Impact of Cyanobacterial Inoculant on Growth and Productivity of Peanut Plants in Sandy Soil

ABSTRACT

During the summer growing seasons of 2021 and 2022, two field experiments were conducted at Ismailia Agricultural Research Center Station(Latitude 30º 35′ 41.901″ N and Longitude 32º 16′ 45. 843″E)to study the effect of cyanobacterial inoculation (*Anabaena oryzae* (*A. oryzae*) and *Nostoc mascarum*(*N. mascarum*)) on peanut yield , quality and certain soil biological activities under various nitrogen fertilization conditions and three types of applications, thefirst treatment was carried out as coating seeds with powder of individual of each cyanobacterial strainandthebefore planted, second treatment seeds were drenched withsuspension of eachcyanobacterial strainindividually andthe last treatment was by foliar doses after 15, 45 and 60 days from seeds planting.Results showed that applying cyanobacteria inoculation to peanut plants generally enhanced peanut plant growth, leading to significantly higher yields of peanut and grains than uninoculated treatments. Treatment of *N.mascarum* + 75% N recorded the highest peanut yield and plant characteristics followed by *N. mascarum*+ 75% N in soil drench application compared to other tested treatments and types of applications. Cyanobacteria enhanced the amount of N, P, Kand Ca in peanut plants overall. By increasing the total chlorophyl, carotenoids, dehydrogenase, urease activities and nutrients in the peanut rhizosphere, cyanobacteria inoculation had a favorable impact on soil fertility. In general, cyanobacteria inoculation with 75% nitrogen amounts can benefit under peanut growth in sandy soil conditions.

Keywords: Cyanobacteria; Anabaena oryzae; Nostoc mascarum; peanut.

1. INTRODUCTION

Peanut (*Arachis hypogaea* L.) is a member of the groundnuts Leguminosae family and extensively cultivated oilseed and food legume in the semi-arid tropics, peanuts grow best in lightwarm, sandy soil, but may be grown in most other soil types provided enough withsuitable fertilizers to loosen the soil. Peanut (nut) pods develop underground. It is one of the most significant and frequently cultivated oil crops, which yearly produces 11% of the protein and 20% of the world's cooking oil [1].

Cyanobacteria are a new type of microbes that can help farmers achieve long-term success. [2] illustrates a theoretical picture of cyanobacteria's possible roles in sustainable agriculture and environmental protection. Because cyanobacteria can fix nitrogen from the air, they could be employed as a biofertilizer in the production of economically significant crops like rice and beans. Chlorophylls, carotenes, xanthophylls, c-phycoerythrin, and c-phycocyanin are among the pigments, with the last two pigments being found in bluegreen algae [3]. They also can help plants with nitrogen deficit, soil aeration, water holding capacity, and vitamin B12 supplementation. They have the potential to fix atmospheric nitrogen, so that could be used as a biofertilizer for the cultivation of economically importantcrops such as rice and beans [4]. Cyanobacterial/microalgal culture for bioremediation can overcome some of the primary restrictions associated with bacteria and fungus that require carbon and other nutrients for growth and development in stoichiometric balance degradation of contaminants.Cyanobacteria can digest a variety of contaminants and play a variety of roles in the soil ecosystem to keep soil fertility high [5].

Several studies have found that cyanobacteria can create extracellular polymeric compounds that aid them in overcoming water stress and binding soil particles to improve soil structure and fertility [6]. Exopolysaccharides-producing cyanobacteria are important in the reclamation and fertilization of desert soils, according to [7]. In the rice crop cultivation area, *Nostoc* sp., *Anabaena* sp., *Aulosirafertilisima*, *Calothrix* sp., *Tolypothrix* sp., and *Scytonema*sp. are the most efficient nitrogen-fixing cyanobacteria [8]. *Anabaena* and *Nostoc* survive on the surface of soil and rocks, fixing up to 20–25 kg of nitrogen from the atmosphere each hectare. *Anabaena* can fix 60 kg of nitrogen per hectare per season and enriches soils with organic matter. Cyanobacteria can grow, mature, and produce useful organic products without the need for a host, whereas cyanobacteria naturallyproduce over 1100 secondary metabolites, includingnon-canonical short peptides, polyketides, terpenes, alka-loids and lipids, although some cyanobacterial species appear to be a major source of carotenoids and antioxidants, unlike chloroplasts, they only produce a moderate amount of aromatic amino acids [9]. Cyanobacteria like as *Anabaena* and *Nostoc* can be found on the surface of soil and rocks, and they are important in the maintenance and build-up of soil fertility, yielding as a natural biofertilizer for different crops such as barley, oats, tomato, radish, cotton. Cyanobacterial algae's main functions are to make porous soil, create sticky compounds, phytohormones (auxin, gibberellins, etc.), vitamins and amino acids excretion [10], increase the water-holding capacity of soil due to their jelly-like texture [11], increasing soil biomass following death and degradation , decreases soil salinity, prevents the growth of weeds [12], Organic acid excretion as a source of soil phosphate [13], Effective heavy metal absorption on the microbial surface (bioremediation) [14].

