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# BIO-ENHANCEMENT EFFECT OF CYANOBACTERIA ON RICE SEEDS GERMINATION AND SEEDLINGS GROWTH

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## ABSTRACT

Beside their ability to fix atmospheric nitrogen, cyanobacteria are known to secrete into the surrounding medium a variety of compounds that could ameliorate or enhance seed germination and growth of rice plants. In an attempt to expound how far the tested cyanobacterial candidates could contribute to rice development, 24-48 hrs and 4 days laboratory incubation experiments in the dark were carried out where three rice cultivars (Giza 177, Giza 178 and Sakha 101) were determined for seed and seedling growth when treated with cell-free extracts (filtrates) of cyanobacterial strains namely, *Anabaena oryzae, Nostoc calcicola, Microchaete tenera* and *Cylindrospermum muscicola*, each separately Results indicate that cyanobacterial culture extracts (filtrates) had stimulated roots and shoots growth of rice seedlings besides increasing seed germination % for the tested rice cultivars *N. calcicola* had recorded the highest percentages germination of 98, 92 and 90 for rice seeds cultivars Giza 178, Sakha 101 and Giza 177 after 48 h incubation in the dark Extending the incubation period to 7 days, again *N. calcicola* supported greater growth pattern of rice roots and shoots than other cyanobacterial candidate

## INTRODUCTION

The importance of nitrogen-fixing cyanobacteria as a source of combined nitrogen to paddy crop, has been appreciated ever since De (1939) had demonstrated the capacity of certain cyanobacteria to fix atmospheric nitrogen in rice fields. Since then many workers have paid attention to the study of cyanobacteria flora in rice fields and the effect of those cyanobacteria on rice plants (Singh, 1961). He has reported that rice plants fertilized with *Aulosira fertilissima*, gave rice yield three times greater than the control. Gupta and Lata (1965) while working on cyanobacteria of paddy fields, have observed that *Fischerella, Scytonema* and *Nostoc* sp. accelerate the germination of seeds.

Besides nitrogenous compounds, Cyanobacteria have other biologically active constituents like vitamin $B_{12}$ and auxins, which may also contribute appreciably to their nitrogen fixing fertilizing activity (Singh and Trehan, 1973). Of the various intracellular amino acids of *Cylindrospermum muscicola*, Cystine, tyrosine and phenylalanine seem to be available to rice plants. Gupta and Shkula (1967) have reported that rice seedlings, treated with an extract of *phormidium* sp., showed a marked stimulation of root and shoot growth.

In a cumulative review, Roger and Kulasooriya (1980) reported that besides increasing nitrogen fertility, cyanobacteria have been said to benefit rice plants by producing growth-promoting substances. More direct evidence for hormonal effects has come primarily from treatments of rice seedlings with cyanobacteria culture or their extracts. Presoaking of rice seeds in cyanobacteria cultures or extracts has decreased losses from sulphate – reducing processes and this has been attributed to the enhancement of germination and a faster seedlings growth due to cyanobacterial exudates. On the other hand, extracts of *Cylindrospermum muscicola* that have given a positive effect on root growth of rice seedlings had an action similar to that produced by vitamin $B_{12}$, which was found to be present in the cyanobacteria cells (1.5 µg $g^{-1}$).

Pathak and Jha (1995) documented that when maize, wheat, mustard (*Brassica juncea*) and lady's finger (*Abelmoschus esculentus)* seeds were inoculated with *Synechocystis aquatilia, Microcystis elabens, Anabaena doliolum* or *Nostoc linkia,* seed germination in wheat and mustard was highest with *S. aquatilis*. In maize and lady's finger, it was highest with *M. elabens*. Maize and wheat yields were highest when inoculated with *A. doliolum*, while the yields of mustard and lady's finger were highest when inoculated with *N. linkia.*

Mule *et al.* (1999) observed that the inoculation of rice plants either with *Nostoc muscorum* or *Tolypothrix tenuis* each alone or combined with urea gave higher seedlings dry weight and shoot length than the control treatment without cyanobacteria inoculation. Shoots were longest with *N. muscorum* + urea indicating that this treatment was more effective than each cyanobacterium alone.

Aref (2001) reported that pre-soaking of rice seeds cv. Sakha 102 in *Nostoc* sp. filtrate had stimulated the seeds germination percentage to reach 90% as compared with water soaked seed. She also added that this stimulation effect occurred with *Nostoc* sp. had appeared with both *Nostoc muscorum* and *Anabaena* sp. in respective to percentage increases of 86.8 and 83.3% over those of water soaked seeds.

