in the soil water extract (1:5)	0.137
Silt %	3.01	O.M%	0.461
Clay %	3.90	Available N (ppm)	18.198
Soil texture: Sandy		Available p (ppm)	2 .189
		Available K (ppm)	71.897

2.4 Statistical Analysis

The experimental data obtained were subjected to analysis of variance (ANOVA), according to the procedures given by Snedecor and Cochran Snedecor [16].

3. RESULTS AND DISCUSSION

3.1 Cyanobacterial Strains Analysis

3.1.1 Morphology of cyanobacterial strains

Anabaena Oryzae: Green, gelatinous, and membranous thallus. Trichomes are short and straight. Cells are barrel-shaped, 1 times as long as they are wide (Fig. 1a), these results were agreed with Naz et al. [17] and Shariatmadari and Riahi [18].

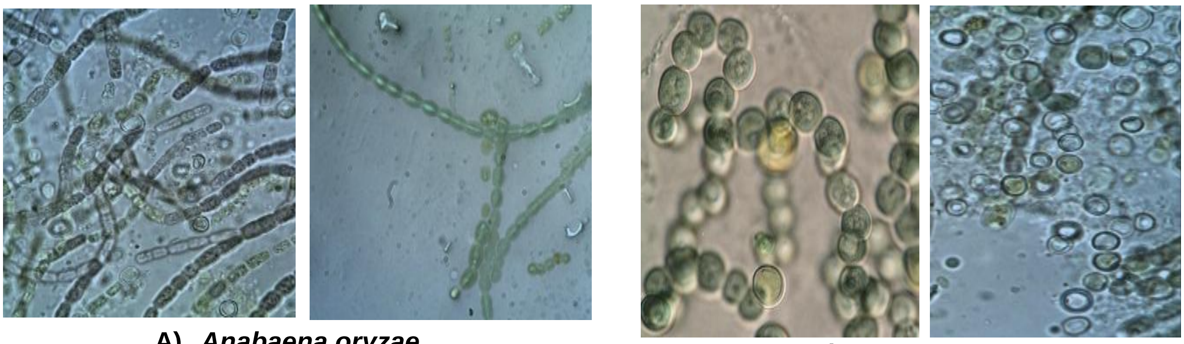
Nostoc muscorum: Culture is dark green trichomes had no implications. They were uniseriate, single, and aggregated, with no polarity or tapering. There was no sheath formed. Yellowish-brown rounded cells (Fig. 1b). There were only a few heterocysts found. They had a single occurrence and two positions, intercalary and terminal [19].

Nostocacae is a widely distributed heterocystous filamentous cyanobacterial family with a high potential for N_2 fixation in a variety of ecological and agricultural conditions [20]. These resultus are consistent with El-Sawah [21] which demonstrated that the ability of cyanobacteria to fix N2 and the most active cyanobacteria for nitrogenase activity strains *Anabaena oryzae* and *Nostoc* spp. *Nostoc* can form a N_2-fixing symbiotic association with a wide variety of host plants, including liverworts and hornworts [22] or it can form a free-living (non-symbiotic) association. The formation of an artificial association between *Nostoc* strains and rice plants revealed that *Nostoc* has a positive chemotaxis toward plant roots [23].

3.1.2 Nutrients contents in cyanobacterial cultures

Data in Table 2 reported pH and some nutrient contents in cyanobacterial cultures filtrates, The pH of both strains was close to 7, with *A. oryzae* having a lower pH than *N.muscroum*.,while organic matter percentage in *A. oryzae* was 26.27 higher than *N. muscroum.* and nutrient content of *A. oryzae* strain (N,P and K) (168.40, 11.68 and 6.74) respectively, was greater than *N. muscroum* (238.27, 10.87 and 4.16) respectively.