Due to the previous advantages of cyanobacteria, the current study was curried out to investigate the effect of two cyanobacterial strains *A. oryzae* and *N.mascarum* as individual strain on growth and productivity of peanut plant with different ratios of nitrogen and compare between using full nitrogen fertilization and replace it with cyanobacteria by 50 ,75 % nitrogen ratios, in order to exchange chemical N fertilizers with biofertilizers and improve the biological characteristics of these sandy soilswhich are available in Ismailia Research Center stationandproduce clean agricultural productsin suitable atmosphere.

2. MATERIALS AND METHODS

The current study was carried out in a field experiment in sandy soil at the Ismailia Agricultural Research Station (ARC), Ismailia Governorate, Egypt, in two consecutive summer seasons 20201 and 2022. Three various methods of adding treatments of cyanobacterial strains and different nitrogen fertilization ratios were achieved. Agric. Microbiol. Dept., Soils, Water and Environ. Res. Inst., ARC, Giza, Egypt, donated of A.*oryzae* and *N. muscorum*c yanobacterial strains. Area of experiment was 10.5 m^2 and contained 5 ridges: 60 cm apart and 3.5 m long. During soil preparation recommended doses of potassium and phosphorous fertilization was added as potassium sulfate (48 % K_2SO_4) and monocalcium super phosphate (15.5% P_2O_5) form and nitrogen as ammonium nitrate (33.5%), thenitrogen 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. Treatments of cyanobacteria were added by three different ways as following, first, seeds were drenched with suspension of *A. oryzae* and *N. muscorum* individual of each strain, another treatment was carried out as coating seeds with powder of individual of each cyanobacterial strain, before planted, the last treatment was by foliar doses after 15 ,45 and 60 days from seeds planting. Seeds were planted by hand on one side of the ridges (3-4 seed in each hill) and diluted to 2 plants $hill^{-1}$ after 15 days of planting. Cyanobacterial treatments were applied three times during experiment, first at zero time of planting, second was after 30 days and the last was after 45 days of planting. All recommended agricultural practices were adopted throughout both growth seasons. Samples of plants were taken randomly at harvest time after 75 days to determine different soil and plant analysis.

2.1 Plant Analysis

2.1.1 Total chlorophyll and carotenoids

Pigments were extracted from 1 g fresh young leaves in a dimethylformamide (DMF) soln. Overnight at 4□ to estimate the mass of chlorophyll a, chlorophyll b, total chlorophyll and Carotenoids per leaf. At wavelengths of 663, 470, and 647 nm, the pigments were calculated using Moran's equation and a spectrophotometer Beckman Du 7400 [15].

2.1.2 Seeds quality

The peroxidenumber, saponification, iodine value, and saponifiable value were determined in seeds oil percentage content, Total carbohydrates percentages determined according to Dubios et al., Protein content in the peanut seedsaccording to standard methods [16].

2.2 Soil Analysis

2.2.1 Soil characteristics and available nutrients N,P, K and Ca

Soil samples were collected, air dried, gently ground and passed through 2mm sieve to determining, the particle size distribution using international pipte method and hexameta phosphate as dispersing agent. Soil organic matter (OM) content was determined using the modified walkelt and Blak method. pH using electrod pH meter. Water soluble cations and anions were determined by Collins Clcimeter and available N,P and K according to [17]. Soil exchangeable Ca^{2+} was extracted with 1 mol/L ammonium acetate (pH 7.0) [18].

Table 1. Physical and chemical properties of experimental soil

Soil characteristics	Value	Soil characteristics	Value
Particle size distribution%:		**Soluble cations (soil paste mmolc L^{-1}):**	
Coarse sand	62.35	Ca^{2+}	2.92
Fin sand	30.08	Mg^{2+}	2.18
Silt	3.45	Na^{+}	1.30
Clay	4.12	K^{+}	2.34
pH (1:25 soil: water suspension)	7.65		
ECe (dS m^{-1}, soil paste extract)	0.87	Soluble anions (soil paste, mmolc L^{-1}):	
$CaCO_3$ %	0.18	CO_3^{2-}	0.00
Organic matter %	0.21	HCO_3^{-}	2.47
Available nutrients (mg Kg-^{1})		Cl^{-}	3.53
Nitrogen	34.67	SO_4^{-2}	2.74
Phosphorus	2.1		
Potassium	147		

2.2.2 Dehydrogenase and urease activity

Casida's method for determining dehydrogenase activity was used to examine the samples [19], while urease activity was measured using colorimetric method [20].