This work is to evaluate the effect of cyanobacteria filtrate on the seed germination and the growth performance of three different rice cultivars (Giza 177, Giza 178 and Sakha 101).

## MATERIALS AND METHODS

Four cyanobacterial strains (*Anabaena oryzae*, *Nostoc calcicola, Microchaete tenera* and *Cylindrospermum muscicola*) are kindly supplied by Professor Dr. F. M. Ghazal, Agricultural Microbiology Department, Soils, Water and Environment Institute, Giza, Egypt. These cyanobacteria strains were grown on BG11 medium (Rippika *et al.,* 1979) under continuous illumination (2500 Lux) at temperature of 28 – $32^0$ C up to their appropriate logarithmic phase, each of them was then filtered and their filtrates were used to evaluate their extract influence on the germination and the growth performance of rice seeds cultivars Giza 177, Giza 178 and Sakha 101 supplied by Rice Research Institute, Agricultural Research Center, Giza. Egypt.

The surface of rice seeds were sterilized by soaking in a saturated sodium hypochlorite solution (2%v/v) for 2 h, with periodical agitation, and then washed thoroughly with sterile distilled water. The seeds were allowed to germinate in a petri – dish of 10 cm diameter containing 25 mL watery agar (1.2 g agar 100 $mL^{-1}$ distilled water).

Each petri – dish was supplied with 10 seeds and supplemented with 2 mL crude filtrate of cyanobacteria as extract.

The plates were incubated for 24, 48 h and 7days old at 28 –30$^{0}$C in the dark. As sterility check plates without seeds were also incubated. In the control petri – dishes cyanobacterial filtrate was substituted with distilled water and/or BG11 medium for comparison, each treatment was repeated in triplicates and arranged in complete randomized design as described by Gomez and Gomez (1984). The percentage of germination of rice seeds were calculated as follows:

$$\text{Germination \%} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

After 7 days of incubation, rice seedlings were measured for shoots and root lengths in comparison with those of the control treatments.

## RESULTS AND DISCUSSION

Data in Table (1) and illustrated by Figures (1, 2 and 3) indicate the bio-stimulation effect of the cyanobacteria filtrates (extracts) on rice seeds germination after 24 and 48 hours incubation in the dark.

**Table (1): Effect of cyanobacteria filtrate on rice seed germination after 24 and 48 hrs**

| Rice cultivars | Additives | Germination % of total seeds | |
|---|---|---|---|
| | | 24 hrs. | 48 hrs. |
| Giza 177 | (Distilled water control) | 10.00 | 60.00 |
| | BG 11 medium | 15.00 | 66.00 |
| | Filtrate of : | | |
| | *Anabaena oryzae* | 20.00 | 80.00 |
| | *Nostoc calcicola* | 30.00 | 90.00 |
| | *Microchaete tenera* | 47.00 | 87.00 |
| | *Cylindrospermum muscicola* | 30.00 | 70.00 |
| Giza 178 | (Distilled water control) | 15.00 | 64.00 |
| | BG 11 medium | 20.00 | 80.00 |
| | Filtrate of : | | |
| | *Anabaena oryzae* | 40.00 | 86.00 |
| | *Nostoc calcicola* | 35.00 | 98.00 |
| | *Microchaete tenera* | 45.00 | 88.00 |
| | *Cylindrospermum muscicola* | 37.00 | 79.00 |
| Sakha 101 | (Distilled water control) | 13.00 | 72.00 |
| | BG 11 medium | 18.00 | 80.00 |
| | Filtrate of : | | |
| | *Anabaena oryzae* | 45.00 | 85.00 |
| | *Nostoc calcicola* | 29.00 | 92.00 |
| | *Microchaete tenera* | 48.00 | 89.00 |
| | *Cylindrospermum muscicola* | 34.00 | 74.00 |

Within 24 hrs of water moistening, 10% of rice cv. Giza 177 were germinated increased to 60 % after 48 hrs with distilled water treatment.

