Role of blue-green algae in managing damping-off and charcoal rot diseases of bean

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Abstract-- Bean (*Phaseolus vulgaris* L.) is a very important legume crop in Egypt for local consumption and exportation. Many soil-borne pathogenic fungi can infect bean roots; from those *Macrophomina phaseolina* (Tassi) Goid ; the causal of damping-off and root-rot (charcoal-rot).

Ninety nine fungal isolates representing six fungal species were isolated from bean diseased roots and stems showing the typical symptoms of damping-off, root- rot, wilt and stem- rot diseases. The isolated fungi were purified and identified as *Aspergillus niger, Fusarium oxysporum, F.solani , Macrophomina phaseolina* , *Pythium debaryanum* and *Rhizoctonia solani.* All the isolated fungi, except *A. niger* were pathogenic and caused different degrees of damping-off when used to test their pathogenic potentialities using Nebraska bean cv. Data indicated clear variations in respect to the ability of the tested fungi to attack bean. Severe symptoms were appeared on plants grown in soil infested with each of *R. solani, F.solani, M. phaseolina*.

In this study, blue-green algae were used as bioagents against the pathogenic fungi. Culture filtrate of the 3 blue green algal species, *i.e. Spirulina platensis*, *Nostoc muscorum*, *Anabaena flos-aquae* was used to test its effect on *M. phaseolina*; the causal of charcoal - rot *in vitro* and in the greenhouse. It was observed that the tested concentrations of the three cynaobacterial filtrates resulted in significant inhibitory effect to the fungal growth of *M. phaseolina* . The reduction in the linear growth was increased gradually by increasing the concentration of the filtrate. In addition, the tested cyanobacteria significantly reduced the infection by *M. phaseolina* and improved crop parameters of bean plants .

Key words---Bean, blue green algae , *Macrophomina phaseolina* , bioagent , damping-off , root-rot and management .

1 INTRODUCTION

Bean is grown commercially in Egypt for producing green pods or dry seeds, it has a great nutritional value especially the dry seeds which contain high protein content and this makes a broad sector in Egypt depend on it as a cheap protein source. (Davidson, 1975).

Bean plants are liable to infection by many diseases within the growing season which decreases the total production; charcoal rot caused by *Macrophomina phaseolina* is one of these diseases which decreases the yield, has a host range more than 500 plant species (Purkayastha *et al.*, 2006); inciting a stem canker disease in many crops that is often referred to as charcoal rot, due to the charcoal type coloration imparted to the colonized plant tissues. *M. phaseolina* is primarily soil borne in nature, with heterogeneous host specificity, that is, the ability to infect monocots as well as dicots and non-uniform distribution in the soil . Controlling this fungus in the soil is very difficult because of the high formation rate of sclerotia which help the fungus to survive in the hard living conditions.

In this study, we tried to evaluate blue- green algae filtrate in controlling *M. phaseolina*.

Cyanobacteria or blue–green algae are photoautotrophic microorganisms largely distributed in nature. Some of them have been used as human food for many years because of their high protein content (35–65%) and nutritional value. *Arthrospira (Spirulina*) is the best known genus and it was consumed by the Aztecs in Mexico Valley and by the Chaad lake population in Africa. At present, some countries are culturing it on a large scale (Steven and Russell, 1993). Cyanobacteria also, are considered chief biological agents that have been studied for the control of plant pathogens, particularly soil borne fungi (Papavizas and Lumsden, 1980; Abdel-Kader, 1997 and Hewedy *et al.*, 2000). This is mainly due to producing various biologically active compounds, those could operate in biological control of plant pathogens (Kulik, 1995 and Schlegel *et al.*, 1998). These biologically active compounds include antibiotics and toxins (de Caire *et al.*, 1987, 1990; Bonjouklian *et al.*, 1991; Carmichael, 1992; Frankmolle *et al.*, 1992a, b and Kiviranta *et al.*, 2006). De Caire *et al.* (1990) reported that extra-cellular products from *Nostoc muscorum* is promising as a biological control of soybean seedlings damping off. *Nostoc muscorum* has the ability to fix atmospheric nitrogen, this making them good candidates for environments with low nitrogen rates.

Also, *Anabaena flos-aquae* is filamentous cyanobacteria that is known for their nitrogen fixing abilities, and they form symbiotic relationships with certain plants, such as the mosquito fern.

Spirulina platensis is filamentous , undifferentiated, non- toxigenic cyanobacteria that has been used as food since ancient times. It possesses other biological functions such as antiviral, antibacterial, antifungal, and anti-parasite activities (Khan *et al.*, 2005).

This study was conducted with the objectives of determining the causative fungi of bean root rot and damping-off diseases. Evaluation the effect of filtrates of some cyanobacteria on the improvement resistance of bean plants to infection with *Macrophomina phaseolina* , in addition to their effect on some plant parameters.

