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PNITROGEN-FIXING ABILITIES OF SOME CYANOBACTERIA IN SANDY LOAM SOIL AND EXUDATE EFFICIENCY ON RICE GRAIN GERMINATION

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Abstract

Cyanobacterial collections (eleven strains) have been used to screen their growth and nitrogen fixing abilities in both sterile and non-sterile soils under different incubation periods. The effect of cyanobacterial exudates on germination of rice grains were also investigated. The maximum growth of cyanobacteria represented by their biomass was recorded for *Nostoc rivulare* and *Nostoc microscopicum* after 60 day old cultures in sterile soil. However, in non-sterile soil, the greatest biomass was detected for *Calothrix brevissima*, *Nostoc microscopicum* and *Nostoc carneum* at the same incubation period. The most potent strains for nitrogen fixation in sterile soil were *Anabaena variabilis*, *Nostoc spongiaeforme* and *Nostoc linckia* after 90 days of incubation. Meanwhile, *Nostoc muscorum* and *Calothrix brevissima* recorded the highest values of nitrogen fixation in non-sterile soil at the same incubation period. Pretreatment of rice grain with cyanobacterial exudates of *Nostoc linckia*, *Nostoc muscorum* and *Anabaena flos-aquae* stimulated the germination by 25,14 and 10%, respectively. The results revealed that nitrogen-fixing abilities and rice grain germination appeared to be strain-specific. Moreover, the persistence of *Nostoc muscorum* and *Calothrix brevissima* in non-sterile soil for 90 days with a maximum activities indicate that both of them can be used as non-indigenous strains

Key words: cyanobacterial biomass, cyanobacteria, germination, nitrogen fixation, rice grain .

Introduction

Global interest in biological nitrogen fixation is a direct consequence of this necessity to provide some economic assistance to the small and marginal farmers and to introduce a judicious combination of linear and cyclic fertilization of soils (Banerjee and Kumar 1992). Many trials have been conducted to increase rice yield by cyanobacterial inoculation (algalization) (Roger and Kulasooriy 1980). Algalization has been reported to have a benifical effect on grain yield (Dong *et al.* 1995; El Ayouty 1998). However, other reports indicated failure of algalization under widely different agroclimatic conditions (Roger and Watanabe 1981). Cyanobacterial fertilizers are a promising alternative to avoid soil pollution caused by agrochemical. They may recover the nutrient content and structure lost after harvesting as they bring to soil combined exopolysaccharide, that aggregate soil particles which helps to reduce soil erosion and improve soil structure (Oesterreicher 1990), and bioactive substances that enhance seedlings growth (de Caire *et al.* 1997; de Mule *et al.* 1999). The increases in crop yield as a result of cyanobacterial application cannot be attributed solely to cyanobacterial nitrogen fixation and persumably a variety of other biological substances synthesized and liberated by these cyanobacteria may have a role to play. The agricultural importance of cyanobacteria lies in their capacity to fix and

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metabolize the molecular nitrogen thus, liberating a part of the fixed nitrogen and possibly growth regulators as extrametabolites. Also, some investigators (Goyal 1989; Roger and Kulasooriya 1980) reported the solubilizing of the insoluble phosphate, addition of organic matter and improving the physical and chemical nature of the flooded rice soil. The excretion of growth promoting substances and soluble photosynthetic products enhances the development of bacteria and can stimulate crop growth (Oesterreicher 1990). The objective of the present study was to examine the ability of some non-indigenous strains of cyanobacteria to fix nitrogen and the efficiency of their exudates on rice grain germination.

Materials and Methods

Organisms and growth conditions: The eleven cyanobacterial strains (Table 1) used in this study were obtained from the Botany Department, Faculty of Science, Zagazig University . The cells were grown in a medium described by Watanabe (1951) at a constant temperature 25±1°C, illuminated continuously with fluorescent tubes maintaining the desired light intensity (800 lux). Cyanobacteria at the exponential growth phase were used as inoculum to the following experiments:

a) **Estimation of cyanobacterial growth:** Chorophyll a of the different samples were extracted with 90% acetone according to Lynn (1972) and the biomass was estimated by Lynn and Bogelsberg (1974) method. The difference between inoculated and non-inoculated sample (control) was represented in Table 1.

