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Impact of Cyanobacteria Inoculation on some Physical and Chemical Properties of Soils with Different Texture

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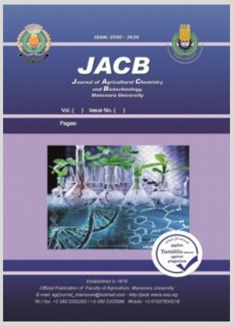

ABSTRACT

The impact of cyanobacterial species (*Nostoc lichenoides*, *Nostoc indistinguendun*, *Nostoc favosum*, and their mixtures) on soil characteristics such as water holding capacity (WHC), aggregate size distribution (%) to calculate the mean weight diameter (MWD), organic carbon (OC), total nitrogen content, and total counts of cyanobacteria in sandy and clay loam soils. The results showed that, compared to the un-inoculated soil, inoculation of the soil with cyanobacteria and their mixtures enhanced the soil WHC, MWD, OC, and total nitrogen in both soil types. After 60 days of incubation, the cyanobacterial mixtures produced the best WHC% results. In the case of MWD, the results were more pronounced with cyanobacterial mixtures at 60 days compared with the control in both sandy and clay loam soils, respectively. The organic carbon content of soils increased with increasing incubation period, reaching the highest value in the two soil types at 60 days incubation period. Also, the inoculation with different cyanobacteria species significantly increased the total nitrogen contents of the two tested soils when the incubation period was increased, especially with a mixture of cyanobacteria. At the same time, the results of the total counts of cyanobacteria showed that the highest concentration of the mixture of cyanobacteria was offered 60 days from incubation in clay loam soil (12.00 $\times 10^4$ cfu g^{-1} dry soil). Since there has been a significant development, the implanted cyanobacterial species were shown to cover the soil surface, mainly when a mixture of cyanobacterial species was used to inoculate soils.

Keywords: cyanobacterial species; soil properties

INTRODUCTION

When it comes to protecting and developing soil resources and limiting the environmental effects of agriculture, cyanobacteria offer promising platforms for strengthening soil structure and fertility. More knowledge of various soil types and agroecological regions is required to enhance applications in agricultural settings (Asghari *et al*., 2022). The composition of cyanobacteria crusts includes various species with various characteristics that have helped them thrive in dry and hyper-arid conditions. The damaging impacts of wind and water erosion are diminished by the protective encrusting properties of extracellular polymeric substances (EPS) made by cyanobacteria. EPS has adhesive properties that bind non-aggregated soil particles. To achieve this, the native and dominant cyanobacterial species assessed various soil physicochemical features linked to soil stability and then investigated the relationship between soil characteristics, stability, and cyanobacteria occurrence. We discovered that cyanobacteria could colonize stable soils in arid environments. Nitrogen and soil organic carbon were encouraged by cyanobacteria. Furthermore, EPS increased soil water content and blind soil particles combined in the cyanobacteria-secreted crusts, creating aggregates, and improving surface stability. (Sepehr *et al*., 2019).

The beneficial effects of cyanobacteria on the physical properties of the soil have been demonstrated by studying micro biotic crusts that thus lead to the formation of rigid and entangled superficial structures that enhance the stability of the soil surface and protect it from erosion **(**Costa *et al*., 2018**)**. Cyanobacterial sheaths and extracellular polymeric secretions also play a major role in water storage because of the hygroscopic properties of polysaccharides **(**Joshi *et al*., 2020**)**. Over and above, cyanobacteria have been used as inoculants to enhance soil structure, improve soil fertility, or recover damaged soil crusts, given their beneficial effects on soil **(**Pandey *et al*., 2005**)**. When used for recovering disturbances attributable to trampling, plows, and wildfires, cyanobacteria promoted the establishment of a microbial population, soil stability and increased organic matter **(**Schwinning *et al*., 2008**)**. The most abundant microbial constituents of micro biotic crusts are filamentous cyanobacteria that exert a mechanical effect on soil particles as they form a gluing mesh and bind soil particles on the surface of their polysaccharide sheath material **(**Katra *et al*., 2017**)**. Cyanobacteria also excrete extracellular polymeric secretions, mostly composed of polysaccharides **(**Hu *et al*., 2003**)**. Extracellular polymeric secretions ensure the role of soil particles as binding agents. They contribute to increasing soil water retention capacity **(**Costa *et al*., 2018**)**. This research aims to know the ability of cyanobacteria to improve some physicochemical properties of soils related to water holding capacity (WHC), soil aggregates, mean weight diameter (MWD), organic carbon (OC), total nitrogen content, and total counts of cyanobacteria.

