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PROMOTING EFFICIENCY OF CYANOBACTERIA FILTRATE ON SEED GERMINATION AND PLANT GROWTH OF WHEAT, SOYBEAN AND CLOVER

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ABSTRACT

The effect of cyanobacteria strains filtrates of *Nostoc catcicola* and *Anabaena flos aquae* on the seed germination and / or plant growth of some wheat, soybean and clover crop cultivars were investigated. Soaking the seeds for these crops in the filtrates of both cyanobacteria strains increased the germination percentage over either the control treatment (water) or Watanabe medium. The increase in germination reached 97.49% over the control treatment. Also, the seedling of these crops when treated with the cyanobacteria filtrate seemed to be healthy improved in growth than those treated with water or Watanabe medium only. However, clover seedling treated with *Nostoc* filtrate achieved the highest shoot length increase percentage (100) as compared with other treatments for all tested seedlings crops. Consequently, soybean seedlings tested with *Nostoc* filtrate had recorded 96.67% increase in root length in comparison with all treatments. Generally, both *Nostoc catcicola* and *Anabaena flos aquae* strains had positively stimulated and improved germination and/or plant growth for all tested crops than those of water or Watanabe medium.

INTRODUCTION

Cyanobacteria are abundant in rice fields where their enhancement of soil fertility by means of biological nitrogen fixation has often been studied (Roger and Kulasooriya, 1980), but their beneficial effect is not limited to that. Bently (1958) in studying growth regulator production by phytoplankton showed that some strains of *Anabaena* and *Oscillatoria* exuded auxins-like substances.

Cyanobacterial plant growth-regulator effects have been attributed to production of antibiotics and toxins seeds of, organic acids (Hellebust, 1974), vitamins B (Grieco and Desrochcr, 1978), gibberellic-like substances (Singh and Trehan, 1973) and cytokinin-like substances (Rodgers *et al.*, 1979).

Microalgae are a biochemically diverse assemblage of microorganisms amenable to fermentation and mass culture. Including the cyanobacteria and nearly a dozen eukaryotic classes, microalgae produce a wide array of compounds with biological activity. These include antibiotics, algicide toxins, pharmaceutically active compounds and plant growth regulators (Metting and Pyne, 1986).

Adam (1999) revealed a stimulation effect of the cyanobacterium *Nostoc muscorum* on seed germination of wheat, sorghum, maize and lentil. He also added that the germination of seeds of the tested crop plants either in live cyanobacteria inoculum algal filtrate (exogenous), or boiled algal extract (endogenous) was significantly increased.

Abd El-Aal (1999) showed that soaking 4, rice seeds cultivars viz Giza 171, 172, 178 and 181 in a crude filtrate of the N_2-fixing *cyanobacteria*

Anabaena fertilissima, Calothrix breyissima,, Nostoc muscorum and *Anabaena oryzae* had increased their germination percentage to 100 as compared to 60 for the seeds soaked in water only. Aref (2001) reported that soaking rice seeds cultivar Sakha 102 in *Nostoc sp.* live filtrate had stimulated the seed germination percent age to reach 90 as compared with water soaked seeds. 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.

Mohamed (2001) came to a conclusion that the treatment of rice seedlings with the cyanobacteria filtrates *of Anabaena oryzae, Nostoc calcicola, Microchaetetenera* and *Cylindrospermum muscicola* each alone had increased both shoots and roots lengths than those treated with water only.

This work is a trial to in investigate the effect of live cyanobacteria filtrates of two strains namely *Nostoc calcicola* and *Anabaena flos aquae* on the seeds of wheat, soybean, and clover crops.

MATERIALS AND METHODS

Pure culture of *Anabaena flos aquae* and/or *Nostoc calcicola* were kindly obtained from Agricultural Microbiology Department, Soils, Water and Environment Research Institute, Giza, Egypt. The cultures were then grown in Watanabe medium (1951) under continuous fluorescent white light. The light intensity was kept at 2000 Lux and temperature at 28 ± 2 °C. Seeds of wheat cultivars Giza 5, 68 and 69, soybean cultivars, Giza 22, Giza 82 and Crawford, and clover cultivar Seda were obtained from Agric. Res. Crop Inst., Agric. Res. Center Giza, Egypt.

The growing algae was filtered after desired intervals (log phase) to develop the experiment.

Seeds of wheat (20 seeds), soybean (15 seeds), and clover (25 seeds) were surface sterilized using solimani solution followed by several washing with distilled water. Selected seeds number from each crop was then distributed on water agarized Petri dishes (0.5% agar).