b) **Nitrogen-fixing abilities of inoculated soil:** 1ml of each cyanobacterial suspension (1ml=5000 hormogonial cells) were introduced into 100 g sterile and non-sterile sandy loam soils (38% sand, 36% silt, 26% clay, pH 7.8, total N 0.127%, organic carbon 1.2%, Ca^{++}0.053%, Mg^{++}0.072 and reactive phosphorous 0.047%) in sterilized glass cylinder (6 cm diameter and 10 cm height). Water level was adjusted to 4 cm above the soil surface after well shaking. The inoculated and non-inoculated (control) cylinders were incubated at 25±1°C under light-dark photocycles (15:9) with fluorescent tubes (800 lux) for different incubation periods (30, 60 and 90 days). At the end of each incubation period, all cylinders were thoroughly shaken and left until soil particles were settled down. The supernatent of each sample was siphoned out for determination of the extracellular nitrogen and the wet soils were air dried to be used for estimation of the total fixed nitrogen. Three replicates were set up for each sample. The nitrogen content was estimated as mg/100g soil by the Kjeldahl method (Allen 1953) and the difference between inoculated and non-inoculated sample was represented in Table 1.

c) **Monitoring the efficieny of cyanobacterial exudates:** Homogeneous rice grains (variety Giza, 172) were sterilized by agitation under partial vacuum in H_2O_2 (5%) and tween 80 (1ppm) for 30 minutes, rinsed eight times in sterile demineralized water, soaked in the different cyanobacterial exudates until saturation (36 h). Then washed thoroughly three times with sterile distilled water. Grains were added to Petri dishes with two layers of sterile wet filter paper with daily addition of water. All dishes were incubated at 25±1°C. Percent of germination was measured every 6 h after saturation period (36 h) for 84 hours. Three replicates were used for each sample .

Results and Discussion

Cyanobacterial growth

Data of Table 1 show that the biomass of cyanobacteria, inoculated in both sterile and non-sterile soils, increased with increasing the incubation periods. The maximum growth during the different incubation periods was detected for *Nostoc spongiaeforme*, *Anabaena flos-aquae*, *Nostoc muscorum*, and *Nostoc rivulare* in sterile soil after 60 days of incubation. Among the studied cyanobacterial species, *Nostoc microscopicum*, *Nostoc spongiaeforme* and *Anabaena flos-aquae*, exhibited the highest biomass after the first incubation period (30 days) in sterile soil. Marked increase in the algal biomass was observed for *Nostoc microscopicum*, *Anabaena variabilis*, *Calothrics brevissima*, and *Nostoc carneum* after 90 days of incubation in sterile soil. Sussella and Goyal (1995) were examined forty two cyanobacterial isolates belonging to 8 genera for growth and nitrogen fixation. They reported that *Calothrix* performed much better than *Nostoc* and *Anabaena*. Moreover, Saikia and Bordoloi (1994) state that *Calothrix*, *Anabaena* and *Nostoc* were of common occurrence in forty-five soil samples collected and studied for their cyanobacterial component. The strains of cyanobacteria which had been inoculated in to sterile soil, showed high survival in viability tests (Roger and Ardales 1991). Whitton (2000) mentioned that, 30 N_2-fiixing strains of 136 were lost their viability and growth whereas the remainders were grew and reproduced for 30 months.

Table 1. Cyanobacterial biomass in both sterile and non-sterile sandy loam soil (mg / 100 g soil) amended with some cyanobacteria at different incubation periods. (Results represent the difference between inoculated and non-inoculated soil).

