

Study of soil blue-green algae and their effect on seed germination and plant growth of vegetable crops

مطالعه جلبک‌های سبز - آبی خاکزی و تأثیر آن‌ها بر جوانه‌زنی و رشد محصولات زراعی*

Received: 06.10.2010 / Accepted: 21.04.2011

دریافت: ۱۳۸۹/۸/۱۵ / پذیرش: ۱۳۹۰/۲/۱

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Abstract

Nitrogen fixation blue-green algae make a major contribution to the fertility of soil. It has been suggested that blue-green algae (BGA) assist higher plant growth by supplying growth substances. There are numerous works about roles of blue-green algae in paddy soils and rice growth but little on other plants. In this research, 19 morphospecies belonging to three families of heterocystous and non-heterocystous blue-green algae from several paddy fields in the north of Iran were identified out of which seven species are new to Iran. Among these taxa, three species were used as inoculum in pot culture of cucumber, tomato and squash. The result revealed that addition of all algal extracts can enhance seed germination and plant growth in all treated plants. Statistical analysis showed that there are significant differences in plant height, root length, number of leaves, fresh and dry weight of root, leaf and stem as compared to control.

Keywords: Algal extract, biofertilizer, *Cyanophyta*, Iran, morphospecies

خلاصه

جلبک‌های سبز-آبی تثبیت‌کننده نیتروژن نقش مهمی در حاصلخیزی خاک ایفا می‌نمایند. این گروه از میکرو-ارگانیسم‌ها قادرند به واسطه تولید ترکیبات تسریع‌کننده رشد موجب افزایش رشد گیاهان عالی گردند. تا به امروز مطالعات متعددی در زمینه بررسی نقش جلبک‌های سبز-آبی در بهبود رشد گیاه برنج صورت پذیرفته است، اما اطلاعات اندکی راجع به تأثیر آن‌ها بر رشد سایر گیاهان موجود است. طی این تحقیق ۱۹ ریخت گونه متعلق به سه تیره از جلبک‌های سبز-آبی دارای هتروسیست و فاقد هتروسیست از شمال ایران مورد شناسایی قرار گرفت که هفت گونه از آن‌ها برای نخستین بار از ایران گزارش می‌شوند. سپس به منظور ارزیابی نحوه اثرگذاری عصاره جلبکی بر رشد گیاهان عالی، سه گونه از جلبک‌های سبز-آبی دارای هتروسیست جداسازی شده به عنوان ماده تلقیحی در کشت گلدانی سه گیاه خیار، گوجه فرنگی و کدو مورد استفاده قرار گرفتند. نتایج نشانگر تسریع جوانه‌زنی بذرها و افزایش معنی‌دار رشد رویشی گیاه در حضور عصاره جلبکی بود.

واژه‌های کلیدی: ایران، سیانوفیتا، ریخت‌گونه، کود زیستی، عصاره جلبکی

* بخشی از رساله دکتری نگارنده اول به راهنمایی دکتر ریاحی ارائه شده به دانشگاه شهید بهشتی