After 14 days of cultivation, cyanobacterial extract was applied to cultures. This period marked the end of exponential growth and the start of the stationary phase of growth, which was characterized by increased cell differentiation into specialized cells [24]. Prokaryotic organisms and phototropic in nature include *Nostoc*, *Anabaena*, *Oscillatoria*, *Aulosira*, *Lyngbya*, and others. They contribute significantly to the enrichment of paddy field soil by fixing atmospheric nitrogen and supplying vitamin B complex and growth promoting substances that allow the plant to grow vigorously [25]. These findings are consistent with those obtained by El-zawawy [26], who reported that the ecological effect on the activation of growth and rates of nitrogen fixation by different cyanobacterial isolates. Anabaena sp. is involved in regulation of nitrogen fixation and cellular differentiation.



A) ***Anabaena oryzae***

B) ***Nostoc muscorum***

Fig. 1. Microscopic appearance of cyanobacterial strains at 40 X magnification under and Nikon digital microscope (Eclipse80i), A: *Anabaena oryzae strain* , *B. Nostoc muscorum* strain

Table 2. Values of few selected chemical characteristics of culture filtrate

Analysis characteristics	*Anabaena oryzae*	*Nostoc muscorum*
pH	6.86	7.10
Organic matter %	26.27	20.65
Total nutrients mg.l^{-1}		
Nitrogen	168.40	238.27
Phosphorus	11.68	10.87
Potassium	6.74	4.16

3.1.3 Pigment content and phosphate solubilizing potential

Cyanobacteria are photosynthetic organisms that can synthesise chlorophyll both cyanobacterial strains produced chlorophyll in this study, but *A. oryzae* recorded (24.75µg ml^{-1}) more than *N. mascourum* which recorded (20.68 µg ml^{-1}**).** Furthermore, both cyanobacterial strains were capable of dissolving phosphate on agar plates and forming clear zones around colonies.

Tanaka et al. [27] revealed that chlorophyll a is the most significant pigment in photosynthetic organism, due to the highly fluorescent and covalently bound phycobiliproteins, cyanobacteria frequently provide a wide variety of colored pigments [28]. Results of this study were agreed with Jaywalk et al. [29], which revealed that cyanobacteria, particularly the *Anabaena*, and *Nostoc*, genera, are blue green algae with a focus on nitrogen fixation and some aspects of photosynthesis. According to Salama [30], cyanobacterial strains can produce phosphatase enzyme, which is responsible for dissolving insoluble phosphate. Thus, cyanobacteria also contribute to phosphate decomposition in agriculture by transforming insoluble phosphate compounds into soluble form, which can be readily absorbed by the crop [31].Additionally, it has been shown that cyanobacteria from the genera *Nostoc*, *Phormidium*, and *Oscillatoria* degrade specific herbicides and an organo-phosphorus insecticide, increasing the soil's suitability for agriculture, the decay of cyanobacteria also contributes organic matter, nitrogen, and phosphorus to the soil [32].

3.2 Experimental Results

3.2.1 Plant analysis

All growth and production characteristics improved in all treatments that received cyanobacteria inoculant supplemented by 75% N in both single and mixed applications, but mixed applications improved more than single applications. Tables (4 and 5) displayed plant heights, pod counts, seed weight per plant, pod yield fed^{-1}(kg),100-pod weight (g),100-seed weight (g). The greatest plant height and seed weight per plant in a single application were 28.38 cm and 27.6 g, respectively, recorded by *A. oryzae* treatment in soil drench application, while the greatest number of pods was 26.26 pods recorded by *A. oryzae* with 75% N in seed coating application. In mixed application, *A. oryzae* (soil drench) + *N. muscorum* (Foliar) mixed with 75% N treatment produced the highest plant height, number of pods, and seed weight per plant, measuring 31.2 cm., 32 pods, and 31.75. g plant $^{-1}$, respectively. Data in Table 5, the greatest pod yield per fed in single applications was 1684.63 which recorded by *N. muscorum* + 75% N in soil drench treatment, while the largest 100-pod weight was 147.13 g recorded by *A. oryzae* + 75% N in foliar application and the highest 100-seed weight was 74 g reported by *N. muscorum* + 75% N in soil drench and foliar applications. In mixed application treatment of *A.oryzae* (S) + *N.muscorum* (F) with 75% N recorded the highest pod yield, 100-pod weight and 100-seed weight, 1867.50Kg fd^{-1}, 169.44 g and 87.12g, respectively.