Strains \ Soil	Cyanobacterial biomass (mg / 100 g soil)					
	Sterile			Non-sterile		
	Age (days)			Age (days)		
	30	60	90	30	60	90
Nostoc microscopicum Carm.	131 ± 4	209 ± 6	177 ± 4	22 ± 4	100 ± 9	28 ± 4
Nostoc linckia (Roth). and F.	33 ± 2	109 ± 4	106 ± 5	57 ± 5	55 ± 5	24 ± 2
Nostoc spongiaeforme Ag.	102 ± 3	195 ± 5	150 ± 5	59 ± 3	41 ± 6	31 ± 3
Anabaena variabilis Kütz.	41 ± 2	118 ± 3	165 ± 7	88 ± 5	62 ± 2	19 ± 4
Anabaena flos-aquae (Lyngb.) Breb.	117 ± 3	177 ± 5	156 ± 7	26 ± 6	36 ± 4	62 ± 2
Nostoc muscorum Ag.	42 ± 3	138 ± 5	103 ± 5	39 ± 2	59 ± 3	85 ± 3
Nostoc piscinale Kütz.	52 ± 2	76 ± 2	13 ± 1	55 ± 4	60 ± 7	7 ± 2
Nostoc rivulare Kütz.	84 ± 2	262 ± 8	80 ± 3	42 ± 5	55 ± 5	63 ± 7
Calothrix brevissima West, G.S.	10 ± 1	39 ± 2	171 ± 8	15 ± 3	125 ± 6	82 ± 5
Anabaena spiroides Kleb.	32 ± 4	39 ± 2	45 ± 3	61 ± 7	58 ± 5	48 ± 4
Nostoc carneum Ag.	75 ± 2	112 ± 4	180 ± 7	44±5	73±7	12±3

Values are mean ± SD of three replicate experiments.

Results showed that the great ability of all investigated strains to survive in sterile soil for as long as 90 days except for *N. piscinale* and *N. rivularae.* may be related to the tolerance and good adaptability of cyanobacterial species to dominate (Brock 1973; Metting 1981).

Regarding the non-sterile soil, the highest biomass was recorded for *N. microscopicum* after 60 days of incubation, for *C. brevissima* after 60 and 90 days and

finally for *N. muscorum* after 90 days of incubation. Much of the success of *Nostoc* in terrestrial habitat is related to its ability to remain desiccated for months or years and fully recover metabolic activity within hours to days after rehydration with liquid water (Doods *et al.* 1995). In addition, *Nostoc* is somewhat resistant to predation, this probably is related to production of large amounts of sheath material, synthesis of microcystin-like toxins by some strains, and formation of colonies that are too large for many algivores to consume. The gelatinous sheath material of half the cyanobacterial species studied by Lange (1976) was able to chelate elements essential for their growth and Belnap and Harper (1995) considered the possibility that the sheaths may also influence the availability of elements to other organisms. Cyanobacteria have the ability to synthesis, identity and function of extracellular polysaccharides (Philippis and Vincenzini 1998; Adhikary 1998). These biopolymers regulate the loss and uptake of water from cells, serve as a matrix for the immobilization of other components of the cell which may offer protection and may protect cell walls from damage during swelling and shrinkage (Caiola *et al.* 1993; 1996). On the other hand, the biomass appeared to be progressively decreased with increasing the incubation periods with *N. linckia*, *N. spongiaeforme*, *A. variabilis*, and *A. spiroides* in non-sterile soil. This reduction is probably due to the presence of predators and cyanobacterial grazers in the soil which diminish and reduce the flourishent of indogenous algae (Grant *et al.* 1985; Reddy *et al.* 1986). In several soil systems cyanobacteria are the most important organisms that increase the biological activity of the soil surface layer. This may lead to an increase in the mineralisation of native soil organic matter (Rao and Burns 1990) and its nitrogen content (Sprent and Sprent 1990) thus increasing the concentration of plant-available nutrients.

Influence of cyanobacterial incubation period on the nitrogen-fixing abilities

The obtained data (Tables 1, 2 and 3) indicate a parallel relation between growth of each strain and its efficiency in the processes of nitrogen-fixation. The total nitrogen-fixing abilities of the different strains in sterile soil was higher than that in non-sterile one (Tables 3 and 4). As apparent from the results the efficiency of the tested cyanobacteria to fix nitrogen increased with increasing the incubation period in sterile soil. Present results agree with those found by Arora *et al.* (1994) who reported the increased fixation of nitrogen with the age of cyanobacterial culture. Generally, the most potent strains for nitrogen-fixation in a decline order, were *A. variabilis* > *N. spongiaeforme* > *N. linckia* > *N. carneum* in sterile soil.

