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POTENTIAL OF INOCULATION WITH SOME TYPES OF CYANOBACTERIA ON IMPROVING SOIL CHARACTERISTICS

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ABSTRACT

The effect of soil inoculation with different cyanobacterial species (*Nostoc commune*, *Anabaena* sp., *Nostoc calcicola.* and *Anabaena variabilis*) and/ their mixture on some soil characteristics in terms of water holding capacity (WHC) %, organic carbon (OC), mg/kg soil; total nitrogen, mg/kg soil; dehydrogenase activity, TPF 100g; evolved CO_2 and viable count of cyanobacteria for sandy, clay loamy and calcareous soils. Results revealed that soil inoculation with cyanobacteria and/or its mixture increased the soil WHC%, organic carbon content, mg/kg soil, total nitrogen, mg/kg soil dehydrogenase activity, TPF 100g; evolved CO_2 and total cyanobacteria count over un- inoculated control. This trend was true along with increasing the inocubation periods and was more pronounced with clay loamy followed by calcareous and then sandy soils at 60 days incubation period. Since in this case a heavy growth of the inoculated cyanobacterial species were observed to cover the soil surface especially in case of the inoculation with the mixture of cyanobacterial species.

INTRODUCTION

Cyanobacteria (Blue green algae) are photoautotrophic organisms possess the twin abilities of photosynthesis and biological nitrogen fixation. These organisms, endowed with tremendous genome plasticity, are distributed in all possible biotypes of the world. The soil environment provides suitable conditions for the growth and multiplication of blue green algae with respect their requirements for light, water, high temperature and nutrients. Water stable aggregates has been considered as a major factor that influence soil productivity, it can be considered the key of soil fertility. The formation, size and stability of soil aggregates are influenced by the various physical and environmental condition of soil (Omar, 1990). The soil ecosystem represents an unique aquatic-terrestrial habitat, which provides a favorable environment for growth and activity of cyanobacteria, meeting their requirements for light, water, temperature and nutrient availability. This, in turn, considered one of the major reasons for the relatively maintenance and stable yield of soil under flooded conditions (Roger *et al.* 1993). Cyanobacteria also add organic matter, synthesize and liberate amino acids, vitamins and auxins, reduce oxidizable matter content of the soil, provide oxygen to the submerged soil ameliorate salinity, buffer the pH and solubilize phosphates. (Mandal *et al.* 1998 and Kaushik 2004)

The aim of the work to study the effect of soil inoculation with some cyanobacterial species namely *Nostoc commune*, *Anabaena* sp., *Nostoc calcicola.* and *Anabaena variabilis* individually or in a mixture on properties of different soil types; sandy, clay loamy, calcareous in terms of water holding capacity, organic carbon, total nitrogen, soil dehydrogenase activity, evolved CO_2 and viable count of cyanobacteria.

MATERIALS AND METHODS

Materials

1- Microbial types

cyanobacteria species

- *Nostoc commune*
- *Anabaena* sp.
- *Nostoc calcicola*
- *Anabaena variabilis*

2-Medium used

Modified Watanabe medium;(Watanabe *et al*., 1951)

This medium; was modified by El –Nawawey *et al*. (1958), its composition is as follows (g /L):

K_2HPO_4	0.30	
Mg $SO_4.7H_2o$	0.20	
K_2SO_4	0.20	
$CaCO_3$	0.10	
Glucose	2.00	
Fe Cl_3 1%(Freshly prepared)	0.20	Ml
Micro-nutrient solution	1.00	Ml
Distilled water up to	1000	Ml

The micro-nutrient solution is prepared as follows (g /L)

H_3BO_3	2.8	
Zn $SO_4.7H_2O$	0.22	
Cu $SO_4.5H_2O$	0.08	
$MnCl_2$	1.80	
Molybdic acid	0.02	
Distilled water	1.00	L