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MATERIALS AND METHODS

A laboratory experiment with a completely randomized design with six replicates was conducted at the Agriculture Faculty, Al-Azhar University, Nasr City, Cairo, Egypt, in 2021 to evaluate the impact of cyanobacteria inoculation species on some physical and chemical properties of soils with different textures. Two types of soil with contrasting particle size distributions were collected from two areas of Egypt. These soils were: (1) sandy and (2) clay loam. The sandy and clay loam soil samples were collected from the surface layer (0–30 cm) of the Cairo-Alexandria Desert Road near Sadat City and El-Gharbia Governorates, respectively. Each soil sample was spread on clean paper sheets, air-dried, and mixed thoroughly. The different soil samples were sieved through a 2 mm sieve to get rid of debris and then stored in airtight-plastic bags for physical and chemical analyses, according to Page *et al.* (1982) and Klute (1986). Some physical and chemical characteristics determined by the soil used are shown in Table (1).

Table 1. Some physical and chemical properties for the soils used

Soil property	Soil 1*	Soil 2*
Particle size distribution:		
Coarse sand (%)	7.74	4.91
Fine sand (%)	82.76	19.79
Silt (%)	4.31	35.63
Clay (%)	5.19	39.67
Texture class	Sandy	Clay loam
pH (1:1 suspension)	8.09	7.93
EC_e (1:2.5 extract, dS m^{-1})	1.25	2.13
Water holding capacity (WHC, %)	9.00	31.00
Mean weight diameter (MWD, mm)	0.457	0.502
Soil organic carbon (mg/kg)	115	322
Total nitrogen content (mg/kg)	2.00	8.00

*** Each value represents the average of six replicates.**

Cyanobacterial species used

Nostoc lichenoides, *Nostoc indistinguendun*, and *Nostoc favosum*, were isolated and identified by using modified Watanabe medium (Watanabe *et al*., 1951). These cyanobacterial species were tested for their ability to fix nitrogen El-Nawawy *et al*. (1958) and maintained for this study.

Laboratory experiment

The current experiment employed two soil types: sandy and clay loam. Two kg of dry soil from each soil type was placed in rectangular polyethylene trays (50 30 10 cm). These trays were inoculated with isolated cyanobacteria such as *Nostoc lichenoides* (C1), *Nostoc indistinguendun* (C2), *Nostoc favosum* (C3), and mixture (C4), with one strain for each tray. The soil moisture was held at 100% of the field capacity. The inoculum was delivered to the soil surface using a hand-held spray. Each inoculated tray was in four replicates. Water was added when needed to compensate for the daily evaporated water. The cyanobacteria count in soil-based inoculants was determined using the number of colonies formed per soil unit (cfu g^{-1} dry soil).

Determination of water holding capacity (WHC)

Water holding capacity was determined according to Klute (1986).

Determination of mean weight diameter (MWD)

The experiment, soil samples were taken for aggregate size distribution by dry sieving to calculate the mean weight diameter (MWD) according to Six *et al.* (2002) as follows:

$$\mathbf{MWD} = \sum_{i=1}^{n} X_i W_i$$

Where:

X_i: Mean diameter of the considered aggregate size (mm),

W_i: weight percentage of the dry aggregate size class with respect to the total sample.

Determination of organic carbon

Organic carbon was determined using the modified Walkey-Black method (Black, 1965).

Determination of total nitrogen

Total nitrogen in the cyanobacteria were determined using the micro-kjeldahl method according to Jackson (1973). Results were expressed as mg nitrogen/100 ml culture.