However, the highest efficiency of nitrogen-fixation in the non-sterile soil was recorded for *A. variabilis*, *A. flos-aquae* after 30 and 60 days of incubation, *C. brevissima* after 60 and 90 days and finally, *N. muscorum* which has the greatest value after 90 days of incubation. Begum (1993) reported that the most abundant nitrogen-fixing cyanobacteria in rice soil were the species of *Nostoc* and *Calothrix*. *Nostoc* has the ability to survive in terrestrial habitats and fix nitrogen in symbiotic interactions with fungi (lichen), liverworts, hornworts, mosses, ferns, cycads, and the angiosperm *Gunnera* (Doods *et al.* 1995). Cyanobacteria show the presence of ascorbic acid as a constituent of the algal cell, it may participate in processes of nitrogen fixation and nitrate reduction, ultimately influencing plant growth.

Table 2. Fixed nitrogen estimated as total nitrogen (by Kjeldahl) in sterile sandy loam soil (mg / 100 g soil) amended with some cyanobacteria at the different incubation periods. (Results represent the difference between inoculated and non-inoculated soil).

Strains \ Days / Fixed nitrogen (mg/100g soil)	30			60			90		
	Cellular nitrogen	Extra-cellular nitrogen	Total fixed nitrogen	Cellular nitrogen	Extra-cellular nitrogen	Total fixed nitrogen	Cellular nitrogen	Extra-cellular nitrogen	Total fixed nitrogen
Nostoc microscopicum Carm.	22.6 ± 1.5	0.97 ± 0.20	23.57 ± 1.7	26.3 ± 1.6	1.87 ± 0.05	28.17 ± 2.1	33.7 ± 1.5	1.75 ± 0.02	35.45 ± 1.8
Nostoc linckia (Roth). and F.	33.2 ± 1.3	0.07 ± 0.01	33.27 ± 1.6	53.6 ± 2.1	1.46 ± 0.03	55.06 ± 3.2	76.7 ± 3.7	1.33 ± 0.04	78.03 ± 2.9
Nostoc spongiaeforme Ag.	27.6 ± 1.0	1.79 ± 0.80	29.39 ± 1.8	40.1 ± 1.8	1.64 ± 0.04	41.74 ± 2.9	80.2 ± 3.2	1.50 ± 0.03	81.70 ± 3.5
Anabaena variabilis Kutz.	25.7 ± 1.6	0.17 ± 0.04	25.87 ± 1.6	60.7 ± 2.0	0.36 ± 0.01	61.06 ± 3.1	87.9 ± 4.1	0.32 ± 0.05	88.22 ± 2.3
Anabaena flos-aquae (Lyngb.) Breb.	15.3 ± 1.2	1.15 ± 0.10	16.45 ± 1.3	17.4 ± 1.9	1.43 ± 0.06	18.83 ± 2.1	18.2 ± 0.8	1.43 ± 0.05	19.63 ± 0.7
Nostoc muscorum Ag.	21.5 ± 1.4	2.18 ± 0.20	23.68 ± 1.9	28.4 ± 1.2	1.41 ± 0.07	29.81 ± 2.6	35.7 ± 1.3	0.55 ± 0.02	36.25 ± 0.8
Nostoc piscinale Kutz.	12.6 ± 1.5	0.97 ± 0.08	13.57 ± 1.6	16.4 ± 1.1	2.32 ± 0.09	18.72 ± 1.4	20.3 ± 1.1	1.64 ± 0.08	21.94 ± 2.6
Nostoc rivulare Kutz.	21.8 ± 1.3	0.68 ± 0.05	22.48 ± 1.4	25.6 ± 1.5	1.12 ± 0.05	26.72 ± 2.7	31.6 ± 1.5	0.97 ± 0.07	32.57 ± 3.1
Calothrix brevissima West, G.S.	28.2 ± 1.8	0.97 ± 0.07	29.17 ± 1.9	30.3 ± 1.4	0.58 ± 0.03	30.88 ± 2.5	27.9 ± 0.9	0.82 ± 0.07	28.72 ± 2.4
Anabaena spiroides Kleb.	23.4 ± 1.7	0.73 ± 0.10	24.13 ± 1.8	24.5 ± 1.6	1.27 ± 0.04	25.77 ± 1.3	36.4 ± 1.9	1.26 ± 0.06	37.66 ± 1.2
Nostoc carneum Ag.	25.8 ± 1.7	0.74 ± 0.06	26.54 ± 1.8	63.6 ± 2.3	0.79 ± 0.02	64.39 ± 2.0	49.7 ± 1.7	1.61 ± 0.06	51.31 ± 2.3

Values are mean ± SD of three replicate experiments.