Cyanobacterial filtrate (10 ml) added to Petri dishes to obtain two sets for either *Anabaena flos aquae* or *Nostoc calcicola.* The plates were incubated for 48 hrs at 28 ± 2 °C in the dark. In the control Petri dishes cyanobacterial filtrates were substituted with water and/or Watanabe medium for comparison. Each treatment was repeated in triplicates and arranged in complete randomized design. The percentage of seed germination was calculated as follows:

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

Well developed 5 days old seedlings of all crops were then transferred to 500 ml capacity bottles containing 250 ml semiliquid water agar (1%) and exposed to same treatments executed in Petri-dishes germination test. Bottles were incubated in the growth chamber but under continuous light (5000 Lux) for 10 days. Developed seedlings for the tested crops were then measured for root and shoot lengths in centimeters.

RESULTS AND DISCUSSION

Germination percentage of wheat, soybean and clover seeds increased when soaked for 48 hrs in the filtrate of the cyanobacteria strains *Nostoc calcicola* and/or *Anabaena flos aquae* (Table 1), when compared with seeds soaked in either water or Watanabe media. Soaking the tested seeds in *Nostoc* filtrate gave better germination percentages than those soaked in *Anabaena* filtrate when compared with soaking in water the control treatment. The highest percentage increases over the control treatment had achieved by soybean cv. Crawford (97.49) followed by 93.33, 88.57, 83.33 and 48.33% for soybean cv. Giza 22, soybean Giza 82, clover cv. Seda and wheat cv. Giza 68, respectively. However, amongst wheat cultivars, seeds of Giza 68 gave the highest seed germination increase percentages over the control treatment (48.33) followed by cv. Giza 5 (47.69) and cv. Giza 69 (42.86) when the seed soaked all seeds in *Nostoc* .filtrate. Soybean seeds treated with *Nostoc* filtrate caused percentage germination to be arranged as 97.49 (cv. Crawford), 93.30 (cv. Giza 22) and 88.57 (cv. Giza 82). Soaking the seeds of the tested crops in *Anabaena* filtrate had achieved increases in the germination percentages over the control treatment. These increases were slightly lower than those obtained with soaking in *Nostoc* filtrate. However, soaking the tested seeds in Watanabe medium free from cyanobacteria had slightly increased the germination percentages over the control treatment (Tables 1&2).

Table (1): Seed percentage germination of wheat, soybean and clover as affected with cyanobacteria filtrates after 48 hrs.

Crop treatments	Germination %						
	Wheat			Soybean			Clover
	Giza 5	Giza 68	Giza 69	Giza 22	Giza 82	Crawford	Seda
Control (water	65.0	60.0	70.0	40.00	46.67	50.00	48.00
Media	68.0	65.0	80.0	53.00	73.33	70.00	56.00
Nostoc	96.0	89.0	100.0	77.33	88.00	93.33	88.00
Anabaena	92.0	77.0	93.0	60.67	78.67	85.33	80.00

The growth rate of the tested crop plants due to cyanobacterial culture filtrates are demonstrated in Tables (3&4). All seedlings treated with the cyanobacterial culture filtrates had increased in both shoots and roots lengths. *Nostoc* filtrate caused both shoots and roots seedlings lengths to be superior to those achieved by *Anabaena* filtrate when compared with the control treatments. However, the highest shoot or root lengths increase percentages due to cyanobacterial seedlings treatment were noticed for clover shoot (100) and soybean cv. Giza 22 root (96.67) when treated with *Nostoc* filtrate. The highest shoot lengths percentage increases achieved by using *Anabaena* filtrate were also with clover cv. Seda (73.68) followed by soybean cv. Crawford (60.87), soybean cv. Giza 22 (52.00), soybean cv. Giza 82(44.00) and wheat cv. Giza 68 (32.00). Same trend noticed with *Anabaena* filtrate with shoot lengths was observed with root lengths when compared with the control treatments.