Introduction

Cyanobacteria represent a small taxonomic group of photosynthetic prokaryotes which some of them are able to N₂ fixation and also possess a tremendous potential for producing a wide range of secondary metabolites. Cyanobacteria have drawn much attention as prospective and rich sources of biologically active constituents and have been identified as one of the most promising groups of organisms capable of producing bioactive compounds (Fish & Codd 1994, Schlegel *et al.* 1999). Production of bioactive molecules such as auxins, production of secondary metabolites linked to biocontrol of bacterial and fungal diseases as well as improving soil structure and porosity through secretion of polysaccharides aiding in soil aggregation are the most important functions of these microorganisms (Karthikeyan *et al.* 2007, Sergeeva *et al.* 2002). De (1939) attributed the natural fertility of flooded rice field soil and its maintenance to the process of biological nitrogen fixation by cyanobacteria. This was the first report, which recognized the agronomic potential of cyanobacteria in India. The widespread application of single element fertilizers (especially N in Asian countries) in the cultivation of major crops has led to accelerated exhaustion of other major and minor nutrients leading to nutrient imbalances and poor soil fertility. In the current scenario therefore, an urgent need has been felt to deploy microbial biofertilizer which are multifaceted such as cyanobacterial biofertilizer. As yet for substitution of chemical fertilizers by microbial biofertilizers many studies have been done. Gupta & Shukla (1967) studied the algal influence on growth, yield and protein content of rice plants and showed that pre-soaking rice seeds with BGA cultures or extracts enhances germination, promotes the growth of roots and shoots, and increases the weight and protein content of the grain. Svircev *et al.* (1997) also reported that plant growth was enhanced in the presence of cyanobacterium, even without organic N fertilizer application. Beneficial effects of cyanobacterial inoculation were reported, not only for rice, but for other crops such as wheat, soybean, oat, tomato, radish, cotton, sugarcane, maize, chili, bean,

muskmelon and lettuce (Venkataraman 1972, Rodgers *et al.* 1979, Singh 1988, Arif *et al.* 1995, Thajuddin & Subramanian 2005, Saadatnia & Riahi 2009, Maqubela *et al.* 2008, Karthikeyan *et al.* 2007). Several reasons have been proposed for beneficial effects of cyanobacteria on the growth of different plants. The capacity for biosynthesis of growth promoting substances such as auxins, amino acids, sugars and vitamins (Vitamin B₁₂, Folic acid, Nicotinic acid and Pantothenic acid) was reported by Misra & Kaushik (1989 a, b) that can enhance growth of plant. Additionally, cyanobacteria excrete complex organic carbon compounds that bind to the soil particles and improve soil aggregation, hence improve soil structure, soil permeability and water holding capacity of soil (Kaushik 2007). However, to date, the effect of single species cyanobacteria biofertilizer on plant growth has not yet been fully investigated. The primary aim of this research was to study cyanobacteria species isolated from soil and the second aim was pointing out the role of cyanobacteria as a biofertilizer in vegetables such as cucumber, tomato and squash plants.

Materials and Methods

Soil samples were collected from the depth of 0–5 cm on two paddy fields in Gilan and Mazandaran provinces in the north of Iran (Rangaswamy 1996).

- Isolation of cyanobacteria

Soil samples were transferred to sterile Petri dishes and added to them sterilized BG-11 medium with pH: 7.1. The Petri dishes were placed in a culture chamber at 25° C and a 12/12 h light dark cycle at artificial illumination (2000–2500 Lux) for two weeks. After colonization, for purification, identification and multiplication of colonies, a part of each colony was removed by a loop and transferred to a new plate. After purification of taxa, taxonomic determination was carried out by light microscopy and based on Desikachary (1959), Prescott (1970) and Wehr *et al.* (2002), and corrected based on algaebase website (www.algaebase.org).

In this study, out of 19 morphospecies identified and three dominant species, *Anabaena vaginicola*, *Nostoc* sp. and *Nodularia harveyana*, were selected for further study.

- Preparation of algal extract

Three selected heterocystous cyanobacteria were grown in 500 mL flasks containing nitrate free BG-11 medium for 14 days at artificial illumination (2000–2500 Lux) at 25° C, with constant stirring and aeration. The cultures were harvested and the cells washed with distilled water. Cell extracts were made by grinding the algae in distilled water with a pestle and blender. An algal suspension containing 5.0 g fresh algal material in 500 mL of distilled water is referred to as a 1% extract.

- Germination of seeds

Air-dried seeds of squash, tomato and cucumber plants were soaked in algal extracts for 24h. Seeds, without algal extract, served as control. Percentage of germination was estimated by spreading 30 seeds on filter papers placed in glass Petri-dishes containing 5.0 mL of a cell extract. Petri dishes containing seeds with 5.0 mL of distilled water served as a control (Nanda *et al.* 1991). Four replications were made for each treatment. The Petri-dishes were placed at natural illumination at 25° C.