Methods

Experimental work

A greenhouse experiment was conducted to investigate the effect of soil inoculation with different species of cyanobacteria on some soil characteristics in terms of water holding capacity (WHC), organic carbon (OC), total nitrogen, dehydrogenase activity, CO_2 evolved and total viable count of cyanobacteria. For this purpose, three soil types, i. e., sandy, clay laomyand calcareous soil were distributed in three sets of polyethylene trays each with a dimension of 60x35x10 cm. One set of trays was devoted for each soil. Each tray in every soil set received 2 kg dry 2ml sieved soil. The trays were then watered with sterile distilled water to keep the soil moisture content up to 85% of soil water holding capacity. Each soil set was inoculated with four cyanobacteria species namely *Nostoc commune*, *Anabaena* sp., *Nostoc calcicola.* and *Anabaena variabilis* individually and in a mixture. The inoculum was applied in concentrate suspension of frssh biomass in sterile distilled water to each tray corresponded to about 0.18 g/ tray biomass. Biomass determination was used to estimate the cyanobacteria biomass of the cyanobacterial culture suspension used for soil inoculation. Water was added when needed to compensate the evaporated water. Numbers of cyanobacteria in soil based inoculants were determined using the colony formed unit per gram of soil (cfu) (Allen and Stanier, 1968). Thus, each soil set contained four treatments as follows:

1-Control (Un-inoculated)
2-Inoculation with *Nostoc commune*
3-Inoculation with *Anabaena* sp.
4-Inoculation with *Nostoc calcicola.*
5-Inoculation with *Anabaena variabilis*
6- Inoculation with the mixture of all the above mentioned cyanobacterial species. The soil trays for each set were randomly distributed in a complete randomized design as described by Gomez and Gomez (1984).

The cyanobacteria species were isolated from the soil and identified through the study of Ph-D of El-zawawy (un-published according to EL-Ayouty and Ayyad (1972)

The soil samples were collected initially and every 15 days up to sixty days to determine water holding capacity (Richards, 1954), organic carbon (Walkley and Black, 1934), total nitrogen (Jackson (1973)) dehydrogenase activity (Casida *et al.* 1964), soil capacity for CO_2 (Pramer and Shmidt, 1964), and total cyanobacterial count colony formed unit per gram of soil (cfu) (Allen and Stanier, 1968).

Statistical analysis

Data obtained were subjected to the analysis of variance and treatment means were compared using the L.S.D method according to Steel and Torrie(1980).

RESULTS AND DISCUSSION

Water holding capacity (WHC)%:

Data of Table (1) showed the differences in WHC values due to inoculation of soil with different species of cyanobacteria compared to un-inoculated control. The data exhibited slight increases in WHC values due to the inoculation with the different species of cyanobacteria through all sampling dates. The best date gave increases for WHC of soil was 60 days after inoculation. On the other hand, the inoculation with the mixture of cyanobacteria gave the best result for improving soil WHC, at all sampling dates and for the different soil types. Values of WHC of inoculated soils compared to control were 9.25 and 9.0 for sandy soil; 31.3 and 31.0 for clay loamy soil and 29.31 and 29.1 for calcareous soil respectively. In general, the inoculation with cyanobacterial species solitary or in a mixture caused improve in WHC% of the different soil types and at each sampling date. The data revealed low increases due to inoculation this may regard to the shoot period of inocubation Such results supported the idea that cyanobactyeria incorporation to soil as a biofertilizer plays an important role in improving soil properties without any consideration to other different treatments employed. This may be attributed to the extracellular polysaccharides polymers from cyanobacteria which maintain higher water contents (Mashhour *et al.,* 2009 and Doudle and Williams 2010), or the effect of cyanobacteria on biological crust formation of the soil surface, which holding soil particles together and thereby offering higher WHC%. This is in an agreement with those of Hill *et al*. (2002). Taha (2000) reported that the increasing soil water holding capacity was positively related with the increase in soil aggregate stability, confirming the importance of polysaccharides in the promoting of soil aggregation. Although polysaccharides are considered to be an important factor in soil aggregate for mation.

Table(1):Effect of cyanobacterial inoculation on soil water holding capacity (WHC) % .

Soil type	Treatments	Inoculation periods(days)			
		15	30	45	60
Sandy	Control	9.00	9.00	9.00	9.00
	Nostoc commune	9.02	9.08	9.01	9.13
	Anabaena sp.	9.05	9.09	9.14	9.17
	Nostoc calcicola	9.05	9.10	9.16	9.19
	Anabaena variabilis	9.06	9.11	9.17	9.21
	Cyanobacteria mixture	9.08	9.13	9.19	9.25
Clay loamy	Control	31.00	31.00	31.00	31.00
	Nostoc commune	31.05	31.10	31.15	31.20
	Anabaena sp.	31.07	31.11	31.18	31.21
	Nostoc calcicola	31.08	31.13	31.20	31.24
	Anabaena variabilis	31.10	31.15	31.23	31.25
	Cyanobacteria mixture	31.13	31.20	31.25	31.30
Calcareous	Control	29.10	29.10	29.10	29.10
	Nostoc commune	29.12	29.15	29.18	29.20
	Anabaena sp.	29.15	29.17	29.20	29.22
	Nostoc calcicola	29.17	29.19	29.23	29.25
	Anabaena variabilis	29.18	29.20	29.24	29.27
	Cyanobacteria mixture	29.20	29.25	29.28	29.31