Table (2): Effect of cyanobacteria filtrate (*Nostoc calcicola* and /or *Anabaena cquae*) on the germination of wheat, soybean and clover*

Crop	Wheat						Soybean						clover	
	Giza 5		Giza 68		Giza 69		Giza 22		Giza 82		Crawford		Seda	
Treatments	Mean No. of germination	%increase	Mean No. of germination	% increase	Mean No. of germination	% increase	Mean No. of germination	%increase	Mean No. of germination	%increase	Mean No. of germination	% increase	Mean No. of germination	% increase
ontrol (water)	13.0	-	12.00	-	14.0	-	6.0	-	7.0	-	7.8	-	12.0	-
Media	13.60	4.62	13.00	8.33	16.0	14.29	8.0	33.33	11.0	57.14	10.5	34.62	14.0	16.67
Nostoc	19.20	47.69	17.80	48.33	20.0	42.86	11.6	93.30	13.2	88.57	14.0	97.49	22.0	83.33
Anabaena	18.40	41.54	15.40	28.33	18.6	32.86	9.1	51.67	11.8	68.57	12.8	64.10	20.0	66.67

* Seed were soaked for 48 hrs in cyanobacterial filtrates of *Nostoc calcicola* and /or *Anabaena flos aquae.*

Table (3): Effect of cyanobacteria filtrate (*Nostoc calcicola* and /or *Anabaena flos aquae*) on shoot lengths in cm of wheat, soybean and clover*

Crop	Wheat						Soybean						clover	
	Giza 5		Giza 68		Giza 69		Giza 22		Giza 82		Crawford		Seda	
Treatments	Mean No. of germination	%increase	Mean No. of germination	% increase	Mean No. of germination	% increase	Mean No. of germination	%increase	Mean No. of germination	%increase	Mean No. of germination	% increase	Mean No. of germination	% increase
Control (water)	16.00	-	15.00	-	14.10	-	10.0	-	20.00	-	9.2	-	1.9	
Media	17.00	6.25	16.00	6.67	15.70	11.35	12.70	27.0	24.20	21.0	11.5	25.00	2.6	36.84
Nostoc	21.20	32.50	20.40	36.00	19.20	36.17	17.90	79.0	30.10	50.5	16.8	82.61	3.8	100.0
Anabaena	19.50	21.88	19.80	32.00	18.10	28.37	15.20	52.0	28.80	44.0	14.8	60.87	3.3	73.68

* Seed were soaked for 48 hrs in cyanobacterial filtrates of *Nostoc calcicola* and or /*Anabaena flos aquae.*

Table (4): Effect of cyanobacteria filtrate (*Nostoc calcicola* and /or *Anabaena cquae*) on root lengths in cm of wheat, soybean and clover*

Crop	Wheat						Soybean						Clover	
	Giza 5		Giza 68		Giza 69		Giza 22		Giza 82		Crawford		Seda	
Treatments	Mean No. of germination	%increase	Mean No. of germination	% increase	Mean No. of germination	% increase	Mean No. of germination	%increase	Mean No. of germination	%increase	Mean No. of germination	% increase	Mean No. of germination	% increase
Control (water)	11.7	-	9.7	-	8.6	-	3.0	-	9.0	-	10.7	-	4.30	-
Media	13.6	16.24	12.5	28.87	12.1	40.70	3.8	26.67	12.0	33.33	12.0	12.150	6.00	39.53
Nostoc	15.6	33.33	14.8	52.58	15.0	74.72	5.9	96.67	16.0	77.78	16.2	51.40	7.90	83.72
Anabaena	15.0	28.21	13.6	40.21	14.1	63.95	5.5	83.33	15.1	67.77	15.8	47.66	6.90	60.47

* Seed were soaked for 48 hrs in cyanobacterial filtrates of *Nostoc calcicola* and or */Anabaena flos aquae.*

Seedlings treated with *Nostoc* filtrate gave the highest root length increase percentages over the control treatments as 96.67 for for soybean cv. Giza 22 followed by 83.72, 77.78, 74.72 and 52.58 for clover cv. Seda, soybean cv. Giza 82, wheat cv. Giza 69 and wheat cv. Giza 68, respectively. While, the corresponding highest increase percentages for the seedling treated with *Anabaena* were in the order of 83.33 (soybean cv. Giza 22), 63.95 (wheat cv. Giza 69), 60.47 (clover cv. Seda), 47.66 (soybean cv, Crawford) and 40.21 (wheat cv. Giza 68).

Generally, this work may lead to a conclusion that it could be useful to pre-soak the crop seeds in the cyanobacteria filtrate before sowing. This process may help in decreasing the period of seed dormancy.

In the present work, cyanobacterial culture filtrates have stimulated both root and shoot growth for the tested crops besides increasing seed germination percentages as compared with both the control or Watanabe medium treatments.