3. RESULTS AND DISCUSSION

3.1 Chlorophyll and Carotenoids

Both cyanobacterial strains are greatly improved total chlorophyll , carotenoids, contents in peanut plants, data in Table 2 indicated that, the effect of treatment with individual cyanobacterial strains, and with different nitrogen rates by different types of applications on total chlorophyl and carotenoids of peanut plants at 70 days during two seasons 2021 and 2022, the highest chlorophyll contents were 1.55 and 1.44 mg g^{-1} F.W, recorded by *N. muscurum* +75% Ntreatmentin seed coating and soil drench application respectively, while the largest content of carotenoids were 52.82 and 51.53 mg g^{-1} F.W recorded by *A.oryzae and A.oryzae*+ 50 % N treatments in soil drench and foliar application respectively . Generlly total chlorophyll and carotenoids contents increased in treated peanut plants in the presence of both cyanobacterial inoculants supplemented with nitrogen in different types of applications.

Moreover, data in (Table 3) showed the effect of treatment with cyanobacterial as individual strains and with different nitrogen rates by various types of applications on oil percentages, iodine number, peroxidase number, saponification value total carbohydrates percentage and protein solubility percentage in peanut seeds after harvest during two seasons 2021 and 2022. A slightly significant variation in the peanut oil percentage content was observed among different applications and treatments, the highest oil percentage was 45% reported by *N. muscorum*+75 % N treatment in soil drench application type followed by 43.75% reported by treatment of *A. oryzae* + 75% N in the same type of application, then *N.muscorum*+75 % N and *A. oryzae*+ 75% N treatments in the other two types of application. Iodine number for peanut seeds was measured to define the degree of unsaturation and stability of peanut oil samples of peanut seeds , that affect the quality of seeds and decrease the percentage of rancidity, the highest iodine value was 97.42 recorded by treatment of *A. oryzae* followed by 97.39, 97.28 measured by *N. muscarum*and *A. oryzae + 50%* N respectivelyin seed coating application type , but foliar application type iodine number was less than seed coating 97.36 recorded by *A. oryzae* treatment and 97.29 recorded by *N. muscarum* treatment while soil drench application type was the least Iodine number at all. Peroxidase number. The peroxide number of different varieties of peanut oil has significantly the highest value of 3.63 mEq was found in *A. oryzae* treatment, followed by 3.32 and 3.26 mEq which recorded by *A. oryzae* +50% N and *N. muscarum* treatments respectively in seed coating application type, had peroxide value of other treatments in all application type was in acceptable values between 2.78 and 3 mEq. Saponification valueis an indication of the size or nature of fatty acid chains esterified to glycerol, the highest saponification values of peanut oil were 193.30 and 192.84 mg g^{-1} which recorded by *A. oryaze* and *N. muscarum* treatments, respectively in seed coating application followed by 192.75 and 192.50 for the *A. oryaze* treatment in foliar and soil drench application, respectively. However total carbohydrate percentage found in soil drench application was the highest percentage 11.36, 11.29 and 11.20 recorded by *N. muscarum* + 75% N, *A. oryzae* +75% Nand *N. muscarum*+50% N treatments respectively. The last recorded parameter was protein solubility percentage , treatment of *Nostoc* + 75% N showed the largest protein percentage 23.29 and *A. oryzae* +75% N 22.74 in foliar and soil drench pplication respectively, followed by 22.94 recorded by treatment of *A. oryzae* +75% N in soil drench application.

3.1.1 Response of Soil pH, Nutrients Contents, Soil Organic Matter Percentages, and Soil Biological Activity to

Data in Table 4 reported the changes in soil pH, organic matter percentage and some nutrient contents such as nitrogen, potassium, phosphorus, and calcium, due to the effect of cyanobacterial treatments and different types of application after 70 days of planting and at harvest time. Results showed that soil pH decreased in *N. muscorum*+75 % N treatment in soil drench application (7.20) than control and other treatments, followed by *A. oryzae* +75 % N treatment (7.23), *N. muscorum*+50 % N treatments (7.30) and *A.oryzae*+50 % N (7.34) treatment, in the same application in 70 days samples. In harvest samples *N. muscorum*+75 % N treatment reported the lowest pH at all (6.87) then *A. oryzae* +75 % N treatment (6.90), *N. muscorum*+50 % N treatments (6.99) and *A. oryzae*+ 50 % N (7.10) treatments, in soil drench applications, soil pH in other treatments and another both types of applications were equal or less than control treatment slightly. In respect to the interaction effect between cyanobacteria treatments and nitrogen rates organic matter percentage increased in both periods, in all treatments and applications than control treatment, the highest organic matter percentage was *N. muscorum*+75 % N treatment (0.3 and 0.34%) in soil drench application at harvest time and 70days respectively then *A. oryzae* +75 % N treatment (0.33 and 0.30 %) at harvest time and 70 days respectively in soil drench application,