- Pot culture

Five healthy seedlings from treated and untreated samples were then grown in 1 liter pots for 40 days. No fertilizer was applied, but soil of treated seedlings was sprayed with 200 mL of algal extract every seven day.

- Statistical analysis

Statistical analysis was performed with one way ANOVA, using SPSS software (Package for the Social Sciences, SPSS Inc., Chicago IL) version 15. Means were separated using the Least Significant Difference (LSD) test at $P < 0.05$.

Results

In the present study, seven taxa of heterocystous and 12 taxa of non-heterocystous cyanophyta were identified. *Nostocaceae* with four genera and seven species, *Oscillatoriaceae* with three genera and six species and *Chroococcaceae* with four genera and six species were

included in the list of isolates (Table 1). The list of these taxa is as follows:

Nostocaceae

Anabaena vaginicola F.E. Fritsch & Rich
Cylindrospermum michailovskoense Elenkin
Nostoc punctiforme (Kützing) Hariot
Nostoc muscorum C. Agardh ex Bornet & Flahault
Nostoc calcicola Brébisson ex Bornet & Flahault
Nostoc sp.
Nodularia harveyana (Thwaites) Thuret

Oscillatoriaceae

Oscillatoria angustissima W. West & G.S. West
 * *Oscillatoria chilensis* Biswas
Phormidium terebriforme (C. Agardh ex Gomont) Anagnostidis & Komárek
 * *Phormidium granulatum* (Gardner) Anagnostidis
 * *Phormidium articulatum* (Gardner) Anagnostidis & Komárek
Lyngbya sp.

Chroococcaceae

* *Aphanothece gelatinosa* (Hennings) Lemmermann
 * *Chroococcus minutus* (Kützing) Nägeli
 * *Chroococcus minimus* (Keissler) Lemmermann
 * *Chroococcus pallidus* (Nägeli) Nägeli
Gleocapsa sp.
Gloeotheca sp.

* New records to Iran

Among these taxa, three species of heterocystous cyanobacteria, *Anabaena vaginicola*, *Nostoc* sp. and *Nodularia harveyana*, which were isolated from paddy field soils, used as a biofertilizer for different vegetables such as cucumber, squash and tomato. The germination of seeds soaked with cyanobacterial extract was faster as compared to seeds soaked in distilled water as control. For untreated seeds of squash and cucumber, germination began after three days, whereas germination of seeds treated with several cyanobacterial inoculum began earlier. Germination of untreated tomato seeds began after six days, whereas treated seeds germinated after four days. In treated seeds, however, seedlings height and roots length were recorded higher than control after 10 days (Table 2, Fig. 1).

In pot culture of squash plant, comparison of control and treatment plants with one way ANOVA showed that treatment groups have a significant difference in root length, plant height, leaf number and weight of fresh and dry root as well as fresh and dry weight of leaf and stem as compared with control (Table 3).

Table 1. Total percent abundance of cyanobacterial genera (summed up over all locations)

Genus	Total No. of species	Percent abundance
<i>Anabaena</i>	1	5.2
<i>Nostoc</i>	4	21
<i>Cylindrospermum</i>	1	5.2
<i>Nodularia</i>	1	5.2
<i>Oscillatoria</i>	2	10.6
<i>Lyngbya</i>	1	5.2
<i>Phormidium</i>	3	16
<i>Chroococcus</i>	3	16
<i>Aphanothece</i>	1	5.2
<i>Gloeothece</i>	1	5.2
<i>Gleocapsa</i>	1	5.2
Total	19	100

Table 2. The effect of cyanobacterial extracts on seedling height (values are means \pm SE)