Germination percentages of 15 and 64, 13 and 72 were obtained in respective to cv. Giza 178 and cv. Sakha 101 treated with distilled water treatment after 24 and 48 hrs, respectively. Seeding on BG 11 medium supported better germination rate of the tested rice cultivars with respective percentages of 15-66 (Giza 177), 20-80 (Giza 178) and 18-80 (Sakha 101) in corresponding to 24 and 48 hrs incubation periods. Cyanobacterial filtrates promoted rice seed germination in rates differed from one strains to another. *N. calcicola* appeared to be the superior stimulant resulting in germination of 98, 92 and 90% of rice seeds cultivars Giza 178, Sakha 101 and Giza 177 after 48 hrs, respectively followed by *M. tenera, A. oryzae* and *C. muscicola* with respective germination percentages of (87, 88 and 89), (80, 86 and 85) and (70, 79 and 74) corresponding to rice cultivars Giza 177, Giza 178 and Sakha 101.

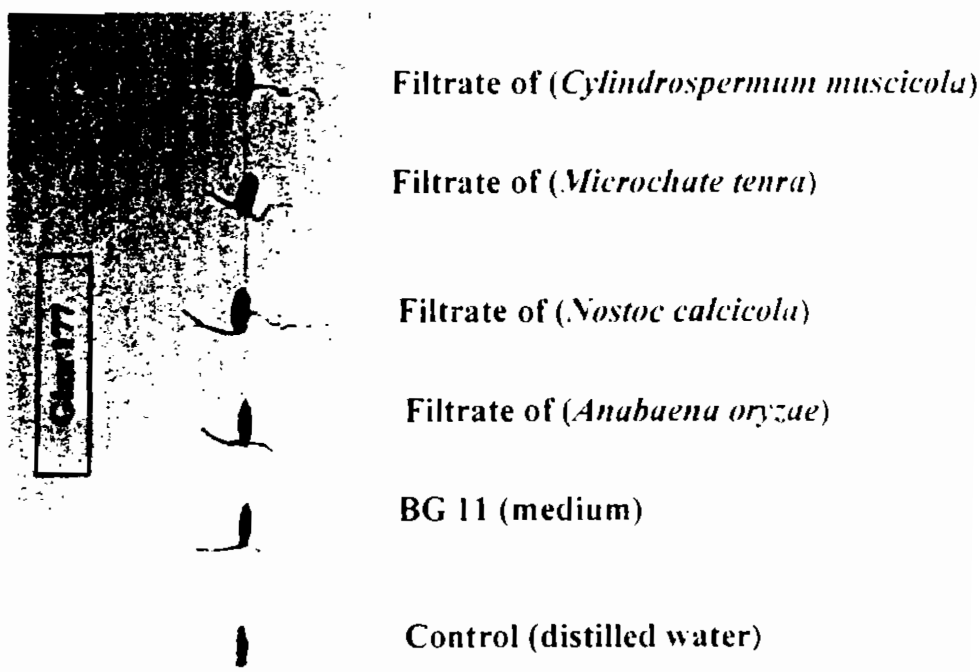



**Fig.(1): Pattern growth of rice seeds under the influence of cyanobacteria filtrate ( cv. Giza 177).**


Fig. (2): Pattern growth of rice seeds under the influence of cyanobacteria filtrate (cv. Giza 178).

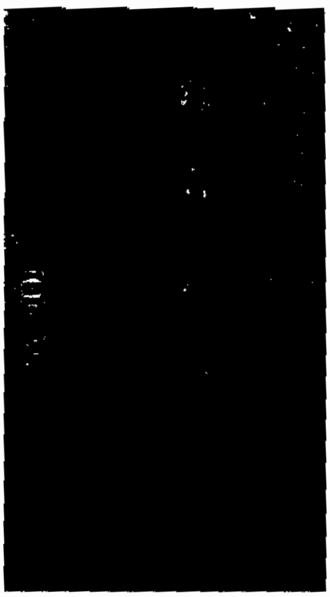


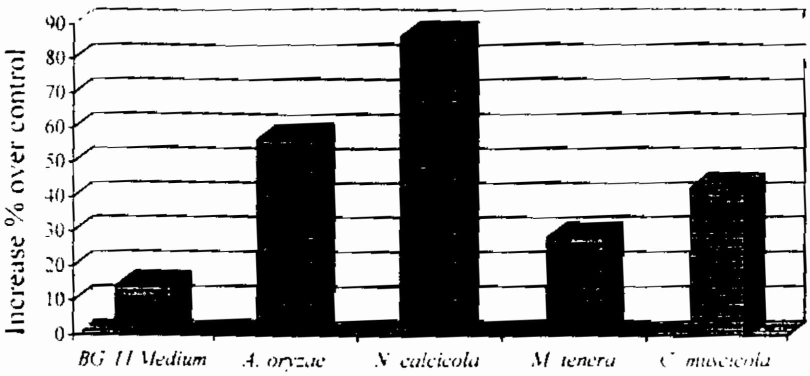

Fig.3: Pattern growth of rice seeds under the influence of cyanobacteria filtrate (cv. Sakha 101).