Table 2. Effect of treatment with individual cyanobacterial strains, and with different nitrogen on total chlorophyl and caroteinoides of peanut plants at 70 days during two seasons 2021 and 2022

Treatments		Total chlorophyll (mg g^{-1} F.W)	Carotenoids (mg g^{-1} F.W)
Control		1.14	23.42
Seed coating	***A. oryzae***	0.81	35.81
	***A. oryzae* +50% N**	1.33	42.77
	***A. oryzae* +75% N**	1.06	46.52
	N. muscarum	1.35	34.62
	***N. muscarum* +50% N**	1.36	38.83
	***N. muscarum* + 75% N**	1.55	47.03
Soil drench	***A. oryzae***	1.10	49.77
	***A. oryzae* +50% N**	1.16	35.12
	***A. oryzae* +75% N**	1.43	43.78
	N. muscarum	1.29	44.52
	***N. muscarum* +50% N**	1.38	40.43
	***N. muscarum* + 75% N**	1.44	48.27
Foliar application	***A. oryzae***	1.26	52.28
	***A. oryzae* +50% N**	1.41	51.53
	***A. oryzae* +75% N**	1.08	44.32
	N. muscarum	1.20	48.02
	***N. muscarum* +50% N**	1.35	46.77
	***N. muscarum* + 75% N**	1.45	37.523

followed by the same treatments in foliar application (0.31 and 0.30%) and the smallest organic matter percenteges were in seed coating applications than control treatment. On the other respect, presence of both cyanobacteria increased nutrient contents in soil compered to uninoculated soil, *N. muscorum*+75 % N treatment was the highest nutrient contents, N (131), P (5.03), K(243) and Ca (1.27) mg.kg^{-1} in soil followed by *A. oryzae* +75 % N treatment N (122), P (4.58) K(227) and Ca (1.15) mg.kg^{-1} in soil in 70 days stage in soil drench application, moreover in seed coating application *N. muscorum*+75 % N treatment recorded N (92), P (4.63), K(217) and Ca (0.65) mg.kg^{-1}, but *A. oryzae* +75 % N treatment N (80), P (4.49),K(209) and Ca (0.60) mg.kg^{-1}in soil in 70 days stage, foliar application recorded the lowest nutrients contents in soil , while harvest time had lowest nutrient contents in all types of applications and all treatments than control.

Global concern surrounds the indiscriminate use of chemical nitrogenous fertilizers in agriculture. Alternatives to nitrogen fertilizers must be sought out immediately due to sustainability concerns. This alternative is provided by biological nitrogen fixation (BNF), a microbiological procedure that transforms atmospheric nitrogen into a form that plants can utilize. Nitrogen-fixing systems provide a financially appealing and environmentally responsible way to reduce external inputs and enhance internal resources [21]. Although groundnuts are legumes and can fix atmospheric nitrogen, they respond best to starting doses of small amounts of nitrogenous fertilizers. Results in Tables 2,3 and 4 indicated that, inoculation with cyanobacteria combined with different ratios of nitrogen significantly increased total chlorophyll and carotenoids, oil content, oil yield and net returns over no inoculation. Cyanobacteria are an emerging type of microorganism for the development of sustainable agriculture. It called diazotrophes are helpful for producing affordable, conveniently accessible biofertilizers that are friendly to the environment. The use ofcyanobacteria to increase crop productivity and soil fertility is covered in several green technology articles. Numerous types of contaminants can be broken down by cyanobacteria, which also plays a variety of roles in the soil ecosystem to maintain soil fertility [2]. Prasad and Prasad [8] reported that, *Nostoc* sp., *Anabaena* sp., *Aulosira* sp., *Calothrix* sp., *Tolypothrix* sp., and *Scytonema* sp. cyanobacteria are the most effective nitrogen-fixing cyanobacteria. They also can supplement plants by vitamin B_{12}, increase soil aeration and water holding capacity, and reduce plant nitrogen deficiency [22].

Chittoraet al. [23] revealed that cyanobacteria and other microalgae's usefulness in a variety of fields has made large-scale cultivation of them necessary. The success of large-scale biomass production. cyanobacteria are essential for maintaining and increasing soil fertility, which makes them a natural biofertilizer [22]. Blue-green algae's main functions are to create sticky compounds and porous soil, excretion of vitamins, amino acids, and phytohormones (auxin, gibberellins, etc.) [11], increase soil's ability to hold water thanks to their distinctive jelly structure, the soil's biomass increased after they died and decomposed, lower salinity of the soil, prevents the growth of weeds, soil phosphate availability due to organic acid excretion, effective heavy metal absorption on the an infectious surface [14].