Species	Control	<i>Anabaena</i>	<i>Nostoc</i>	<i>Nodularia</i>
Squash (<i>Cucurbita maxima</i>)	3 \pm 0.50	6 \pm 0.57*	6 \pm 0.00*	5.83 \pm 0.44*
Cucumber (<i>Cucumis sativus</i>)	5.46 \pm 1.23	10.93 \pm 0.06*	10.76 \pm 0.14*	10.86 \pm 0.06*
Tomato (<i>Solanum lycopersicum</i>)	11 \pm 0.50	14 \pm 0.57*	10 \pm 1.15	11.93 \pm 0.06

* Significant at the 0.05 level

Table 3. The effect of cyanobacterial extracts on squash plant (values are means \pm SE)

Growth parameters	Control	<i>Anabaena</i>	<i>Nostoc</i>	<i>Nodularia</i>
Root length (cm)	20.75 \pm 1.65	32.50 \pm 1.44*	32.25 \pm 1.31*	29.75 \pm 2.25*
Plant height (cm)	32.25 \pm 1.43	46.75 \pm 2.25*	48 \pm 2.12*	44 \pm 2.16*
Leaf number	8.75 \pm 0.75	12 \pm 0.40*	11.25 \pm 0.47*	10.25 \pm 0.47
Weight of fresh root (g)	3.85 \pm 0.38	9.30 \pm 0.56*	6.95 \pm 0.85*	4.55 \pm 0.24
Weight of dry root (g)	1.77 \pm 0.10	5.96 \pm 0.57*	3.15 \pm 0.43*	2.15 \pm 0.08
Weight of fresh stem and leaf (g)	4.17 \pm 0.29	7.85 \pm 1.39*	6.17 \pm 0.26	4.23 \pm 0.46
Weight of dry stem and leaf (g)	0.39 \pm 0.03	0.76 \pm 0.08*	0.63 \pm 0.02*	0.46 \pm 0.05

* Significant at the 0.05 level

Table 4. The effect of cyanobacterial extracts on cucumber plant (values are means \pm SE)

Growth parameter	Control	<i>Anabaena</i>	<i>Nostoc</i>	<i>Nodularia</i>
Root length (cm)	16.50 \pm 2.53	34.25 \pm 3.37*	34.50 \pm 2.62*	31.25 \pm 1.25*
Plant height (cm)	25.25 \pm 2.83	46 \pm 3.24*	45.50 \pm 3.12*	41.25 \pm 1.62*
Leaf number	5.75 \pm 0.25	6 \pm 0.40	6.25 \pm 0.75	5.75 \pm 0.47
Weight of fresh root (g)	0.23 \pm 0.01	0.95 \pm 0.09*	0.99 \pm 0.11*	0.49 \pm 0.05
Weight of dry root (g)	0.07 \pm 0.01	0.43 \pm 0.02*	0.30 \pm 0.01*	0.20 \pm 0.04*
Weight of fresh stem and leaf (g)	0.65 \pm 0.05	1.04 \pm 0.01*	0.86 \pm 0.04	0.7 \pm 0.04
Weight of dry stem and leaf (g)	0.07 \pm 0.00	0.11 \pm 0.00	0.27 \pm 0.14	0.09 \pm 0.00

* Significant at the 0.05 level

Table 5. The effect of cyanobacterial extracts on tomato plant (values are means \pm SE)

Growth parameter	Control	<i>Anabaena</i>	<i>Nostoc</i>	<i>Nodularia</i>
Root length (cm)	11 \pm 1.08	18.75 \pm 0.47*	17 \pm 0.57*	18 \pm 1.47*
Plant height (cm)	16 \pm 1.47	25.75 \pm 0.85*	24 \pm 0.91*	24.50 \pm 0.86*
Leaf number	5.50 \pm 0.28	6.25 \pm 0.25	5.50 \pm 0.28	6.25 \pm 0.25
Weight of fresh root (g)	0.05 \pm 0.00	0.24 \pm 0.11*	0.16 \pm 0.00	0.16 \pm 0.04
Weight of dry root (g)	0.02 \pm 0.00	0.07 \pm 0.00*	0.07 \pm 0.00*	0.07 \pm 0.01*
Weight of fresh stem and leaf (g)	0.54 \pm 0.08	2.27 \pm 0.26*	1.60 \pm 0.25*	1.65 \pm 0.05*
Weight of dry stem and leaf (g)	0.05 \pm 0.00	0.21 \pm 0.00*	0.14 \pm 0.01*	0.16 \pm 0.00*