Interaction SxTxP LSD 0.05= 0.52 LSD 0.01= 0.70

Nitrogen fixation in soil by cyanobacteria :-

Nitrogen fixation by cyanobacteria are shown in Table (2) the inoculation with different cyanobacterial species and its mixture significant increased total nitrogen content (mg/kg soil) of the three soils. This influence was clear and noticeable in case of clay laomy soil, whereas, it attained big increases compared to un-inoculated control soils. The values of nitrogen of inoculated clay laomysoil ranged from 13 to 32 mg/kg soil compared to control (10 mg/kg soil), the calcareous soil values ranged from 9 to 30 mg/kg soil compared to 5 mg/kg soil for the control, while inoculation gave nitrogen content from 5 to 27mg/kg soil compared to 3 mg/kg soil for control in case of sandy soil. Thus, we found that the inoculation with the different types of cyanobacteria had an effective and positive significant influence on increasing nitrogen content of soils and this effect was shown to increase with increasing inoculation period. The rate of increases raised with increasing incubation period and the best period was 60 days after inoculation. In spite of the clear influence of inoculation on total nitrogen of soils, the sandy soils exhibited pronounced increase , at 60 days after inoculation, evaluated by 27 mg/kg soil compared to 3 mg/kg soil for un-inoculated control. These result are similar to those reported by Mfundo *et al*. (2010) that the ability of cyanobacteria to fix N_2 and produce exopolysacharides varies widely among different cyanobacterial strains. Rogers *et al.* (1991) reported that the increases in carbohydrate C, aggregation, total C, total N, and microbial numbers were positively significant in the inoculated soil than control. Most pronounced effect were the > 100000 times increase in actinomycetes and the 444% increase in carbohydrate C. Aggregate stability increased by 21%. The C: N ratio declined from 20:1 to 14:1 during the 300 days. The observed increases in soil N were comparable to those reported by Harper and Pendleton (1993) in a similar study. The observed trends in soil N were also similar to those reported by Skarpe and Henriksson (1987) and Buttars *et al*. (1998) even though their studies used more accurate acetylene reduction method for assessing N_2-fixation. A strong positive association of soil C and soil N indicated close association of C and N dynamics in the inoculated and non-inoculated soils as also reported by Nishar *et al.* (2007). Generally, the increases in N due to inoculation were greater than those of C, which resulted in a narrowing of C: N ratio . In Guquka soil, for example, the C: N ratio dropped from 15:0 in noninoculated soils to 11:4 when soil was inoculated with *Nostoc,* which impacted positively on the mineralization of N . These results clearly indicated that inoculation with *Nostoc.*, enriched the experimental soils with N as a result of atmospheric N_2-fixation.

Table (2): Effect of cyanobacterial species application on soil total nitrogen |(mg/kg soil).

Soil type	Treatments	Inoculation periods(days)			
		15	30	45	60
Sandy	Control	3	3	3	3
	Nostoc commune	5	9	12	18
	Anabaena sp.	7	10	14	19
	Nostoc calcicola	8	10	16	21
	Anabaena variabilis	8	11	19	22
	Cyanobacteria mixture	9	18	24	27
Clay loamy	Control	10	10	10	10
	Nostoc commune	13	15	19	24
	Anabaena sp.	13	16	20	26
	Nostoc calcicola	14	16	21	27
	Anabaena variabilis	16	18	25	29
	Cyanobacteria mixture	17	24	27	32
Calcareous	Control	5	5	5	5
	Nostoc commune	9	11	13	22
	Anabaena sp.	10	13	16	24
	Nostoc calcicola	10	14	18	25
	Anabaena variabilis	12	15	19	27
	Cyanobacteria mixture	15	18	23	30

Interaction SxTxP LSD 0.05= 1.43 LSD 0.01= 1.91

Soil organic carbon (mg/kg) .

Organic carbon content of soil significantly increased as influenced by inoculation with the cyanobacterial species and its mixture (Table 3). The inoculation with *Anabaena variabilis* gave the highest positive influence over all the other used cyanobacterial species. The differences mostly were significant although, the inoculation with the mixture of the cyanobacteria was the best, which attained the highest positive effect compared to the inoculation with the other cyanobacterial species each alone.