The early growth rates of rice seedlings (shoots and roots) and the percentage increases due to cyanobacterial culture filtrates are demonstrated in Table (2) and Figures (4 & 5). Soaking rice seeds cultivars in BG 11 media resulted in increasing both shoots and roots compared with those soaked in distilled water. The corresponding increase percentages were 14.3, 25.0 and 50.0 (shoot) and 100, 100 and 300 (root) for Giza 177, Giza 178 and Sakha 101, respectively. Similarly, seeds of all rice cultivars did positively responded to cyanobacterial culture filtrates and gave different increases for both shoots and roots compared with those soaked in distilled water.

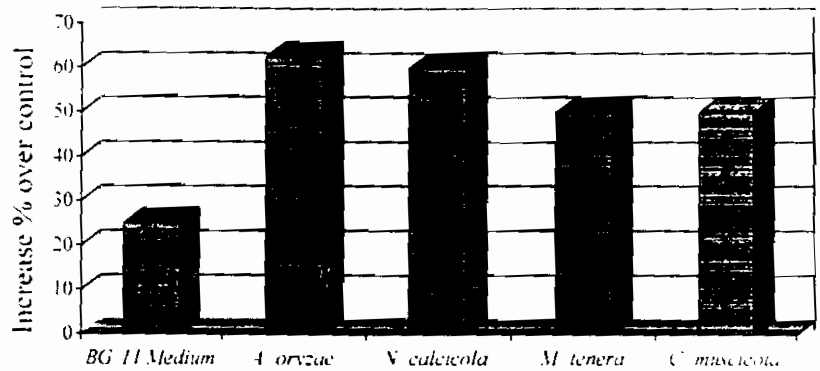
Treatment of rice seed cultivars with *A. oryzae* filtrates supported percentage increases of 57.5, 62.5 and 80.0 in respective to rice cultivars Giza 177, Giza 178 and Sakha 101. The corresponding roots increase percentages were 850, 150 and 500. In respect to other tested cyanobacterial strains, *N. calcicola* recorded percentage increases of 85.7, 90.5 and 90 for shoots against 900, 450 and 850 for roots in corresponding to Giza 177, Giza 178 and Sakha 101 rice cultivars. *M. tenera* gave such increase percentages as 28.6, 50.0 and 75.0 (shoots) and 300, 100 and 600 (roots) in respective to Giza 177, Giza 178 and Sakha 101.

**Table (2) : Effect of cyanobacteria filtrate on rice seedlings growth after 7 days incubation**

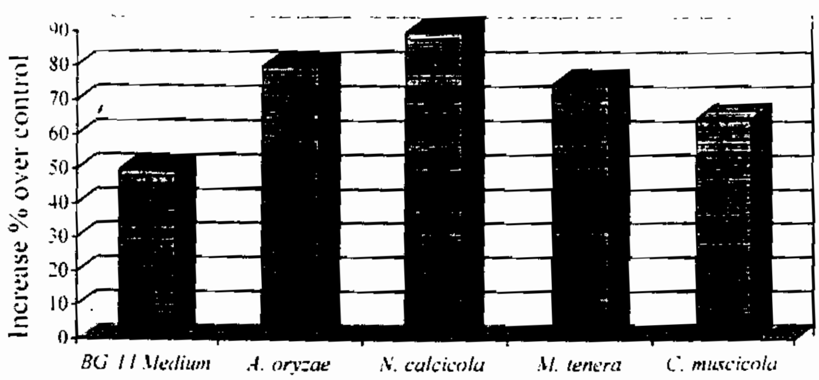
| Rice cultivars | Additives | Seven days old seedlings | |
|---|---|---|---|
| | | Shoot length cm | Root length cm |
| Giza 177 | (Distilled water control) | 0.70 | 0.20 |
| | BG 11 medium | 0.80 | 0.40 |
| | Filtrate of : | | |
| | *Anabaena oryzae* | 1.10 | 1.90 |
| | *Nostoc calcicola* | 1.30 | 2.00 |
| | *Microchaete tenera* | 0.90 | 0.80 |
| | *Cylindrospermum muscicola* | 1.00 | 1.60 |
| Giza 178 | (Distilled water control) | 0.80 | 0.20 |
| | BG 11 medium | 1.00 | 0.40 |
| | Filtrate of : | | |
| | *Anabaena oryzae* | 1.30 | 0.50 |
| | *Nostoc calcicola* | 1.52 | 1.10 |
| | *Microchaete tenera* | 1.20 | 0.40 |
| | *Cylindrospermum muscicola* | 1.20 | 0.60 |
| Sakha 101 | (Distilled water control) | 0.40 | 0.20 |
| | BG 11 medium | 0.60 | 0.80 |
| | Filtrate of : | | |
| | *Anabaena oryzae* | 0.72 | 1.20 |
| | *Nostoc calcicola* | 0.76 | 1.90 |
| | *Microchaete tenera* | 0.70 | 1.40 |
| | *Cylindrospermum muscicola* | 0.66 | 1.30 |