Total count of cyanobacteria

The counts of cyanobacteria in soil-based inoculants were determined using the colony formed per unit/g soil (cfu g^{-1} dry soil) according to Allen and Stanier (1968).

Statistical analysis

A completely randomized design with six replicates was performed. The collected data were subjected to the analysis of variance (ANOVA) according to the procedure outlined by Steel and Torrie (1980). The differences among the means were compared using the least significant difference (LSD) at 5%.

RESULTS AND DISCUSSION

Ability of cyanobacteria nitrogen fixation

Results in Table (2) indicated that the three species of cyanobacteria varied in their capacity to produce intracellular and extracellular nitrogen, increasing gradually with an increasing incubation period. The highest values of intracellular and extracellular nitrogen secreted were recorded at 35 days of growth with *Nostoc lichenoides*, while the lowest nitrogen content was found in *Nostoc favosum* isolate.

Table 2. The amounts of biomass (mg dwt/100 ml) culture and fixed nitrogen (mg N /100 ml) culture by the three cyanobacterial species during incubation periods (days)

Cyanobacterial isolates	mg dwt/100 ml culture					mg N /100 ml culture				
	Incubation period (days*)									
	7	14	21	28	35	7	14	21	28	35
Nostoc lichenoides	62	110	161	230	348	9.07	9.85	12.33	15.18	16.68
Nostoc indistinguendun	62	110	152	220	318	5.55	7.58	9.48	12.58	16.29
Nostoc favosum	61	99	150	205	310	4.35	5.33	10.30	14.41	16.28
LSD 0.05						16.79				

*** Each value represents the average of six replicates.**

Effect of cyanobacteria on physical and chemical properties

Soil water holding capacity (WHC, %)

The data in Table (3) showed a discrepancy in WHC values because of soil inoculation with different soils of cyanobacteria compared to non-inoculated controls. The results showed non-significant increases in WHC values due to inoculation with varying types of soil cyanobacteria during all sampling periods. However, the 60-day period gave the best WHC results. On the other hand, inoculation with the cyanobacterial mixture gave the most significant result for soil WHC improvement in all sampling periods and different soils. The WHC values for soil inoculated with cyanobacteria compared with the control were *Nostoc lichenoides*, (9.15 %), *Nostoc indistinguendun*, (9.18%), *Nostoc favosum*, (9.22%), and mixture (9.23%), compared to the initial time of (9.0%) for sandy soil. *Nostoc lichenoides*, (31.20%); *Nostoc indistinguendun*, (31.25%); *Nostoc favosum*, (31.27%), and mixture (31.28%) to initial time (31.00%) for clay loam soils, respectively. In general, inoculation with solitary cyanobacterial species or a combination improved the WHC ratio for different soil types and at each sampling date. The data revealed low increases due to pollination, which may relate to the shooting period. These results support the idea that incorporating cyanobacteria into the soil as biofertilizers is essential for improving soil properties without considering the different treatments used. These results are like those reported by Doudle and Williams (2010), El-Zawawy (2016) and Ghazal *et al.* (2018).

Aggregate size distribution and mean weight diameter (MWD, mm)

Data of the aggregate size distribution and their mean weight diameter in Figs. (1 to 4) show that all treatments improved the aggregate size distributions at different diameters and MWD with soil inoculation of other species of cyanobacteria compared to the soil uninoculated. The data exhibited increases in MWD values due to the inoculation with the different species of cyanobacteria through all sampling dates. Data also indicated that it reached the maximum value of the MWD after 60 days of inoculation. The improvement in MWD was more pronounced for inoculating soil with a cyanobacteria mixture, with a value of 1.093mm compared with the control of 0.502mm in clay loam soil. On the other hand, the value obtained in sandy soil when the soil was inoculated with a cyanobacteria mixture was 0.797 mm, compared with the control value of 0.497 mm. On the other hand, inoculating soil with different species of cyanobacteria positively improved soil aggregates and mean weight diameter, especially under cyanobacteria mixture, followed by *Nostoc favosum* followed by *Nostoc indistinguendun* and lastly, *Nostoc lichenoides* treatments for both soils. These results are explained by Caire *et al.* (1997), Sepehr *et al.* (2019), and Asghari *et al.* (2022).