3.1.2 Response of microbial community at different growth stages of peanut

Generally, data in Table 5 revealed that the presence of cyanobacteria mixed at different rates of nitrogen increased the soil biological activity as represented by the amount of DHA and urease activities after 70 days of planting. This increase in soil biological activity resumed to increase because of cyanobacteria inoculation till 70 days, then it highly decreased at harvest time due to reducing of water, inoculum and nutrients in all treatments and application types as well as control treatment. *N. muscorum*+75 % N treatment reported the highest activities in both enzyms in soil drench and seed coating applications 131.15, 115 µl H_2 (DHA) and 61.86, 52.4 µl NH_4 (urease) respectively, followed

Table 3. Effect of treatment with cyanobacterial strains with different nitrogen rateson oil percentages, pH, iodine number, peroxidase number, emulsification total carbohydrates percentage and protein contents in peanut seeds during two seasons 2021 and 2022

Treatments	Oil %	Iodine number	Peroxide number	Saponification value (mg g^{-1}) mg g-1	Total carbohydrate %	Protein %
Control	41.40	97.18	3.46	192.50	10.72	20.63

Seed coating	***A.oryzae***	37.55	97.42	3.63	193.30	10.55	19.55
	***A.oryzae* +50% N**	40.21	97.28	3.32	192.28	10.69	20.08
	***A.oryzae* +75% N**	41.84	96.92	3.00	191.75	10.81	21.64
	N. muscorum	39.10	97.39	3.42	192.84	10.63	19.63
	***N. muscorum* +50% N**	40.88	97.09	3.05	191.96	10.77	21.32
	***N. muscorum* + 75% N**	42.56	96.82	2.93	190.98	10.90	22.35
Soil drench	***A.oryzae***	39.60	97.22	3.15	192.50	10.70	21.00
	***A.oryzae* +50% N**	41.72	96.99	2.87	191.75	11.00	22.50
	***A.oryzae* +75% N**	43.75	95.77	2.44	189.55	11.29	22.94
	N.muscarum	40.18	97.15	3.00	192.10	10.89	21.33
	***N.muscarum* +50% N**	42.83	96.86	2.48	190.63	11.20	22.75
	***N.muscarum* + 75% N**	45.00	95.71	2.35	188.94	11.36	23.29
Foliar application	***A.oryzae***	38.80	97.36	3.26	192.75	10.61	20.28
	***A.oryzae* +50% N**	40.57	97.18	3.06	191.89	10.80	21.12
	***Anabeana* +75% N**	42.50	96.86	2.84	190.88	11.00	22.60
	N.muscarum	39.68	97.29	3.18	192.22	10.68	20.55
	***N.muscarum* +50% N**	41.53	97.00	2.97	191.24	10.95	21.79
	***N.muscarum* + 75% N**	43.50	96.78	2.78	190.55	11.15	22.74

Table 4. Effect of each cyanobacterial strains by different nitrogen rates on N,P, K and Ca contents in soil through growth peanut plants at 70 days and at harvest tomeduring two seasons 2021 and 2022