Table (3): Effect inoculation of different soil types with of cyanobacterial species on soil organic carbon (mg/kg soil)

Soil type	Treatments	Inoculation periods(days)			
		15	**30**	**45**	**60**
Sandy	Control	119	119	119	119
	Nostoc commune	195	315	360	486
	Anabaea sp.	273	350	420	513
	Nostoc calcicola	312	350	480	567
	Anabaena variabilis	312	385	570	594
	Cyanobacteria mixture	351	630	720	729
Clay loamy	Control	399	399	399	399
	Nostoc commune	507	525	570	648
	Anabaena sp.	507	560	600	702
	Nostoc calcicola	546	560	630	729
	Anabaena variabilis	624	630	750	783
	Cyanobacteria mixture	663	840	810	864
Calcareous	Control	198	198	198	198
	Nostoc commune	351	385	390	594
	Anabaena sp.	390	455	480	648
	Nostoc calcicola	390	490	540	675
	Anabaena variabilis	468	525	570	729
	Cyanobacteria mixture	585	630	690	810

Interaction SxTxP LSD 0.05= 86.74 LSD 0.01= 115.37

The improving effect for cyanobacterial inoculation on the different soils varied from soil type to another. Whereas, the sandy soil was the most soil type improved for its content of organic carbon than the other tow soil types which contains 729 mg/kg soil compared to 119 mg/kg soil for un-inoculated control at incubation period of 60 days then fallowed by the calcareous soil which greatly improved also and gave organic carbon value 810 mg/kg soil compared to 198 for control. The clay laomy soil was the lowest improved one. The organic carbon content of soils was noticed to increased with the increasing incubation period and the highest organic carbon values was found at 60 days incubation period. These findings are in agreement with those of Six *et al*. (2006) and Mashhour *et al*. (2009) where they reported that soil amino sugars, which are components of microbial cell walls, have been used to assess the microbial contribution to soil organic matter (SOM) accumulation and turnover in soil. The increase in soil carbohydrate was positively correlated with the increase in soil aggregate stability, confirming the importance of polysaccharides in the promotion of soil aggregation. Although polysaccharides are considered as an important factor in soil aggregate formation (Benzig-Purdie and Nikrforuk, 1989), some authors (Martens and Frankenburger, 1992) have suggested that their is no direct effect of polysaccharides on soil organic matter, but might be affected the soil organic matter through their microbial degradable products. In general, as important, soil organic matter ploughing increases the rate of organic matter mineralization, by exposing previously inaccessible organic matter (bound in aggregates) to microbial attack and rendering the soil more susceptible to crusting.

Cyanobacteria dehydrogenase activity (µg TPF/ 100 g dry soil^{-1} h^{-1})

Data represented in Table (4) showed that the dehydrogenase activity values (µg TPF/ 100 g dry soil^{-1} h^{-1}) varied with the variation of soil type, cyanobacterial species and inoculation period. Whereas, activity of the enzyme was showed to increase with the increasing inoculation period. The incubation period of 60 days was the best in this respect, on the other hand, the enzyme activity varied with the variation of soil types, as the clay laomy soil exhibited the highest activity followed by calcareous soil, then the sandy soil. The data, also, revealed that the inoculation with the different studied cyanobacterial types notably varied in their effect on the dehydrogenase enzyme activity. The best values were shown with *Anabaena variabilis* which gave 19.4, 25.6 and 23.5 at incubation periods 60 days for sandy, clay loamy and calcareous soil, respectively. While the inoculation with the mixture of cyanobacterial species exhibited the highest values of dehydrogenase enzyme activity than all other cyanobacterial species alone which gave 19.7, 29.2 and 25.5 at 60 days inoculation periods for sandy, clay laomy and calcareous soil, respectively. These result are similar to those reported by Elsherif *et al*.(2013) studied the response of faba bean to inoculation with Azolla and cyanobacteria individually or mixed under saline soil and water condition. Results revealed that Rizobium inoculation with application of *Azolla* and Cyanobactiria as dry T7 (*Azolla* 500 g + cyanobacteria 200 g) or foliar T6 (*Azolla* + cynobacteria) recorded the highest significant increases in nodule numbers and its dry weight as compared to control. The all treatments of *Azolla* and Cyanobacteria increasing the soil total Cyanobacteria, as well as CO2 evolution and dehydrogenase activity. Wlodarczyk *et al*. (2002). Fu and Dexter of have transformed *Synechocystis* strain PC 6803 by inserting pyruvate dehydrogenase and alcohol dehydrogenase II genes from *Zymomonas mobilis* to produce ethanol.