Table 3. Pigmentcontnent and phosphate dissolving in cyanobacterial culture medium

Strain	Chlorophyll a (µg ml^{-1})	Phosphate Dissolving
Anabaena Oryzae	24.75	**positive**
Nostoc muscorum	20.68	**positive**

Table 4. Averages of plant height^1, No. of podsplant^{-1} and seed weight plant^{-1} as affected by alga types, N fertilization levels in and their mixed application

Treatments		**Plant height (cm)**		**Number of Pods plant $^{-1}$**		**Seed weight plant^{-1} (g)**	
Control		**27.75 (a)**		20**.44 (ab)**		**23.299 (c)**	
			Single Application				
N%		**50**	**75**	**50**	**75**	**50**	**75**
Seed coating (C)	*A.oryzae*	22.50(bc)	26.5 (bc)	23.64 (bc)	26.26(bc)	20.83(ef)	22.86(cd)
	N. muscorum	21.58(bc)	24.60 (bc)	19.38 (c)	21.63(bc)	19.95(f)	22.48(cd)
Soil drench (S)	*A.oryzae*	24.9(a)	28.38 (a)	22.5 (a)	26.00(a)	21.20(e)	27.60(a)
	N.muscorum	23.13(a)	27.13 (a)	21.84 (ab)	24.63(a)	21.25(e)	26.31(b)
Foliar (F)	*A.oryzae*	24.38 (b)	28.25 (b)	21.38 (d)	23.47(bc)	22.06(cd)	24.41(c)
	N.muscorum	23.88(b)	26.75 (b)	18.29 (d)	20.31(bc)	23.40(c)	25.60(b)
L.S.D. (0.05)		**3.20**		**2.71**		**3.30**	
			Mixed Application				
N%		**50**	**75**	**50**	**75**	**50**	**75**
A.oryzae (S) + *N.muscorum* (C)		28.00 (c)	30.44(b)	26.88 (de)	29.38 (bc)	27.6(b)	28.28(ab)
A.oryzae (S) + *N.muscorum* (F)		29.63 (b)	31.20(a)	28.89 (cd)	32.00 (a)	27.43(b)	31.75(a)
A.oryzae (C) + *N.muscorum* (S)		26.38(d)	28.00(c)	24.85 (f)	27.5 (d)	25.91(c)	27.21(b)
A.oryzae (C) + *N.muscorum* (F)		29.25(bc)	30.88(ab)	27.63(d)	30.25(ab)	26.32(bc)	27.99(ab)
A.oryzae (F) + *N.muscorum* (S)		27.38(cd)	30.13(a)	27.22 (d)	29.63 (b)	27.42(ab)	27.33(b)
A.oryzae (F) + *N.muscorum* (C)		27.00(cd)	30.00 (bc)	27.10(de)	29.5 (b)	27.38(ab)	29.8(ab)
L.S.D. (0.05)		3.40		1.75		2.75	

Biofertilizers are recommended for agricultural use as a renewable natural nitrogen fixing biofertilizer for a variety of crop plants. They are nonpolluting, low-cost, and rely on renewable resources in addition to free solar energy, atmospheric nitrogen, and water [33]. Cyanobacteria are a valuable bioresource that has primarily been used in agriculture as a biofertilizer due to their well-established role as diazotrophs, proficiency in diverse soil ecologies, and ability to compete with native flora and fauna [34]. Furthermore, the nutrients fixed by cyanobacteria are made available to plants and microbial life in the soil. These are used as inoculants to improve soil fertility and structure, as well as crop yields, particularly in rice and other plants [35]. Cyanobacteria, whether in solid or liquid form, might be a beneficial potential biofertilizer. They also may photosynthesize and fix nitrogen in a wide range of soil types [36]. Biofertilizers have the potential to improve long-term crop productivity and soil fertility while reducing the environmental impact of fertilizer production and nutrient leaching into groundwater deposits [37]. Crop plants can only use fixed nitrogen when it is available extracellularly, either as extracellular products or through mineralization of their intracellular contents after death via microbial decomposition, because of the slow release of fixed and metabolized nitrogen, crop plants can use more nutrients from the soil in the presence of algal inoculation. The nitrogen fixation by blue-green algae has a 'on' switch that activates when the combined nitrogen level falls below a threshold level (40 ppm) due to progressive utilization and loss from the soil atmosphere [38].