Treatments		Soil at 70 days						Soil at harvest time					
		pH	OM*	N	P	K	Ca	pH	OM*	N	P	K	Ca
			%		mg.kg^{-1} soil				%		mg.kg^{-1} soil		
Control		6	0.21	82(a)	4.18(a)	173(d)	0.45(g)	7.43	0.23	36 (c)	3.21(d)	151(d)	0.45(a)
Seed coating	*Anabena*	7.50	0.22	44 (g)	4.21(f)	178(e)	0.47(f)	7.41	0.23	32(e)	3.25(e)	155(c)	0.25(e)
	Anabeana +50% N	7.47	0.23	66(e)	4.37(e)	192(c)	0.55(d)	7.38	0.26	37(c)	3.29(d)	159(b)	0.25(d)
	Anabeana +75% N	7.43	0.24	80(c)	4.49(c)	209(a)	0.60(b)	7.27	0.29	39(b)	3.43(b)	163(a)	0.29(c)
	Nostoc	7.49	0.22	48(f)	4.21(f)	183(d)	0.49(e)	7.40	0.24	34(d)	3.25(d)	156(c)	0.27(d)
	Nostoc +50% N	7.44	0.24	74(d)	4.45(d)	205(b)	0.58 (c)	7.35	0.27	39(bc)	3.32(c)	163(e)	0.27(d)
	Nostoc + 75% N	7.41	0.26	92(b)	4.63(b)	217(f)	0.65(a)	7.26	0.30	42(a)	3.56(a)	167(a)	0.31(b)
Soil drench	*Anabena*	7.43	0.24	47(g)	4.25(f)	182(e)	0.86(f)	7.39	0.25	35(e)	3.42(e)	158(c)	0.54(e)
	Anabeana +50% N	7.34	0.28	86 (e)	4.47(e)	210(c)	0.97(d)	7.10	0.28	40(c)	3.49(e)	165(b)	0.58(d)
	Anabeana +75% N	7.23	0.31	122(c)	4.58(c)	227(a)	1.15(b)	6.90	0.33	43(e)	3.55(d)	171(a)	0.63(c)
	Nostoc	7.42	0.26	52(f)	4.28(f)	194(d)	0.92(e)	7.37	0.26	37(d)	3.44(b)	160(c)	0.65(d)
	Nostoc +50% N	7.30	0.30	109(d)	4.55(d)	223(b)	1.05(c)	6.99	0.31	43(bc)	3.57(c)	168(e)	0.60(d)
	Nostoc + 75% N	7.20	0.34	131(b)	5.03(b)	234(f)	1.27(a)	6.87	0.36	46(a)	3.73(a)	174(a)	0.67(b)
Foliar application	*Anabena*	7.52	0.22	39(g)	4.15(f)	175(e)	0.56(f)	7.41	0.23	29(e)	2.95(e)	152(c)	0.24(e)
	Anabeana +50% N	7.5	0.25	59(e)	4.22(e)	189(c)	0.62(d)	7.41	0.26	33(c)	3.12(d)	153(b)	0.26(d)
	Anabeana +75% N	7.49	0.29	78(c)	4.28(c)	198(a)	0.70(b)	7.39	0.30	36(e)	3.22(d)	157(a)	0.29(c)
	Nostoc	7.52	0.23	41(f)	4.15(f)	178(d)	0.59(e)	7.41	0.23	30(d)	2.97(d)	153(c)	0.27(d)
	Nostoc +50% N	7.50	0.27	65(d)	4.25(d)	195(b)	0.66(c)	7.40	0.29	33(bc)	3.15(c)	156(e)	0.27(d)
	Nostoc + 75% N	7.49	0.31	80(b)	4.33(b)	206(f)	0.76(a)	7.38	0.33	38(a)	3.27(a)	158(a)	0.30(b)
L.S.D. (0.05)				2.59	0.040	2.82	0.037			2.48	0.040	2.66	0.026

**OM : Organic Matter*

Table 5. Some microbial enzymes activities in rhizosphere of peanut beans plants treated with both cyanobacterial strains during two seasons

Treatments		Dehydrogenase (mg TPF g^{-1} dry rhizosphere $soil^{-1}$ day^{1})		Urease (mg NH_4-N $g^{-1}day^{-1}$)	
		70 days	Harvest	70days	Harvest
Control		40.4	10.5	10.2	4.11
Seed coating	***A. oryzae***	20.6	11.1	11.21	6.18
	***A. oryzae* +50% N**	57.60	29.50	29.50	8.10
	***A. oryzae* +75% N**	100.90	14.90	42.00	8.70
	N. muscarum	109.10	13.21	26.50	7.25
	***N. muscarum* +50% N**	35.40	13.71	32.50	8.42
	***N. muscarum* + 75% N**	115.00	15.00	53.40	8.84
Soil drench	***A. oryzae***	35.45	12.45	15.70	5.27
	***A. oryzae* +50% N**	66.70	13.70	22.80	7.28
	***A. oryzae* +75% N**	110.00	14.60	50.8	9.18
	N. muscarum	112.90	12.92	26.2	6.72
	***N. muscarum* +50% N**	71.10	14.10	35.7	8.70
	***N. muscarum* + 75% N**	131.15	14.77	61.86	9.89
Foliar application	***A. oryzae***	15.05	5.05	7.12	2.12
	***A. oryzae* +50% N**	57.00	7.00	26.5	4.25
	***A. oryzae* +75% N**	81.33	8.23	22.8	4.98
	N. muscarum	46.27	6.77	9.25	3.75
	***N. muscarum* +50% N**	67.75	7.75	21.5	4.75
	***N. muscarum* + 75% N**	102.00	8.43	27.21	6.21

by *A. oryzae* +75 % N treatment 110 µl H_2 (DHA) and µl 50.8 NH_4 (urease) in soil drench application and in seed coating application were 100.9 µl H_2 (DHA) and 42 µl NH_4 (urease). However, all treatments in all application types combined with cyanobacteria inoculation reported higher enzymes activities than all treatments without nitrogen and control treatments after 70 days of planting also at harvest time.