2 MATERIALS AND METHODS

Isolation , purification and identification of the isolated fungi :

Bean materials affected with charcoal rot were frequently encountered in the Experimental Station , Fac. of Agic. ., Cairo Univ. Samples of naturally infected bean plants showing the typical symptoms of any of damping-off of seedlings , root rot, stem rot and wilt diseases were collected also from different fields located in Giza and Qalyubia governorates . Diseased plants were uprooted and placed in plastic bags and kept in a cool container during transportation. The plant samples were kept in a refrigerator at 5^0c for further studies.

Infected roots and stems were washed thoroughly in tap water and cut into small pieces (0.5-1.0cm), then surface sterilized with 2% Clorox solution for 2 minutes. Pieces were then washed several times with sterilized water and dried between folds of sterilized filter paper. Pieces were transferred onto the surface of potato dextrose agar (PDA) in Petri-dishes and incubated at 25^0c for 10 days. Observations were daily carried out and the emerged fungi were picked up and cultured on PDA slants. All the isolated fungi were purified using the single spore and/or the hyphal tip techniques described by *Dhingra and Sinclair* (1985). The purified fungi were identified according to their morphological features either to the generic or to the species level using the descriptions of *Snyder and Hansen* (1940); *Booth* and *Waterston* (1964); Gilman(1957); Booth (1971) and Barnett and Hunter (1972).

Stock cultures were maintained on PDA slants under paraffin oil in a refrigerator at $5\text{-}10^{0c}$ and were subcultured on fresh medium every 6-8 weeks .

Proper identification of *Macrophomina phaseolina* can be often problematic due to the presence of two asexual sub-phases. So , a histo-pathological study was carried out for the occurrence of pycnidia on bean infected tissue. Infected parts of bean stems cv. Nebraska were treated according to the following schedule : fixation was in formalin-acetic –alcohol solution (FAA), dehydration was in a series of ethyl alcohol and n-butyl alcohol and embedding was in paraffin wax as described by Johansen (1940) and Sass (1940), the samples were sectioned 15 µ thick by means of a rotary microtome and stained with crystal violet and erythrosin .

Preparation of blue green algal biomass :

Blue green algae (*Nostoc muscorum* and *Anabaena flos-aquae*) were grown on media described by Watanabe(1951) at the Lab. Of Phycol. , Dept. of Botany, Fac. of Sci., Cairo Univ.., Giza, Egypt , under continuous fluorescent white light whose intensity was kept at 200 LUX and tempretaure 28 ºC, while *Spirulina platensis* was grown on Zarrouk media (Zarrouk 1966) under temperature 30^0c and continous fluorescent light . After 21, days we started to separate the biomass from the cultural medium by centrifuging (40 min , 800 g , 10 c) , the supernatant was sterilized using 0.25 µm syringe filter.

Inhibitory effect of cyanobacteria filtrates :

This study was performed using Petri dishes containing PDA medium and stock with different concentrations from each of the cyanobacteria filtrates, plates containing PDA without cultural filtrates were used as check . The plates were incubated at 30 ^{0}C , the linear fungal growth was measured when the fungal growth completely covered any Petri dish of any treatment by measuring the mean of growth diameters (Cobb *et al.*, 1968).

Dry weight of the tested fungus :

M. phaseolina was grown in 100 ml conical flasks filled with 30 ml potato dextrose broth and each was inoculated with a disc taken from a 7-day-old PDA culture. Cultures were incubated at 30°C for 14 days. Fungal biomass dry weight was determined after filtration and drying at 70°C for 48 hrs.

Green house experiments:

Pathogenicity test :

This experiment was carried out to throw light on the pathogenic potentialities of the isolated fungi. The experiment was carried out in pots kept in the greenhouse of Dep. Of Plant Pathology, Fac.of Agric., Cairo Univ.The soil and pots (30cm in diameter) used in the greenhouse experiments were treated with formalin solution made up at the rate of 1 liter of concentrated solution (36-40% formaldehyde) to 50 liter of water. The soil was covered with plastic sheets for 7 days to retain the gas. The soil was not planted until the odour of formaldehyde had disappeared.