3.2.2 Nitrogen (N), potassium (K), phosphorus (P) contents in plants

Table (6) showed that the ratio of nitrogen, phosphorus and potassium contents in peanut plant and seeds. In respect of presence of cyanobacteria, single and mixed treatments in the presence of 75% nitrogen had higher contents than other treatments and control. The largest nitrogen, phosphorus, and potassium ratios were 1.35, 0.46, 3.10 respectively recorded by *A. oryzae* (S) + *N. muscorum* (F) mixed treatment with 75% nitrogen , followed by *A.oryzae* (F) + *N. muscorum* (S) mixed treatment with 75% , 1.31, 0.44 and 2.93 respectively. On the other hand, single applications with 75% nitrogen was higher than single applications with 50% nitrogen, *A.oryzae* treatment in foliar application recorded the highest nitrogen, phosphorus, and potassium ratios , 1.16,0.38 and 2.70 respectively. The levels of nitrogen, phosphorus, and potassium in seeds followed a similar trend, the largest nitrogen, phosphorus, and potassium contents were 328.50,89.47 and 351.30 mg Kg^{-1}seed respectively, recorded by *A.oryzae* (S) + *N.muscorum* (F) mixed treatment with 75% nitrogen , followed by *A.oryzae* (F) + *N.muscorum* (S) mixed treatment with 75% , 326.70, 86.91 and 349.19 mg Kg^{-1}seed respectively. Additionally, seeds with a single application of cyanobacteria and 75% nitrogen had higher nitrogen, phosphorous, and potassium contents than seeds with a single application and 50% nitrogen addition. The largest nitrogen, phosphorus, and potassium were 268.30,61.08 and 302.30 mg Kg^{-1}seed respectively which reported by *A. oryzae* treatment in foliar application.

The widespread use of chemical nitrogenous fertilizers in agriculture is a worldwide concern. Alternatives to nitrogen fertilizers must be found urgently due to environmental concerns. This alternative is biological nitrogen fixation (BNF), a microbiological process that converts atmospheric nitrogen into a plant-usable form. Nitrogen-fixing systems are an economically and environmentally sound way of reducing external inputs while improving internal resources [38]. It has also been proposed that cyanobacteria can improve phosphorus bioavailability to plants by solubilizing and mobilizing insoluble organic phosphates in the soil using phosphatase enzymes [35].

Results of this study agreed with Ghazal et al. [33] who revealed that, plant growth-promoting rhizobacteria (PGPR), potassium solubilizer, phosphorus solubilizer, and nitrogen fixer (N-fixer) are the biofertilizers that are most frequently used. The effects of organic and mineral fertilisation on plants growing in sandy soil were examined by Ahmed et al. [39]. The production of cereals, wheat straw, and peanut pods all increased via biofertilizers. The metabolic cost hypothesis can be investigated using cyanobacteria as models. Increased nitrogen fixation may be to blame for this, as it helped the plants absorb and utilize all of their nutrients more efficiently. This resulted in higher levels of N, P, and K in the kernel and haulm, which in turn caused higher levels of N, P, and K uptake [40].