In the present study, the inoculation of cyanobacteria increased the soil's biological activity in terms of dehydrogenase (DHA) and urease activities. cyanobacterial inoculation, indicating their potential as plant growth and soil fertility improving rhizobacteria in other crops, including wheat, have benefited from [24] plant colonisation can be indirectly assessed by estimating microbial activity and soil enzyme parameters during crop growth. In the absence of soil enzymes, which govern the transformation of elements in soil, the fertility of agricultural land falls. Microbial activity and soil fertility are often linked because nutrient cycling occurs via bacteria, resulting in variances in yields and changes in many soil characteristics. Because it represents the mineralization of key organic compounds, soil microbial biomass is critical in maintaining soil functions (Frankenberger and Dick 1983). Zulpa et al. [25] investigated the impact of soil nutrients and microbilogical activity on the cyanobacteria such as *Nostoc muscorum* and *Anabaena oryzae*, both strains produce exudates and biomass which capable of improving the microbial activity in soil.Dehydrogenase enzyme activity (DHA), which denotes energy transfer, serves as a measure of total microbial activity in soil. It has been suggested that using soil enzyme activities to consider the economic and sustainability impacts of agricultural operations and even to diagnose different types of soil [26]. Urease (urea amidohydrolase, EC 3.5.1.5), one of numerous soil enzymes, is a crucial enzyme that is directly related to the transformation, biological turnover, and bioavailability of nitrogen [27]. 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 [28] and have a one-of-a-kind ability to fix nitrogen from the atmosphere by linking photosynthesis with nitrogen fixation. Bio-fertilizers are often environmentally friendly, less expensive, and can provide the nutrient requirements of crops [21]. Certain cyanobacteria have been discovered to not only flourish in such hostile environments, but also to improve the soil's physico-chemical qualities by supplementing it with carbon, nitrogen,available phosphorus, and other nutrients. The beneficial effects of using organic fertilizers in combination with mineral -N fertilizers on increasing nutrient concentration and content of peanut seeds could be due to their effect on providing plants with their nutritional requirements from various nutrients for a longer period, as well as their effect on increasing the availability of nutrients in the soil for uptake by plants and improving the nutritional status of the plants. The use of organic N in combination with bio fertilizers as a partial replacement for chemical fertilizers was very efficient in increasing nutrient concentration and content in the peanut plants. These results could be attributed to biofertilizer's ability to transport important nutrients like N and P as well as secrete plant growth-promoting compounds like nitrate [29]. Microbial inoculants like cyanobacteria can help to restore thefertility of soil but are effective under specific environments and specific crops due to low efficiency nitrogen fertilisers especially in sandy soils due to leaching, its poor organic matter content, and weak water retention which, cause a great loss for nitrogen fertilizer [30].

4. CONCLUSION

From the previous results, recommendation is that using cyanobacteria as a biofertilizer combined with 75% nitrogen ratio may be useful for the quality and productivity of peanut plants on sandy soil, while soil drench application type is more beneficial than other types of applications .To confirm and genuinely endorse this study, it must be done again with peanut and some other cereal crops.

ACKNOWLEDGEMENT

The authors would like to thank the project of ”Establishing a National Network for Conversation and Handling Microbial Culture Biofertilizers Strains and Bacterial Animal Diseases” ARC branch for providing us with potent strains chemical and equipments to carry out this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shen X, Xiao X, Dong Z, Chen Y. Silicon effects on antioxidative enzymes and lipid peroxidation in leaves and roots of peanut under aluminum stress. Acta Physiologiae Plantarum. 2014 Nov;36:3063-9.
2. Singh S P, Pathak J., Sinha R P, Cyanobacterial factories for the production of green energy and value-added products: an integrated approach for economic viability. Renew. Sustain Energy Rev. 2017;69:578-595.
3. Meena M, Swapnil P , Barupal T, Sharma K. A review on infectious pathogens and mode of transmission. J. Plant Pathol. Microbiol. 2019;10:1-4.
4. Chittora D, Meena M, Barupal T, Swapnil P, Sharma K. Cyanobacteria as a source of biofertilizers for sustainable agriculture. Biochem. Biophysics Reports. 2020;22:100737.
5. Subashchandrabose SR, Ramakrishnan B, Megharaj M, Venkateswarlu K, Naidu R. Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organicpollutant degradation. Environment International. 2013;51:59-72.
6. Singh JS, Kumar A, Rai AN, Singh DP. Cyanobacteria: A precious bio-resource in agriculture, ecosystem, and environmental sustainability. Front. Microbiol. 2016;7:1-19.
7. Singh R, Parihar P, Singh M, Bajguz A, Kumar J, Singh S, Singh VP, Prasad SM, Uncovering potential applications of cyanobacteria and algal metabolites in biology, agriculture and medicine: current status and future prospects, Front. Microbiol. 2017;8:515.
8. Prasad RC, Prasad BN. Cyanobacteria as a source biofertilizer for sustainable agriculture in Nepal, J. Plant Sci. Bot. Orient. 2001;1:127-133.
9. Dittmann E, Gugger M, Sivonen K, Fewer DP. Natural product biosynthetic diversity and comparative genomics of the cyanobacteria. Trends in Microbiol. 2015;23:642-52.
10. Rodríguez AA, Stella AM, Storni MM, Zulpa G, Zaccaro MC, Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in *Oryza sativa* L. Saline Systems. 2006;1448:2-7.
11. Thajuddin N, Subramanian G, Cyanobacterial biodiversity and potential applications in biotechnology. Curr. Sci. 2005;89:47-57.
12. Saadatnia H, Riahi H. Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plants. Plant Soil Environ., 2009;55:207-12.
13. Wilson LT, Cyanobacteria: a potential nitrogen source in rice fields. Texas Rice. 2006;6:9-10.
14. Ibraheem IB, Cyanobacteria as alternative biological conditioners for bioremediation