* Significant at the 0.05 level

In pot culture of cucumber plant, comparison of control and treatments with statistic analysis showed that there is a significant difference between control and treatment groups in root length, plant height, weight of fresh and dry root and fresh weight of leaf and stem but no significant difference was observed in leaf number and weight of dry leaf and stem (Table 4). Also in pot culture of tomato plant, comparison of control and treatments with one way ANOVA showed that there is a significant difference between control and treatment groups in root length, plant height, dry weight of root as well as fresh and dry weight of leaf and stem (Table 5).

In other words the results revealed that there was a significant difference in most measurement factors in different plants treated with cyanobacterial inoculum's as compared to controls. However, effect of algal culture is not the same for all parts of plants and in different plants. In addition, effect of different algal inoculum was not the same in different plants. For example among these cyanobacteria genera, *Anabaena* showed more positive effect on most vegetative characters of tomato plant and some characters of other studied plants, whereas *Nodularia* showed less positive effect on vegetative characters of studied plants. Also among several studied

vegetative characters, leaf number showed the least difference in treatments as compared to controls and root length and weight showed the most difference in treatments. Root as an absorptive organ and producer of several substances such as phytohormones is an important part of plants (Hamidi *et al.* 2010). A positive effect of PGPR (Plant growth-promoting rhizobacteria) on root growth parameters such as root length, total surface of root, dry weight of root and rootlet density was reported by Pan *et al.* (1999). Zahir *et al.* (2000) showed that PGPR increased length and dry weight of maize root. The results of present study also showed that growth parameters of root such as root length, dry and fresh weight of root increased significantly in treated plants. Increase in dry weight of root in treated plants represent that the root growth was increased and as a result water and nutrition uptake to gain strength. Improvement of water and nutritional elements uptake from soil can improve total plant growth.

Discussion

The review of literatures showed that, there are only a few studies on similar subjects, especially on vegetable crops; nevertheless results of other studies on other plants confirm the results of this study. The results obtained in the first part of this work showed that pre-soaking seeds by algal extract accelerates seed germination and seedling height (Fig. 1). Previously, Nanda *et al.* (1991) showed that, pre-soaking of pumpkin and cucumber seeds in *Westiellopsis prolifera* extract can accelerate seed germination and spraying extracts of this cyanobacterium to emerged seedling during their subsequent cultivation led to significant increase in growth and development of both crops. They suggested that, the supply of nitrogenous nutrients to the seeds is important. Jacq & Roger (1977) also showed that in addition to N-contributions, pre-soaking of rice seeds in BGA culture has decreased losses from sulphate

reducing processes and this has been attributed to the enhancement of germination and faster seedling growth due to algal exudates. The second part of this research revealed that algal extract can enhance plant growth (Fig. 2).

Statistical analysis confirm that there is a significant difference in plant height, root length, number of leaf, fresh and dry weight of root, leaf and stem in treated plants as compared to control. Venkataraman & Neelakantan (1967) showed that the production of growth substances and vitamins by the algae may be partly responsible for the greater plant growth and yield. The capacity for biosynthesis of growth promoting substances such as auxins, amino acids, sugars and vitamins (Vitamin B₁₂, Folic acid, Nicotinic acid and Pantothenic acid) also can enhance plant growth. The other reason that can suggest for increased plant growth by using cyanobacterial extract is that, the growth of BGA in soil seems to influence the physical and chemical properties of soil. The water stable aggregate significantly increase as a result of algal growth and thereby improves the physical environment of the plants. Results of this study showed that these heterocystous cyanobacteria (*Anabaena vaginicola*, *Nostoc* sp. and *Nodularia harveyana*) have ability to promote vegetable growth and they are appropriate candidate for the formulation of a biofertilizer. Study also showed not all heterocystous cyanobacteria can be used as a fertilizer. For example, some species of the genus *Nodularia* may have a negative effect on plant growth since they produce toxins such as nodularin. This genus needed more study for application as a biofertilizer.