Table 5. Averages of pod yield fed.$^{-1}$ (kg), 100-pod weight (g), 100-seed weight (g), and 100-pod weight (g) as affected by cyanobacteria types, N fertilization levels and their mixed application

Treatments		**pod yield fed.$^{-1}$ (kg)**		**100-pod weight (g)**		**100-seed weight (g)**	
Control		1670.75 (ab)		138(a)		60.76 (ab)	
		Single Application					
N%		**50**	**75**	**50**	**75**	**50**	**75**
Seed coating (C)	*A. oryzae*	1489.64 (bc)	1681.75(a)	126.50 (bc)	145.00 (b)	56.63(c)	63.83(b)
	N. muscorum	1187.33 (cd)	1677.37(a)	123.50 (bc)	138.13(b)	51.13(d)	66.00(ab)
Soil drench (S)	*A. oryzae*	1308.00 (c)	1521.75(ab)	120.00(ab)	138.38(ab)	49.88(de)	63.88(b)
	N. muscorum	1197.88 (cd)	1684.63(a)	125.38(ab)	140.00(ab)	50.38(de)	74.00 (a)
Foliar (F)	*A. oryzae*	1411.38 (bc)	1677.13(ab)	127.63(cd)	147.13(b)	54.75(cd)	65.75(ab)
	N. muscorum	1197.88 (cd)	1684.63(a)	125.38(cd)	140.00(bc)	50.38 (de)	74.00 (a)
L.S.D. (0.05)		307.47		4.68		4.67	
		Mixed Application					
N%		**50**	**75**	**50**	**75**	**50**	**75**
A.oryzae (S) + *N.muscorum* (C)		1659.75 (e)	1803.00 (b)	147.00 (d)	164.00(b)	73.13(ef)	84.28(ab)
A.oryzae (S) + *N.muscorum* (F)		1712.20(d)	1867.50 (a)	153.22 (c)	169.44 (a)	75.00(e)	87.12(a)
A.oryzae (C) + *N.muscorum* (S)		1664.5(e)	1799.57 (bc)	149.88 (cde)	166.50(ab)	74.75(e)	85.64 (ab)
A.oryzae (C) + *N.muscorum* (F)		1623.13(e)	1783.25 (c)	146.13 (de)	168.00(a)	71.25 (fg)	82.25 (c)
A.oryzae (F) + *N.muscorum* (S)		1658.88(ef)	1776.65 (cd)	141.38(f)	164.63(b)	68.75(g)	81.25(cd)
A.oryzae (F) + *N.muscorum* (C)		1672.50(de)	1807.30(b)	150.14(cd)	165.37(ab)	72.46(f)	85.30(ab)
L.S.D. (0.05)		132.44		114.15		3.02	

Table 6. Averages of N, P and K percentages in peanut plants as affected by cyanobacteria treatments under different N fertilization levels in and their mixed types of applications of cyanobacteria