Table 3. Effect of cyanobacterial inoculation on soil water holding capacity (WHC, %)

Soil type	Treatments	incubation periods (days)*		
		30	45	60
Sandy	*Nostoc lichenoides*	9.08	9.07	9.15
	Nostoc indistinguendun	9.09	9.15	9.18
	Nostoc favosum	9.10	9.16	9.22
	Cyanobacteria mixture	9.12	9.18	9.23
Clay loam	*Nostoc lichenoides*	31.15	31.15	31.20
	Nostoc indistinguendun	31.12	31.19	31.25
	Nostoc favosum	31.14	31.25	31.27
	Cyanobacteria mixture	31.175	31.26	31.28
LSD 0.05		5.85		

*** Each value represents the average of six replicates.**

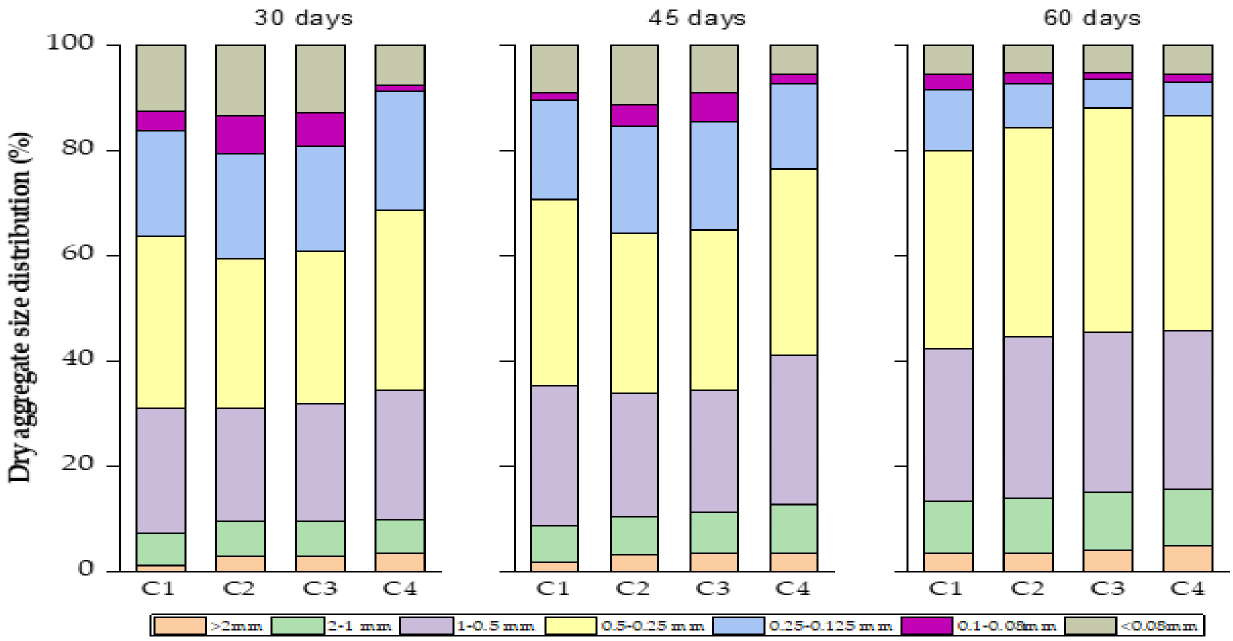

Fig. 1. Effect of different cyanobacteria species inoculation; *Nostoc lichenoides* (C1), *Nostoc indistinguendun* (C2), *Nostoc favosum* (C3), and cyanobacteria mixture (C4) on aggregate size distribution of sandy soil using the mean of six replicates.