Table 3. Fixed nitrogen estimated as total nitrogen (by Kjeldahl) in non- sterile sandy loam soil (mg / 100 g soil) amended with some cyanobacteria at the different incubation periods. (Results represent the difference between inoculated and non-inoculated soil).

Strains / Days / Fixed nitrogen (mg/100g soil)	30			60			90		
	Cellular nitrogen	Extra-cellular nitrogen	Total fixed nitrogen	Cellular nitrogen	Extra-cellular nitrogen	Total fixed nitrogen	Cellular nitrogen	Extra-cellular nitrogen	Total fixed nitrogen
Nostoc microscopicum Carm.	5.3 ± 0.2	2.10 ± 0.09	7.40 ± 1.1	7.4 ± 1.5	0.83 ± 0.08	8.23 ± 1.3	2.30 ± 0.10	0.23 ± 0.04	2.53 ± 0.1
Nostoc linckia (Roth). and F.	7.2 ± 0.5	0.97 ± 0.07	8.17 ± 1.6	5.6 ± 1.3	0.62 ± 0.03	6.22 ± 1.4	4.10 ± 0.40	0.46 ± 0.06	4.56 ± 0.4
Nostoc spongiaeforme Ag.	3.5 ± 0.2	0.37 ± 0.02	3.87 ± 0.7	3.5 ± 0.9	0.50 ± 0.05	4.00 ± 1.0	3.20 ± 0.20	0.50 ± 0.10	3.70 ± 0.7
Anabaena variabilis Kutz.	14.7 ± 0.9	0.37 ± 0.03	15.07 ± 0.9	10.1 ± 2.1	0.68 ± 0.06	10.78 ± 1.7	5.70 ± 0.60	0.37 ± 0.06	6.07 ± 0.2
Anabaena flos-aquae (Lyngb.) Breb.	10.4 ± 0.8	0.55 ± 0.02	10.95 ± 0.8	11.3 ± 1.4	0.32 ± 0.04	11.62 ± 1.1	4.40 ± 0.20	0.24 ± 0.03	4.64 ± 0.4
Nostoc muscorum Ag.	9.3 ± 0.4	0.40 ± 0.05	9.70 ± 0.6	6.2 ± 1.6	1.02 ± 0.06	7.22 ± 1.1	22.3 ± 1.50	1.39 ± 0.60	23.69 ± 3.1
Nostoc piscinale Kutz.	3.8 ± 0.2	0.51 ± 0.02	4.31 ± 0.2	6.4 ± 0.8	1.53 ± 0.08	7.93 ± 0.9	1.90 ± 0.01	0.20 ± 0.02	2.10 ± 0.2
Nostoc rivulare Kutz.	1.3 ± 0.1	0.15 ± 0.03	1.45 ± 0.5	4.4 ± 0.7	1.31 ± 0.20	5.71 ± 0.8	1.20 ± 0.09	0.18 ± 0.04	1.38 ± 0.3
Calothrix brevissima West, G.S.	4.2 ± 0.3	0.22 ± 0.04	4.42 ± 0.4	12.3 ± 0.9	1.20 ± 0.40	13.50 ± 0.9	12.30 ± 1.10	1.92 ± 0.30	14.22 ± 0.5
Anabaena spiroides Kleb.	3.4 ± 0.3	0.70 ± 0.05	4.10 ± 0.6	2.8 ± 0.3	0.63 ± 0.04	3.43 ± 0.9	1.20 ± 0.10	0.50 ± 0.06	1.70 ± 0.2
Nostoc carneum Ag.	2.3 ± 0.5	0.22 ± 0.03	2.52 ± 0.9	5.4 ± 1.3	3.20 ± 0.2	8.60 ± 1.8	0.81 ± 0.05	0.12 ± 0.30	0.93 ± 0.3

Values are mean ± SD of three replicate experiments.

In general cyanobacteria are recognizied as one of the most important nitrogen-fixing agents in the soil. However, biological nitrogen fixation requires energy generally obtained by catabolism of photosynthetically fixed carbon (photosynthate) (Roger 1985). Trophic independence (synthesizing photosynthate from CO_2 and H_2O) of cyanobacteria, among other nitrogen-fixing microorganisms, make them especially attractive as a biofertilizers (Roger 1985; 1996) in rice field.