Acknowledgments

The authors gratefully acknowledge the financial support of University of Shahid Behashti (Tehran, Iran). Thanks are also due to Dr. Shokri, Ms. Saadatnia, and Ms. Bagheri for their assistance during the research.

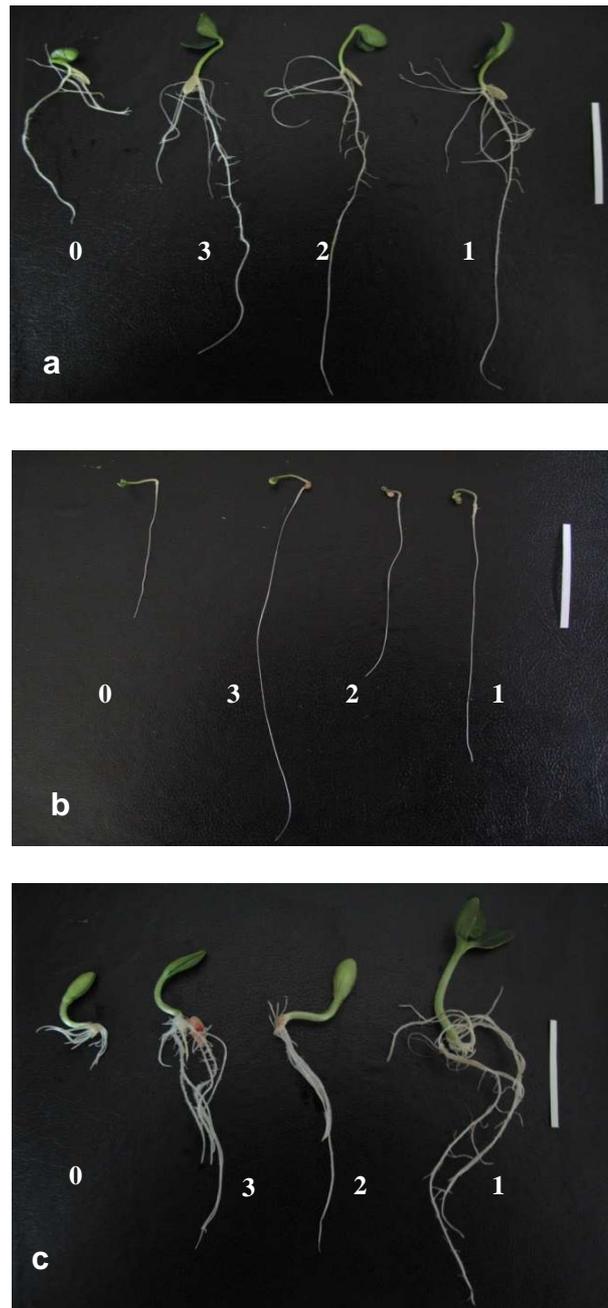


Fig. 1. Seedling height of control and treated plants: a. Cucumber seedling, b. Tomato seedling, c. Squash seedling (0. Control, 1. Plant treated by *Anabaena*, 2. Plant treated by *Nostoc*, 3. Plant treated by *Nodularia* (Bar = 5 cm).



Fig. 2. Plant height and root length of control and treated plants: a–c. Cucumber seedling, d–f. Squash seedling, g–i. Tomato seedling (0. Control, 1. Plant treated by *Anabaena*, 2. Plant treated by *Nostoc*, 3. Plant treated by *Nodularia* (Bar = 5 cm).

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