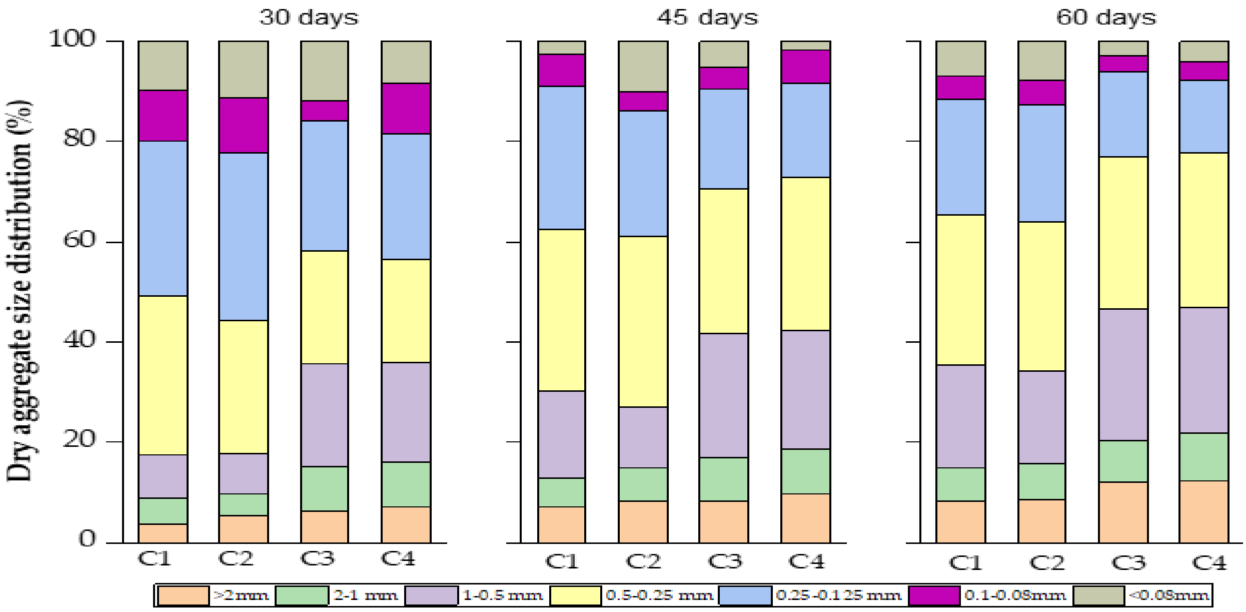

Fig. 2. Effect of different cyanobacteria species inoculation; *Nostoc lichenoides* (C1), *Nostoc indistinguendun* (C2), *Nostoc favosum* (C3), and cyanobacteria mixture (C4) on aggregate size distribution of clay loam soil using the mean of six replicates.

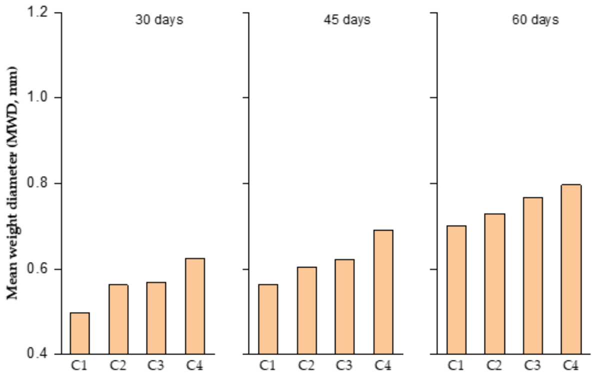

Fig. 3. Effect of different cyanobacteria species inoculation; *Nostoc lichenoides* (C1), *Nostoc indistinguendun* (C2), *Nostoc favosum* (C3), and cyanobacteria mixture (C4) on mean weight diameter (MWD) of sandy soil using the mean of six replicates.