CO_2 evaluated:

Values of CO_2 evolution (mg 100 g dry soil^{-1} h^{-1}) from inoculated soils with different species of cyanobacteria and its mixture notably varied with the variation of soil types, incubation period and microbial species (Table 5). It was noticed that CO_2 amounts consistently increased with the increasing incubation period. Whereas, the incubation period of 60 days gave the highest values. The clay loamy soil exhibited the largest CO_2 amounts, followed by calcareous soil then sandy soil. The inoculation with the different cyanobacterial species revealed different values, whereas *Anabaena variabilis* gave the highest amounts than all others cyanobacterial species. However, the mixture of cyanobacterial species caused evolution of higher amounts of CO_2 than all the used solitary cyanobacterial species the higher amount of CO_2 recorded 275.3 due to the inoculation with the mixture at 60 days from inoculation for clay loamy soil compared to (195) for *Nostoc commune* at 60 days for sandy soil. The CO_2 amount ranged from 101.1 for

Nostoc commune at zero time in sandy soil to 275.3, for the mixture at 60 days inocubation period in clay loamy soil. These result are similar to those reported by Elsherif *et al*.(2013) studied the response of faba bean to inoculation with Azolla and cyanobacteria individually or mixed under saline soil and water condition. Results revealed that Rizobium inoculation with application of *Azolla* and Cyanobactiria as dry T7 (*Azolla* 500 g + cyanobacteria 200 g) or foliar T6 (*Azolla* + cynobacteria) recorded the highest significant increases in nodule numbers and its dry weight as compared to control. The all treatments of *Azolla* and Cyanobacteria increasing the soil total Cyanobacteria, as well as CO2 evolution and dehydrogenase activity. Hegazi (2007) found that the Cyanobacterial inoculation, generally enhanced increasing the CO2 evolution and dehydrogenase activity. N2-fixing cyanobacteria combined with organic amendments significantly increased soil organic matter as well as the available NPK contents than S. platensis. This work led to take in consideration much attention for establishing the technology of cyanobacterial inoculation to vegetable crop production and highlighting both the traditional utilization of the expensive and ecology pollutant mineral fertilizers and the emerging new approach of utilizing organic amendments to meet the market demand for organically grown vegetable.

Table (4): Response of soil dehydrogenase activity (µg TPF 100 g dry soil^{-1} h^{-1}) as affected by inoculation with different types of cyanobacteria species and mixture

Soil type	Treatments	Inoculation periods(days)				
		Zero	**15**	**30**	**45**	**60**
Sandy	*Nostoc commune*	8.5	9.4	9.8	12.1	17.6
	Anabaena sp	9.1	9.3	10.0	12.6	18.1
	Nostoc calcicola	9.1	10.5	11.1	13.0	18.9
	Anabaena variabilis	9.5	10.7	11.3	13.8	19.4
	Cyanobacteria mixture	9.9	11.0	12.7	15.7	19.7
Clay loamy	*Nostoc commune*	8.7	9.6	11.1	12.8	21.8
	Anabaena sp.	9.3	10.9	11.5	12.9	22.9
	Nostoc calcicola	9.3	13.1	13.8	15.9	23.9
	Anabaena variabilis	9.8	13.8	14.0	16.0	25.6
	Cyanobacteria mixture	10.0	14.1	16.2	17.2	29.2
Calcareous	*Nostoc commune*	8.5	9.3	10.7	12.7	19.9
	Anabaena sp.	9.1	10.1	11.0	12.9	20.2
	Nostoc calcicola	9.1	117	12.3	14.2	21.5
	Anabaena variabilis	9.5	11.9	12.8	15.7	23.5
	Cyanobacteria mixture	9.9	12.6	13.7	16.5	25.5

Cynobacteria propagation:

Microbial count in soil was shown to varied with the application of different species of cyanobacteria (Table 6).The count in the clay loam soil were the highest followinged calcareous soil, then sandy soil. Inoculation with *Anabaena variabilis* attained the highest stimulation for viable counts over all other cyanobacterial species. However, the inoculation of different soil types with the mixture of cyanobacterial species gave the largest counts than