Influence of cyanobacterial exudate on rice grain germination.

The nature of positive or negative effect of the different cyanobacterial exudate on rice grain germination was evaluated (Fig.1). The percentage of germination of rice grain treated with the cyanobacterial exudate increased with time. The highest percentage of germination was recorded for the 84 hours-old seedling, with the cyanobacterial exudates of 30 day old culture of *N. muscorum* (14%) and of 60 days for both *N. linckia* (25%) and *A. flos–aquae* (10%) as compared with control. This result coincident with that obtained by Roger and Kulasooriya (1980), who found that presoaking of rice grains with cyanobacterial cultures or extracts enhances germination, promotes the growth of roots and shoots and increases the weight and protein content of the grain. Also, they established that besides increasing nitrogen fertility, cyanobacteria have been assumed to produce growth promoting substances like hormones, vitamins, amino acids or many other components that enhances germination.

The greatest negative effect of the cyanobacterial exudates on germination was observed for that of 90 days old cultures specially of *N. muscorum* (70%), *C. brevissima* (51%) and *A. spiroides* (62%) as compared with the control. The present study revealed that most of the investigated strains had negative effect on germination of rice specially those tested after 90 days.

The nature of positive or negative effect of the cyanobacterial exudate on germination may be due to the action of one or a combintion of factors. Cyanobacteria are known to secrete several different categories of secondary metabolites, such as auxin-like substances (Venkataraman 1981), cytokinin-like substances (Rodgers *et al.* 1979), or gibberellin-like substances (Singh and Trehan 1973), vitamin B (Grieco and Desrochers 1978), organic acids (Hellebust 1974), antibiotics (Moore 1996; Schlegel *et al.* 1999) and toxins (Codd *et al.* 1999; Metting and Pyne 1986). Other secondary metabolites were also recorded, such as phenolics , terpenoids, fatty acids, peptides and alkaloids (Metting and Pyne 1986; Kiviranta *et al.* 1991; Carmichael 1992; Inderjit and Dakshini 1994; Gross *et al.* 1994). Venkataraman (1981) reported that the excretion of ascorbic acid by cyanobacteria is known and play a dual role: (a) as an exadate from cyanobacteria in rice field, it can accelerate growth and development of the plant directly and (b) as a constituent of the cyanobacterial cell, it may participate in processes of nitrogen fixation and nitrate redaction. Also, he established that *Nostoc* exudates show the presence of indol acetic acid and sometimes indol propionic acid, anthrelinic acid and allied compounds. Khan (1975) reported that the internal balance among growth regulators in the seed is the main agent to induce the dormancy or germination. This balance is affected by external factors such as growth-promoting or germination regulators produced by cyanobacteria. In this connection Reddy *et al.* (1986) showed that pretreatment of rice seedlings with a

cyanobacterial culture enhanced rice growth in in-vitro and pot experiments but not significantly increased yield in the field.