Treatments		**Plant**						**Seeds**					
		N %		**P %**		**K %**		**N mg.Kg^{-1} seed**		**P mgKg^{-1} seed**		**K mg Kg^{-1} seed**	
Control		0.98(ab)		0.37 (a)		2.03(c)		267 (a)		60.76(a)		299.90 (a)	
						Single Application							
N%		**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**
Seed coating (C)	*A.oryzae*	0.96 (b)	1.14 (a)	0.29 (ab)	0.36 (ab)	1.79 (de)	2.60 (ab)	119.90 (cd)	235.80 (ab)	23.74 (d)	59.07 (a)	201.3 (d)	292.20 (a)
	N. muscorum	0.90 (bc)	1.11 (a)	0.27 (c)	0.34 (ab)	1.64 (e)	2.47 (bc)	108.6 (d)	228.00 (ab)	21.33 (e)	51.57 (b)	209.30 (cd)	299.80 (a)
Soil drench (S)	*A.oryzae*	0.84 (c)	1.10 (a)	0.27 (b)	0.33 (ab)	1.41 (g)	2.49 (bc)	100.4 (de)	184.70 (bc)	21.18 (e)	54.94 (ab)	189.7 (e)	261.30 (b)
	N.muscorum	0.82 (c)	1.07 (a)	0.26 (bc)	0.28 (bc)	1.52 (ef)	2.42 (bc)	94.9 (e)	179.90 (c)	19.16 (f)	47.74 (c)	194.60 (de)	278.20 (ab)
Foliar (F)	*A.oryzae*	1.02 (ab)	1.16 (a)	0.30 (b)	0.38 (a)	1.95 (d)	2.70(a)	109.8 (d)	268.30 (a)	24.37 (d)	61.08 (a)	207.70 (cd)	302.30 (a)
	N.muscorum	1.00 (ab)	1.14 (a)	0.29 (b)	0.36 (a)	1.76 (de)	2.67(a)	113.2 (cd)	241.40 (ab)	22.55 (de)	53.92 (ab)	218.50 (c)	298.80 (a)
L.S.D. (0.05)		0.61		0.11		0.70		8.11		1.36		27.07	
						Mixed Application							
N%		**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**
A.oryzae (S) + *N.muscorum* (C)		1.19 (ab)	1.28 (a)	0.35 (ab)	0.40 (a)	2.47 (ef)	2.73 (bc)	179.10 (ef)	321.30 (a)	52.43 (f)	82.09 (ab)	2778.00 (e)	328.93 (ab)
A.oryzae (S) + *N.muscorum* (F)		1.20 (ab)	1.35 (a)	0.40 (a)	0.46 (a)	2.58 (d)	3.10 (a)	196.00 (de)	328.50 (a)	57.00 (e)	89.47 (a)	301.80 (bc)	351.30 (a)
A.oryzae (C) + *N.muscorum* (S)		1.18 (ab)	1.27 (a)	0.32 (b)	0.39 (ab)	2.44 (f)	2.83 (ab)	176.90 (ef)	316.0 (b)	50.35 (fg)	79.24 (bc)	282.90 (d)	324.60 (ab)
A.oryzae (C) + *N.muscorum* (F)		1.17 (ab)	1.27 (a)	0.30	0.40 (a)	2.41 (fg)	2.85 (ab)	168.80 (f)	324.3 (a)	49.87 (h)	77.63 (bc)	279.40 (de)	334.80 (a)
A.oryzae (F) + *N.muscorum* (S)		1.19 (ab)	1.30 (a)	0.37 (ab)	0.44 (a)	2.50 (de)	2.93 (a)	199.00 (cd)	326.7 (a)	55.34 (ef)	86.91 (a)	293.80 (c)	349.19 (a)
A.oryzae (F) + *N.muscorum* (C)		1.22 (ab)	1.29 (a)	0.36 (ab)	0.42 (a)	2.40 (fg)	2.78 (b)	175.5 (e)	297.8 (ab)	50.33 (fg)	75 (d)	287.30 (cd)	338.90 (a)
L.S.D. (0.05)		0.51		0.13		0.40		5.44		4.20		82.57	

3.3 Soil Analysis

Table 7 shows the changes in soil pH, organic matter percentage, and some nutrient contents such as nitrogen, potassium, and phosphorus due to individual and mixed cyanobacterial treatments using different types of application and nitrogen ratios after at harvest time. The results revealed that soil pH increased in all treatments when compared to the control, and pH was higher in single applications than in mixed applications. The highest pH was 7.75 recorded by *N. muscorum* + 75% N treatment in foliar single application and 7.61 in mixed application *A. oryzae* (S) + *N. musco rum* (C) + 75% N. In terms of the interaction effect between cyanobacteria treatments and nitrogen rates, the organic matter percentage increased across all treatments and applications, compared to the control treatment, in a mixed application, *A. oryzae* (S) + *N. muscorum* (C) + 75% N treatment showed the highest percentage of organic matter (0.45%), while *A. oryzae* + 75% N treatment reported the highest percentage of organic matter (0.29%) in a single application . In the other respect of the presence of both cyanobacteria soil nutrient contents increased when compared to uninoculated soil, additionally, nutrient contents were higher in mixed applications than single applications. The largest nutrient contents (N, P and K) were reported in mixed application *A. oryzae* (S) + *N. muscorum* (C) + 75% N (141.43, 6.95 and 322.50 mg kg^{-1}soil respectively) , however the largest single application nutrient contents (N, P and K) were reported in treatment of *A. oryzae* +75% N in seed coating application type (99.37, 4.2 and 260.15 mg kg^{-1}soil respectively). Values of few selected characteristics of soil under different treatments.