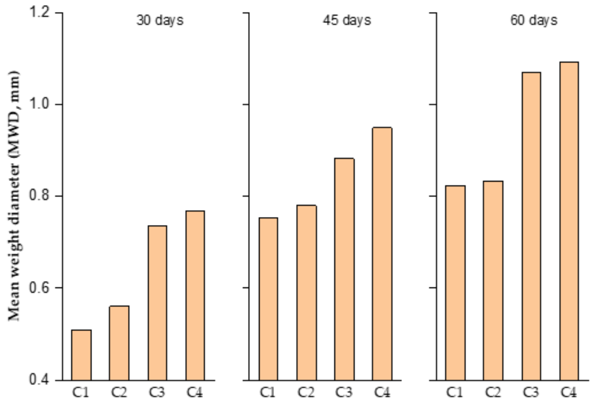

Fig. 4. Effect of different cyanobacteria species inoculation; *Nostoc lichenoides* (C1), *Nostoc indistinguendun* (C2), *Nostoc favosum* (C3), and cyanobacteria mixture (C4) on mean weight diameter (MWD) of clay loam soil using the mean of six replicates.

Soil organic carbon (OC, mg/kg)

Soil organic carbon content significantly increased as influenced by inoculation with the cyanobacterial species and its mixture in Figs. (5 & 6). The inoculation with *Nostoc favosum* gave the most significant positive influence compared to the other tested cyanobacterial species. The differences were primarily substantial, although the inoculation with the cyanobacterial mixture was the best and attained the highest positive effect compared to the inoculation with the other cyanobacterial species, each one alone. The improving effect of cyanobacterial inoculation on the different soils varied from one soil type to another. The organic carbon content of soils increased along with the increasing incubation period, leading to the highest values at 60 days of incubation. In contrast, the clay loam soil improved the content of organic carbon more than the other soil type, which contained 823 (mg/kg) compared to 322 (mg/kg) for un-inoculated soil (control) at an incubation period of 60 days, followed by the sandy soil, the least improved one. These findings agree with those of Doudle and Williams (2010), El-Zawawy (2016) and Ghazal *et al.* (2018).

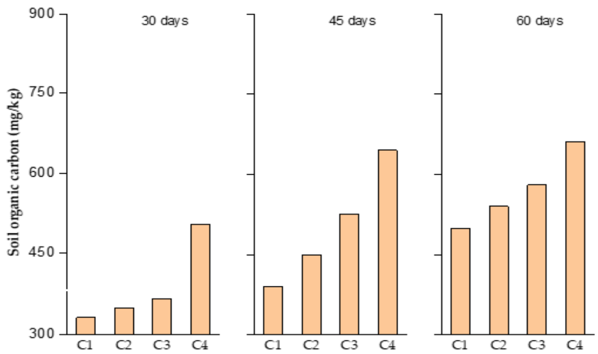

Fig. 5. Effect of different cyanobacteria species inoculation; *Nostoc lichenoides* (C1), *Nostoc indistinguendun* (C2), *Nostoc favosum* (C3), and cyanobacteria mixture (C4) on organic carbon (mg/kg) of sandy soil using the mean of six replicates.

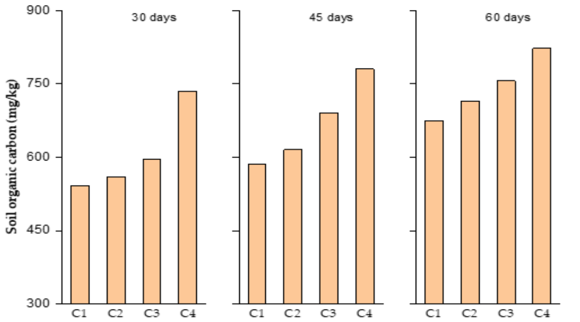

Fig. 6. Effect of different cyanobacteria species inoculation; *Nostoc lichenoides* (C1), *Nostoc indistinguendun* (C2), *Nostoc favosum* (C3), and cyanobacteria mixture (C4) on organic carbon (mg/kg) of clay loam soil using the mean of six replicates.

Nitrogen fixation in soils by cyanobacteria (mg/kg)

Figs. (7 & 8) indicated that the inoculation with different cyanobacterial species and their mixture significantly increased all tested soils of total nitrogen content. This influence was noticeable in the case of clay loam soil, where it attained significant increases compared to un-inoculated (control) soils.