**Giza 177**

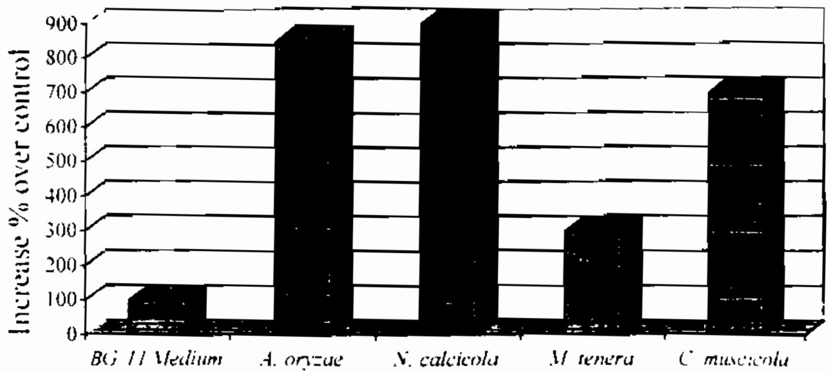



**Giza 178**

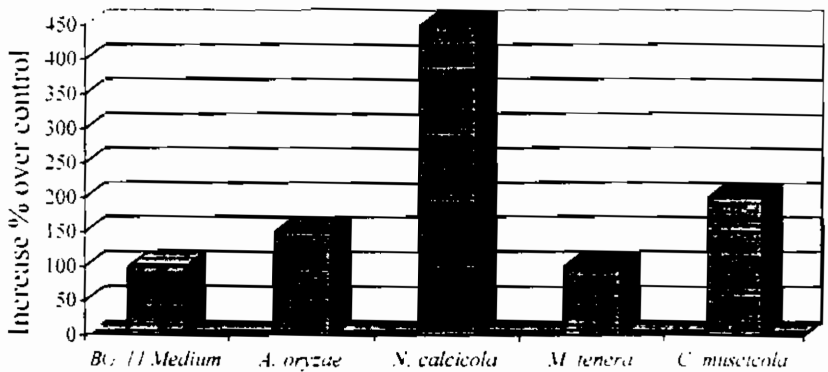



**Sakha 101**

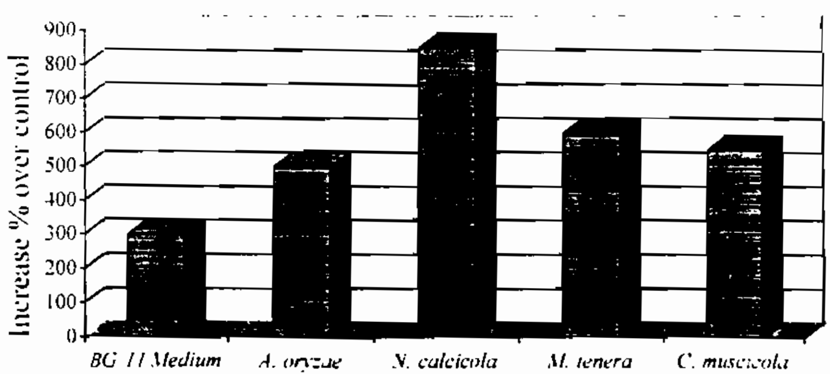
**Fig. (4): Effect of cyanobacterial filtrates on rice shoot seedlings growth after 7 days incubation.**