Cyanobacteria are an emerging type of microorganism for the development of sustainable agriculture [35]. Theoretical illustrations of cyanobacteria's potential roles in environmentally and agriculturally sustainable practises. Cyanobacteria called diazotrophes are helpful for producing affordable, easily accessible biofertilizers that are friendly to the environment. They are able to supplement vitamin B12, improve soil aeration and water holding capacity, and control plant nitrogen deficiency [41]. In spite the fact that groundnuts are legumes and can fix atmospheric nitrogen, they respond best to initial doses of modest nitrogen fertilizer amounts, Results of this study revealed that different ratios of nitrogen inoculation with cyanobacteria caused in a significant increase in soil organic matters and nutrient contents compared to no inoculation, these results were agreement with Ahmed et al. [42].

According to Song et al. [39], cyanobacteria play an important role in the maintenance and development of soil fertility, and thus serve as a natural biofertilizer. The primary functions of blue-green algae are, make adhesive substances are produced in porous soil, excreta of phytohormones (auxin, gibberellins, and so on), vitamins, and amino acids [43].Improve soil water holding capacity by using their unique properties. Structure of jelly increase in soil biomass after their death and composition, reduced soil salinity, controls weed development [44] and soil phosphate availability due to excretion of efficient heavy metal absorption on the organic acids [45]. The cost of producing inorganic nitrogen fertilisers is extremely high. The use of biofertilizer in a sustainable manner addresses nitrogen deficiency in crops. Biofertilizers such as cyanobacteria can fix less than 10 kg/ha nitrogen. Dense mats of cyanobacteria fix approximately 10-30 kg/ha of nitrogen per year [46].

3.4 Response of Microbial Community in Soil

Data in Table 5 revealed that total bacterial and cyanobacterial counts increased as a result of soil biological activities. The microbial community increased more in mixed applications than in single applications when 75 %N was added. *N. muscorum* (C) + 75% N in soil drench single application produced the highest bacterial and cyanobacterial counts of 380 and 63x 10^6 CFU ml^{-1}, respectively. On the other hand, *A. oryzae (C)* + *N.muscorum (S)* + 75 % N in mixed application had the highest bacterial and cyanobacterial counts (490 and 90 x 10^6 CFU ml^{-1}) respectively. These findings were corroborated by Ghazal et al. [47], who noticed that cyanobacterial mixed applications increased soil microbial counts and activities.

In the current study, cyanobacteria inoculation increased the soil's biological activity in terms of the total number of bacteria and cyanobacteria present, soil characteristics, microbial activity and soil fertility are frequently linked. Soil microbial biomass is essential for preserving the functions of the soil because it symbolizes the mineralization of important organic compounds, these findings were in agreement with Shaheen

Table 7. Values of few pH , organic matter percentage , N, P and K mg. kg^{-1}soil in peanut plants as affected by cyanobacteria treatments under different N fertilization levels in and their mixed types of applications