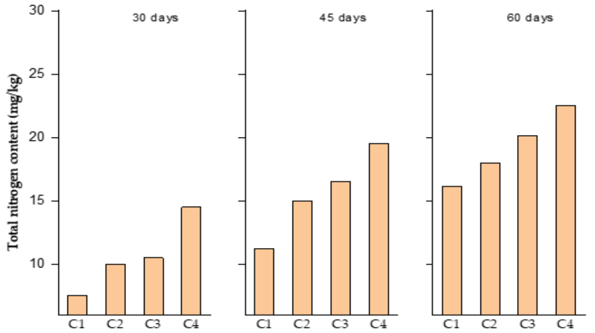

Fig. 7. Effect of different cyanobacteria species inoculation; *Nostoc lichenoides* (C1), *Nostoc indistinguendun* (C2), *Nostoc favosum* (C3), and cyanobacteria mixture (C4) on total nitrogen content (mg/kg) of sandy soil using the mean of six replicates.

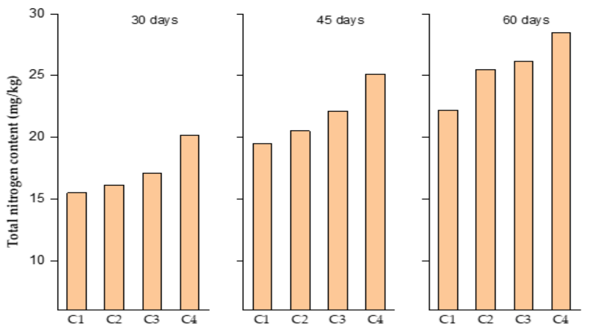

Fig. 8. Effect of different cyanobacteria species inoculation; *Nostoc lichenoides* (C1), *Nostoc indistinguendun* (C2), *Nostoc favosum* (C3), and cyanobacteria mixture (C4) on total nitrogen content (mg/kg) of clay loam soil using the mean of six replicates.

The values of the total nitrogen content of the clay loam soil due to cyanobacteria inoculation registered *Nostoc lichenoides* (22.17 mg/kg), *Nostoc indistinguendun* (25.50 mg/kg), *Nostoc favosum* (26.15 mg/kg) and mixture (28.50 mg/kg) versus control (8 mg/kg). In comparison, cyanobacterial inoculation gave soil nitrogen content *Nostoc lichenoides* (16.12 mg/kg), *Nostoc indistinguendun* (18.00 mg/kg), *Nostoc favosum* (20.15 mg/kg) and mixture (22.50 mg/kg) versus 2 mg/kg for sandy soil. Thus, the present study confirmed that the inoculation with different cyanobacteria species significantly increased the soil total nitrogen contents of all tested soils. Total nitrogen and its rate were increased with the increased incubation period as seen in 60 days (Doudle and Williams 2010, El-Zawawy 2016 & Ghazal *et al.*, 2018).

Total count cyanobacteria species

The cyanobacterial count in soil varied according to the inoculated cyanobacterial species in Table (4). The count in the clay loam soil was higher than in the sandy soil. Inoculation with *Nostoc favosum* attained the highest number of viable counts compared to those recorded by the other tested cyanobacterial species. However, the inoculation of different soils with the cyanobacterial species mixture gave higher microbial counts than any species inoculated alone. On the other hand, the viable count increased with the incubation period. At the incubation period of 60 days, the viable count was the highest. They attained 6.10, 12.00 x 10^4 (cfu g^{-1} dry soil) for sandy and clay loam soils respectively, at the incubation period of 60 days. The growth and establishment of *N. calcicola* in nonsterile soil confirmed the results reported in earlier studies when *N. calcicola* was inoculated onto sterilized soil (El-Zawawy, 2016). Rao and Burns (1990) showed that the survival of the inoculated species for at least 300 days contrasts with findings from other studies using phototrophic inoculate, in which several cyanobacterial species, including *Nostoc muscorum*, were inoculated onto a flooded brown silt loam soil. By day 147, the numbers of introduced cyanophyceae did not differ from those in the non-inoculated soils. The survival of *Nostoc muscorum* was not dependent on soil saturation as the soil was held at 60% water holding capacity and not flooded. However, the soil moisture content is usually critical for phototroph survival. These results agree with Drew and Anderson (1977) and Dhar *et al.* (2015).