Giza 177




Giza 178




Sakha 101

**Fig. (5): Effect of cyanobacterial filtrates on rice root seedlings growth after 7 days incubation.**

However, also the positive response of tested rice cultivar to the treatment with *C. muscicola* is expressed by the shoot increases of 42.9% (Giza 177), 50% (Giza 178) and 65% (Sakha 101). The respective increases in root lengths were 700%, 200% and 550%. Again, culture filtrates of *N. calcicola* supported greater growth pattern of rice shoots and roots than other cyanobacterial candidate (Table, 1 and Figs. 1, 2 & 3).

In this work, cyanobacterial culture extracts have enhanced root and shoot growth of rice seedlings besides increasing seed germination. This might possibly due to the production of growth promoting substances by the microorganisms. Kaptiyeva and Tantsiurkenko (1971) found that water soluble product from 8 *Calothrix* spp., *Anabaena* sp. and *Strtonostoc* sp. had rhizogenous effect and stimulated rice growth. Presoaking of rice seedlings in extracts of *Phormadium* had been shown to accelerate seed germination (Gupta and Lata, 1965), promote the vegetative growth of rice plants and increase the weight and protein content of grains (Gupta and Shukla, 1967). The growth pattern of rice seedlings treated with filterates of *Aulosira fertilisima* resembled seedlings treated with gibbrellic acid (Singh and Trehan, 1973). It has also been demonstrated that amino acids and vitamin $B_{12}$ obtained from cyanobacterial extracts had a rhizogenous influence in rice (Venkataraman and Neelkantan, 1987). Soaking seeds of cucumber and pumpkin in an extract of the $N_2$-fixing cyanobacterium *Westiellopsis prolifica* promoted and enhanced germination and their subsequent growth and development of seedlings. An extract of *Lyngbya sp.* a non-$N_2$-fixing cyanobacterium, had no significant effect (Nanda *et al.,* 1991). The germination of seeds of the some crop plants treated with either live inoculum, algal filtrate (exogenous) or boiled algal extract (endogenous) of the nitrogen fixing cyanobacterium *Nostoc muscorum* was significantly increased due to the nitrogenous compounds as well as nitrate reductase activities and peptides produced in the cyanobacteria filtrate and or other compounds that stimulate growth of crop plants (Adam, 1999). Soaking rice seeds cv. Sakha 102 in *Nostoc* sp. filtrate had stimulated the seeds germination percentage to reach 90% as compared with water soaked seed (Aref, 2001). She also added that this stimulation effect occured with *Nostoc* sp., had appeared with both *Nostoc muscorum* and *Anabaena sp.* in respective to percentage increases of 86.8 and 83.3% over the water soaked seeds.

Tantawi and Mussa (2001) have soaked the seeds of some crops cultivars (wheat, soybean and clover) in the cyanobacterial extract of *Nostoc muscorum* and /or *Anabaena flos aquae* and found that they had positively stimulated and improved both the germination and the plant growth for all tested crops.

However, the production by cyanobacteria of substances that have growth promoting like effect on rice plants is more or less well established, but what is the nature of these substances, this is the question which still needs to find an answer.

## REFERENCES


Adam, M. S. (1999). The promotive effect of *cyanobacterium Nostoc muscorum* on the growth of some crop plants. Acta. Microbiologica. 48:163 – 171.

Aref, Elham, M. M. (2001). Studies on cyanobacteria in Egypt. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Giza , Egypt.

De, P. K. (1939). The role of blue-green algae in nitrogen fixation in rice fields. Proc. R. Soc. Lond., 127 B: 121-139.

Gomez, K. A. and A. Arturo, Gomez. (1984). Statistical procedures for Agricultural research, (2nd ed), pp. 20-29 & 359-387.

Gupta, A. B. and A. C. Shukla. (1967). Influence of algal growth promoting substances on growth, yield and protein content of rice plant. Nature (London), 213: 744.

Gupta, A. B and K. Lata. (1965). Effect of algal growth hormones on the germination of paddy field. Hydrobiol, 24: 430-434.

Kaptiyeva, Zn, P. and O. V. Tantsiurkenko. (1971). Effect of growth of rice seedlings. Microbiol. Zh., 33 : 215-221.