Treatments		**pH**		**O.M%**		**N mg. kg-1soil**		**P mg. kg^{-1}soil**		**K mg. kg^{-1}soil**	
N%		**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**
Control		7.43		0.21		66(c)		3.21(bc)		151(d)	
						Single Application					
Seed coating (C)	*A. oryzae*	7.61	7.4	0.27	0.32	78.66(c)	88.37 (b)	2.51(cd)	3.28 (ab)	179.1 (ab)	260.15 (a)
	N. muscorum	7.68	7.47	0.25	0.28	75.27 (cd)	93.44 (ab)	2.96 (bc)	3.97(a)	168.20 (cd)	181.32 (ab)
Soil drench (S)	*A. oryzae*	7.70	7.58	0.24	0.29	75.24 (cd)	88.31 (b)	2.70(c)	3.38(a)	145.36 (de)	198.57(a)
	N. muscorum	7.73	7.62	0.22	0.26	73.1 (d)	99.67 (a)	2.62(c)	4.2(a)	148.11 (e)	172.18 (bc)
Foliar (F)	*A. oryzae*	7.74	7.69	0.22	0.27	71.33 (de)	90.75 (ab)	2.52 (c)	3.09 (ab)	134.52 (f)	179.35 (ab)
	N. muscorum	7.73	7.75	0.21	0.23	68.35 (e)	84.50 (bc)	2.47(cd)	2.99 (bc)	131.45 (g)	150.25 (d)
L.S.D. (0.05)						4.68		0.79		27.04	
						Mixed Application					
N%		**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**	**50**	**75**
A.oryzae (S) + *N.muscorum* (C)		7.55	7.61	0.36	0.45	111.33 (bc)	141.43 (a)	3.65(cd)	6.95(a)	250.22 (c)	322.50(a)
A.oryzae (S) + *N.muscorum* (F)		7.58	7.15	0.33	0.43	106.23 (c)	126.62 (ab)	3.39(cd)	6.65(a)	241.65 (d)	300.22 (ab)
A.oryzae (C) + *N.muscorum* (S)		7.60	7.25	0.31	0.39	100.26 (d)	114.67 (bc)	3.45(cd)	6.55(a)	182.58 (e)	276.82 (b)
A.oryzae (C) + *N.muscorum* (F)		7.63	7.37	0.25	0.34	92.64 (f)	105.33 (c)	3.02(def)	5.34(b)	164.48 (f)	301.57 (ab)
A.oryzae (F) + *N.muscorum* (S)		7.58	7.28	0.31	0.38	95.67 (df)	116.43 (bc)	3.10(de)	5.53(b)	183.81 (e)	321.50 (a)
A.oryzae (F) + *N.muscorum* (C)		7.62	7.43	0.23	0.32	90.16 (g)	102.12 (c)	2.91(g)	4.20(c)	166.33 (f)	300.64 (ab)
L.S.D(0.05)						5.44		1.70		82.57	

Table 8. Averages of total bacterial count and cyanobacterial count as affected by cyanobacteria treatments under different nfertilization levels and their mixed types of applications of cyanobacteria

Treatments		**Total Cyanobacterial Count (x 10 CFU g dry rhizosphere soil^{-1})**		**Total Bacterial count count (x 10^{6} CFU g dry rhizosphere soil^{-1})**	
Control		50		27	
		Single Application			
N%		50	75	50	75
Seed coating (C)	*A.oryzae*	39	50	14	28
	N. muscorum	42	58	16	30
Soil drench (S)	*A.oryzae*	48	60	17	32
	N. muscorum	52	63	18	38
Foliar (F)	*A.oryzae*	42	57	17	30
	N. muscorum	41	56	15	34
		Mixed Application			
N%		**50**	**75**	**50**	**75**
A.oryzae (S) + *N.muscorum* (C)		60	82	24	39
A.oryzae (S) + *N.muscorum* (F)		70	86	23	45
A.oryzae (C) + *N.muscorum* (S)		78	90	28	49
A.oryzae (C) + *N.muscorum* (F)		82	89	26	42
A.oryzae (F) + *N.muscorum* (S)		80	88	25	44
A.oryzae (F) + *N.muscorum* (C)		79	87	22	43

et al. [48]. *Nostoc muscorum* and *Anabaena oryzae*, two strains of cyanobacteria that produce exudates and biomass capable of improving soil microbial activity, are impacted by soil nutrients and microbial activity [49].

Cyanobacteria, whether solid or liquid, could represent a useful potential biofertilizer. They are able to photosynthesize and fix nitrogen in a variety of soil types [50] and have an exclusive capacity to fix nitrogen from the atmosphere by combining photosynthesis and nitrogen fixation. Bio-fertilizers are often more environmentally friendly, less expensive, and can meet crop nutrient requirements [51]. Certain cyanobacteria have been found to not only succeed in such harsh environments, but also to improve the soil's physicochemical properties by supplementing it with carbon, nitrogen, available phosphorus, and other nutrients.

4. CONCLUSION

It was obvious that using two cyanobacterial individual strains, *Nostoc muscorum* and *Anabaena oryzae*, with different types of applications had a positive effect on peanut plants, while combining applications with both cyanobacterial strains and 75% nitrogen resulted in more increasing in growth characteristics and soil biological activities in peanut plants and their rhizosphere soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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