This might possibly due to the production of growth promoting substances by the microorganisms. Koptiyeva and Tantisur (1971) found that water soluble products from 8 *Calothrix spp., Anabaena* sp. and *Stratonostoc* sp. had a rhizogenous effect and stimulated rice growth. Pre-soaking of rice seedlings in extract of *Phormidium* had been shown to accelerate seed germination (Gupta and Lata, 1964), 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 filtrates of *Aulosira fertilissima* resembled seedlings treated with gibbrellic acid (Singh and Trehan, 1973). Soaking cotton seeds in water extracts of 2 *Chlorella vulgaris* strains for 24 hrs increased percentage germination and seedling growth (Bil'-mes and Shotok, 1988).

Soaking seeds of spring wheat in 1% solution of an extract of growth substances extracted from blue-green alga *Phormidium foveslarum* had accelerated germination(18% increase) and increased the number of seedling by 9-17,8% (Wang *etal.,* 1991).

The cotton seeds pre-soaked in 10, 15 and 20% *Phormidium* extracts alga prepared in water, acetone or boiled water was increased in

germination, total length of seedling and length of radical after 10 days incubation (Likhikar and Tarar, 1995).

Rice seeds germination, seedling elongation and catalase activity in the endosperm and embryo were generally increased due to pre-soaking of rice seeds in algal extract for 24 hrs (Mehta *et al.,* 1999).

Adam (1999) reported that when seeds of wheat, sorghum, maize and lentil exposed to either live *Nostoc muscorum* algal extract or killed algal extract, germination was significantly increased, as were growth parameters and content of nitrogenous compounds compared with control. These increases could be attributed to the nitrogenase as well as nitrate reductase activities of the alga associated with the surface of plants; or the amino acids and peptides produced in the algal filtrate and/or other compounds that stimulate growth of crop plants. Mohamed (2001) showed that soaking rice seeds in cyanobacterial filtrates of *Anabaena oryzae, Nostoc calcicola, Microchaete lenera* and *Cylindrospermum muscicola* each alone had increased germination percentage and both shoots and roots lengths than those treated with water only. He owed this to the growth promoting like-substances secreted by the cyanobacteria strains in their filtrate. Pathak and Jaha (1995) noted that cyanobacteria inoculation increased significantly the percentages of seeds of wheat, maize, lady's finger and mustard. Abd EL-Aal (2006) indicted that the cyanobacterial filtrate extract of *A. oryzae, Nostoc Cacicola, microchate tenera and Cylindrospermum* stimulate the growth of rice seed and increased the lengths of both shoot and roots. They explained that cyanobacteria released into medium growth regulators, amino acids and polysaccharides, which in turn accelerate the growth of rice.

However, the production by cyanobacteria of substances that have growth promoting effect on rice plants is well established, but whether these substances are hormones, vitamins, amino acids or any others, as well as their mode of action is still not clear. Despite, the conclusion that could be derived from this work due how much it is beneficial to utilize the cyanobacterial extracts to accelerate breaking the seeds dormancy and to improve the plants growth, much work is still needed to explore what kind of these substances are responsible in the crop seed germination and/or plant growth improvement.

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التأثير المشجع لراشح للسيانوبكترياعلى انبات ونمو بذور الفمح و فول الصويا والبرسيم

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لقد درس تأثير راشح سلالتين من السيانو بكتيريا هما *Nostoc calcicola* و*Anabaena flos aquae*على انبات بذور اصناف بعض المحاصيل(صنف جيزة وصنف جيزة ٦٨ وصنف جيزة٦٩) وفول الصويا (صنف جيزة ٢٢ و صنف جيزة ٨٢ وصنف Crawford) والبرسيم صنف Seda . حيث نقعت هذه البذور لمدة ٤٨ ساعة فى راشح هذه الطحالب فى الظلام وكذلك عوملت بادرات هذه المحاصيل (عمر خمس أيام) براشح هذه الطحالب وحضنت لمدة عشر ايام تحت الاضاءة المستمرة وقورنت النتائج فى الحالتين بالبذور أو البادرات المعاملة بأى من الماء أو بيئة تنمية الطحالب watmanbae . وأوضحت النتائج أن معاملة البذور براشح الطحالب أدى الى زيادة نمو البادرات لأى من المحاصيل تحت التجربة سواء فى طول السيقان أو الجذور. وقد كان تاثير راشح طحلب*Nostoc* أفضل من تأثير راشح طحلب ال *Anaena*سواء على الانبات أو على نمو البادرات حيث كانت الزيادات المسجلة لل *Nostoc* اعلى منها لل*Anabaena*