Table 4. Effect of cyanobacterial species inoculation on their viable counts in different soil types (x10^4 g dry soil^{-1})

Soil type	Treatments	Growth periods (days)*			
		Initial	30	45	60
Sandy	*Nostoc lichenoides*	0.31	0.40	0.55	4.21
	Nostoc indistinguendun	0.33	0.44	0.84	4.72
	Nostoc favosum	0.36	0.45	1.20	5.35
	Cyanobacteria mixture	0.42	0.53	1.55	6.10
Clay loam	*Nostoc lichenoides*	0.31	0.47	0.63	8.15
	Nostoc indistinguendun	0.33	0.84	1.37	8.95
	Nostoc favosum	0.36	1.30	2.15	10.25
	Cyanobacteria mixture	0.40	1.90	5.70	12.00
LSD 0.05		3.06			

*** Each value represents the average of six replicates.**

CONCLUSION

This study has shown that cyanobacteria positively improve physicochemical properties in both types of soil used, such as water holding capacity, soil aggregates, mean weight diameter, organic carbon, total nitrogen content, and total counts of cyanobacteria when added in a single or mixed form. On the other hand, the highest values were obtained after 60 days of incubation in both types of soil used, especially when added in a mixed form.

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تأثير التلقيح بالسيانوبكتيريا على بعض الخواص الطبيعية والكيميائية للأراضي ذات القوام المختلف

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الملخص

أجريت هذه الدراسة لمعرفة تأثير لقاحات السيانوبكتيريا المختلفة (*N. lichenoides, N. indistinguendun, N. favosum*) بصورة منفردة أو مخلوط وذلك على بعض خواص التربة الطبيعية والكيميائية وهى قدرة التربة على حفظ الماء ، التوزيع الحجمي للتجمعات ومنها حساب متوسط القطر الوزني، ومحتوى التربة من الكربون العضوي ، محتوى التربة من النيتروجين الكلي وعدد السيانوبكتريا بالتربة وذلك في نوعين من الأراضي وهما التربة الرملية والطميية الطينية ، وكانت النتائج المتحصل بالمقارنة بمعاملة الكنترول (الأراضي الغير ملقحة) ان هناك تحسن في الصفات الطبيعية والكيميائية المدروسة للتربة المعاملة بالسيانوبكتيريا سواء كانت في صورة منفردة أو مخلوط حيث زادت قدرة التربة على الإحتفاظ بالماء عند إضافة السيانوبكتيريا بصورة مخلوط بعد 60 يوم من التحضين. وأيضاً بلغت قيم متوسط القطر الوزني إلى 0.797 مم، 1.093 مم بعد 60 يوم من التحضين بالمقارنة بقيم الكنترول 0.457 مم، 0.502 مم وذلك في كلا نوعين الأراضي الرملية والطميية الطينية، على الترتيب. أما بالنسبة لمحتوى التربة من الكربون العضوي فقد حدثت زيادة فيها بزيادة فترة التحضين حيث تم الحصول على أعلى قيمة بعد 60 يوم من التحضين في كلا النوعين من الأراضي. من جهة أخرى، أدى التلقيح بمخلوط السيانوبكتيريا إلى زيادة معنوية في محتوى التربة من النيتروجين الكلي وكذلك في عدد السيانوبكتيريا بالتربة حيث تم الحصول على أعلى القيم بعد 60 يوم من التحضين في كلا النوعين من التربة المستخدمة. وأخيراً، يمكن إستنتاج أن السيانوبكتيريا عند إضافتها للتربة سواء الرملية أو الطميية الطينية كان مناسباً للحصول على أفضل القيم لجميع الصفات التي تم دراستها خصوصا عند إضافتها في صورة مخلوط.