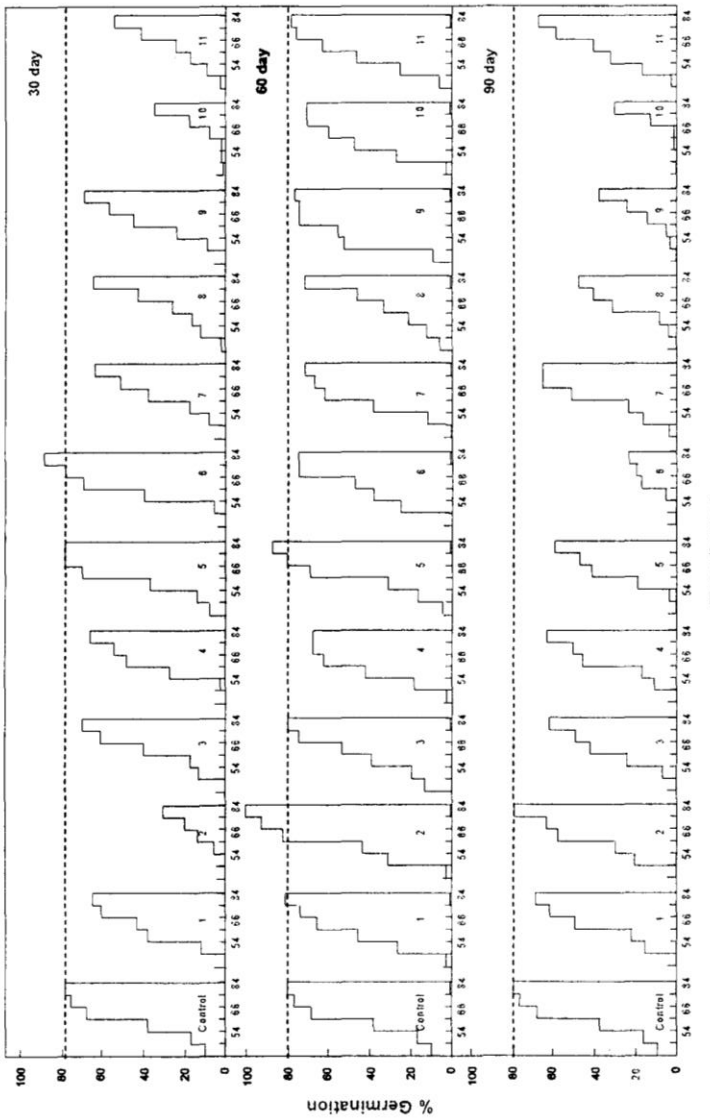

Fig. 1. The percentage of rice grain germination treated with 30, 60, 90 days cyanobacterial exudate at the different incubation hours. 1: *Nostoc microscopicum*, 2: *Nostoc linckia*, 3: *Nostoc spongiaeforme*, 4: *Anabaena variabilis*, 5: *Anabaena flos-aquae*, 6: *Nostoc muscorum*, 7: *Nostoc piscinale*, 8: *Nostoc rivulare*, 9: *Calothrix brevissima*, 10: *Anabaena spiroides*, and 11: *Nostoc carneum*

In conclusion, the results of this investigation have clearly demonstrated that inoculation of soil with some cyanobacterial strains improved its nitrogen status. Also, the presoaking of rice grains in some cyanobacterial exudates stimulated their germination whereas, other strains suppresed the germination.

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كفاءة بعض البكتريا الزرقاء فى تثبيت النيتروجين فى التربة الرملية الطينية وفعالية مستخلاصتها فى إنبات حبوب الأرز.

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تم دراسة ١١ سلالة من البكتريا الزرقاء من حيث النمو معبرا عنه بالكتلة الحيوية، والكفاءة فى تثبيت النيتروجين فىنوعين من التربة إحداهما معقمة والأخرى غير معقمة خلال فترات حضانة مختلفة. وقد تم أيضا معاملة حبوب الأرز بمستخلص هذة البكتريا الزرقاء وسجل اعلى معدل للنمو مع نوستك ريفيولارى و نوستك ميكروسكوبيكم بعد ٦٠ يوم من الحضانة فى التربة المعقمة، ومع كالوثريكس بريفيسيما، نوستك ميكروسكوبيكم، ونوستك كارنيم فى التربة الغير معقمة. وأوضحت النتائج أن أقوى السلالات فىتثبيت النيتروجين هى أنابينا فاريبليز، نوستك سبونجيفورم، ونوستك لنكيا بعد ٩٠ يوم من الحضانة فى التربة المعقمة، أما فى التربة الغير معقمة فكانت نوستك ماسكورم، وكالوثريكس بريفيسيما. وأدت معاملة حبوب الأرز بمستخلص البكتريا الزرقاء نوستك لنكيا،نوستك ماسكورم، وأنابينا فلس أكوا إلى أعلى معدل إنبات بنسبة ٢٥، ١٤، ١٠٪ بالتتابع بالمقارنة بالسلالات الأخرى. وأظهرت الدراسة أن كفاءة تثبيت النيتروجين والإنبات لحبوب الأرز تعتمد على نوع اللقاح الحيوى ويفضل أستخدام نوستك ماسكورم، وكالوثريكس بريفيسيما كسلالات غير محلية نظرا لقدرتهما على البقاء فى حالة حيوية جيدة لمدة ٩٠ يوم فى التربة الغير معقمة.