Mule, Maria. C.Z., Caire, Gloria, Z., Cano, Monica, S. and Colombo, Karina. (1999). Effect of cyanobacterial inoculation and fertilizers on rice seedlings and postharvest soil structure. Commun. Soil Sci. Plant Anal, 30: 97-107.

Nanda, B.; S. K. Tripathy and S. Padhi. (1991). Effect of algalization on seed germination of vegetable crops. World J. Microbiol. And Biotech. 7: 622 – 623.

Pathak, B. and C. N. Jha (1995). Influence of some phytoplanktons on germination and growth of maize, wheat, mustard and Lady's finger. Environ. & Ecology. 13: 728: 731.

Rippika, R.; J. Deruelles; J. B. Waterbury; M Herdman, and R. Y. Stanier. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria J. Gen. Microbiol. 111: 1 – 61.

Roger, P. A. and S. A. Kulasooriya. (1980). Blue-green algae and rice. Ed., International Rice Res. Inst., pp.51-52.

Singh, V. P. and K. Trehan. (1973). Effect of extracellular products of *Aulosira fertilissima* on the growth of rice seedlings. Plant and Soil, 38: 457-464.

Singh, R. N. (1961). Role of blue-green algae in Indian agriculture. I.C. A. R. (New Delhi), {Cf. Singh and Trehan. (1973)}.

Tantawy, S. T. A. and S. A. I. Mussa, (2001). The influence of live cyanobacteria filtrate of *Nostoc calcicola* and *Anabaena flos aquae*. On seed germination abd plant growth of som cultivars (wheat, soybean ands clover). Az. J. Microbiol. 54: 251-272.

Venkataraman, G. S. and S. Neelakantan. (1987). Effect of the cellular constituents of the nitrogen fixing blue- green algae *Cylndrospermum muscicola* on the root growth rice seedlings. J. Gen. Appl. Microbiol., 13: 53 – 61.

**تأثير الحث الحيوى للسيانوبكتريا على انبات بذور الأرز وعلى نمو البادرات**

**السيده على حسن و منى ميخائيل مرقص**
**قسم الميكروبيولوجيا- معهد بحوث الأراضي و المياه والبيئــة- مركــز البحــوث الزراعيــة- الجيزة- مصر**

فى هذه الدراسة أجريت تجربة في المعمل باستخدام أربعة سلالات من السيانوبكتريا كل على حده وكانت هذه السلالات هي:
*Anabaena. oryzae, Nostoc calcicola, Microshaete Tenera, and Cylindrospermum muscicola*
حيث حضنت هذة السلالات النامية على بيئة*BG 11* عند درجة حرارة من ٢٨ – ٣٢°م تحت الإضاءة المستمرة لمدة ٢١ يوم للحصول على طور النمو اللوغاريتمى لها.بعد ذلك رشحت نموات هذة السلالات للحصول على الراشح وذلك لدراسة أثره على انبات أربعة أصناف من الأرز هى جيزة ١٧٧، ١٧٨، سخا ١٠١ وذلك بعد التحضين لحبوب هذة الأصناف النامية على أجــار مائى فى الظلام لمدة ٢٤و ٤٨ ساعة وكذلك أطوال سيقان وجذور البادرات بعد سبعة أيــام مــن التحضين ، ولقد أوضحت النتائج مايلى:-

١- لقد شجع راشح سلالات السيانوبكتريا إنبات تقاوي الأرز لأصناف جيزة ١٧٧، ١٧٨، سخا ١٠١ كما أدى الراشح إلى زيادة طول كل من جذور بادرات الأرز وكذا سيقانها.
٢- حققت السلالة *Nostoc calcicola* أعلى نسبة انبات لأصناف الأرز تحت الدراسة بعد ٤٨ ساعة تحضين فى الظلام ، حيث كانت هذه النسب هى٩٨ % (جيزة ١٧٨) و ٩٢ % (سخا ١٠١) و(جيزة ١٧٧)، وذلك بالمقارنة مع السلالات الأخرى.
٣- كذلك حققت السلالة *Nostoc calcicola* أعلى أطوال لسيقان النباتات وكذا الجذور لجميع أصناف الأرز تحت الدراسة وذلك بعد تحضينه لمدة سبعة أيام فالظلام مع راشح هذة السلالة وذلك بالمقارنة مع محققتة السلالات الأخرى .