of barren soil. Egypt. J. Phycol. 2007;8: 99-116.
15. Moran R. Formulae for determination of chlorophyllous pigments extracted with N, N-dimethylformamide. Plant Physiology. 1982;69:1376-1381.
16. AOAC. 1995. Official methods of analysis. Washington: Association of Official Analytical Chemists.
17. Page AL, Miller RH, Keeney DR. Methods of soil analysis. Part 2. Chemical and microbiological properties. Agronomy, No. 9. Soil Science Society of America, Madison, WI. 1982;1159.
18. Rasmussen C, Heckman K, Wieder WR, Keiluweit M, Lawrence CR, Berhe AA, Blankinship JC, Crow SE, Druhan JL, Hicks Pries CE, Marin-Spiotta E. Beyond clay: Towards an improved set of variables for predicting soil organic matter content. Biogeochemistry. 2018;137:297-306.
19. Casida Jr L, Klein DA, Santoro T. Soil dehydrogenase activity. Soil Sci. 1964;98:371-376.
20. Guo H, Yao J, Cai M, Qian Y, Guo Y, Richnow HH, Blake RE, Doni S, Ceccanti B. Effects of petroleum contamination on soil microbial numbers, metabolic activity and urease activity. Chemosphere. 2012; 87:1273-80.
21. Rashid A, Mir MR, Hakeem KR. Biofertilizer use for sustainable agricultural production. In Plant, Soil and Microbes. Springer, Cham. 2016;163-180.
22. Song W, Teshiba T, Rein K, O'Shea KE. Ultrasonically induced degradation and detoxification of microcystin-LR (cyanobacterial toxin). Environ. Sci. Technol. 2005;39:6300-6305.
23. Chittora D, Meena M, Barupal T, Swapnil P, Sharma K. Cyanobacteria as a source of biofertilizers for sustainable agriculture. Biochemistry and Biophysics Reports. 2020;22:100737.
24. Karthikeyan N, Prasanna R, Sood A, Jaiswal P, Nayak S, Kaushik BD. Physiological characterization and electron microscopic investigations of cyanobacteria associated with wheat rhizosphere. Folia Microbiol. 2009;54:43-51.
25. Zulpa GL, Siciliano MF, Zaccaro MC, Storni MÓ, Palma MA. Effect of cyanobacteria on the soil microflora activity and maize remains degradation in a culture chamber experiment. Int. J. Agric. Bio L. 2008;10:388-92.
26. Tripathi S, Kumari S, Chakraborty A, Gupta A, Chakrabarti K, Bandyapadhyay BK. Microbial biomass and its activities in salt-affected coastal soils. Biol. Fertility Soils. 2006;42:273-277.
27. Liang Y, Yang Y, Yang C, Shen Q, Zhou J, Yang L. Soil enzymatic activity and growth of rice and barley as influenced by organic manure in an anthropogenic soil. Geoderma. 2003;115:149-60.
28. Mishra, Upasana, and Sunil Pabbi. "Cyanobacteria: a potential biofertilizer for rice" Resonance. 2004;9:6-10.
29. Wani PA, Khan MS, Zaidi A. Synergistic effects of the inoculation with nitrogen□fixing and phosphate□solubilizing rhizobacteria on the performance of field□grown chickpea.J. Plant Nutrition Soil Sci. 2007;170:283-287.
30. Ahmed SM, El-Sayed GA, Ghazal FM, El-Rasoul A. Integrated effect of N-forms in mineral and organic with or without cyanobacteria inoculation on improving peanut productivity. J. Soil Sci. Agric. Engin. 2007;32:10769-10781.