Inoculum preparation, growing medium and soil infestation technique :

Each of the tested fungi was allowed to grow in 500 ml milk bottles, each containing 75 gm washed dried barley, 100 gm washed dried coarse sand and 65 ml potato decoction (Attia,1966). After sterilization, a disc (5 mm in diameter) was taken from the margin of 7-days-old culture of the desired fungus and was manipulated to the autoclaved medium in the bottle. After the sufficient growth of the fungi was achieved, the inoculum of each fungus was mixed alone with the soil at the rate of 30 gm/kg soil one week before planting. In check experiments, equal amounts of the uninoculated substrate were added to the soil . Five seeds of bean cv. Nebraska were planted in each pot and each treatment consisted of 3 replicate pots , plants were irrigated as necessary. A randomized block design was followed, having fungi treatments and 3 replicates. Emergence counts were recorded 15 and 30 days after sowing and percentages of pre- and post-emergence damping-off were calculated. Meanwhile, the survived plants were also examined periodically and the number of dead plants due to infection by root rot were counted 60 days after sowing.

Effect of cyanobacteria cultural filtrate on the infection with *M. phaseolina* and some crop parameters :

This experiment was conducted under greenhouse conditions at the Plant Pathology Res. Institute, Agric. Res. Center.

M. phaseolina was grown on barley and sand medium for 15 days at 30°C. Pots (30 cm diameter) were sterilized by dipping in 5%formaline. Soil also was sterilized with 5%formaline , Soil infestation was carried out by adding the inoculum at the rate of 3% of soil weight, irrigated twice within 7 days before sowing to enhance fungal growth. Seeds of beans cv. Nebraska were soaked for 10 min. in the tested cultural filtrate concentrations of the three cyanobacteria, before planting, seeds were then sown in the pots at the rate of 5 seeds / pot, three pots were used as replicates for each

particular treatment. Percentages of pre- and post-emergence damping -off were recorded 15 and 30 days after sowing respectively. Survived plants were counted 60 days after sowing.

Determination of oxidative-reductive enzymes activity :

These analyses were carried out to shed light on the effect of cyanobacteria on the activity of peroxidase and polyphenoloxidase in the tissues of bean plants grown from bean seeds treated with cultural filtrates of the aforementioned cyanobacteria at (75%). Leaf samples representing the second true leaf of the desired treatment were collected to determine the activity of peroxidase and polyphenoloxidase according to the method described by (Lisker *et al* 1983).

Procedure started by cutting off 1 gm from leaves of treated replicates and then crushed them well in 2 ml sodium phosphate buffer 0.1 µ at pH 7.1. The homogenate was filtrated through Whatman No.1 filter paper. The suspension was centrifuged at 6000 rpm at 4oC for 20 min and stored at -18oC until use. One tenth extracted enzyme sample was added to 0.5 ml sodium phosphate buffer 0.1 µ at pH 7.1, 0.1ml H2O2 1% and 0.3 ml pyrogallol 0.05 µ. The mixture was completed to 3 ml using distilled water and color density was read in absorbance spectrophotometer Miltonroy spectronic 601 at 425 nm every 30 second for 10 reads (Kochba *et al.* 1977). Peroxidase activity was calculated as mg/gm fwt.

Polyphenoloxidase activity: Enzyme samples were extracted as mentioned before in peroxidase activity extraction. One tenth extracted sample was added to 0.5 ml sodium phosphate buffer 0.1 ml at pH 7and 0.5 ml catechol 0.001 N. The mixture was completed to 3 ml using distilled water and color density was read in spectrophotometer Miltonroy spectronic 601at 495 nm every 30 second for 10 reads (Lisker *et al.* 1983).

Disease assessment:

For damping-off of seedlings, the number of un-emerged seedlings (pre-emergence damping-off)as well as the number of dead seedlings (post-emergence damping-off) at 15 days and 30 days after planting, respectively were recorded and the percentages of damping-off were calculated. Plants showing root rot symptoms were recorded at 60 days after planting. The number of collapsed or wilted plants was recorded and percentage of disease incidence was calculated. Root rot severity was calculated according to Liu *et al.* (1995). Also, the survived plants were counted after 60 days from planting, uprooted and used for determining the values of some plant parameters, *i.e.* plant height, fresh and dry weight, number of leaves / plant, number of pods / plant.

Statistical analysis :

The data obtained were statistically analyzed using split design block suggested by Snedecor and Cochran (1967). Averages were compared at 0.05 level of probability using least significant difference (L.S.D) as mentioned by Fisher (1948).

3 Results

Isolation, purification and identification of the isolated fungi:

Ninety nine fungal isolates representing six fungal species were isolated from bean diseased roots showing the typical symptoms of damping-off, root rot, wilt and stem rot diseases. The isolated fungi were purified and identified according to their morphological characteristics. The obtained results (Table 1) indicate that *Rhizoctonia solani* Kuhn was the most dominant among those isolated from Giza and Qalyubia governorates showing 38.5% frequency, followed by *Fusarium solani* (Mart.) Sacc. and *Macrophomina phaseolina* (Tassi.) Goid , being 23.2 and 16.2% on the average, respectively. Other fungi, *i.e. Pythium debaryanum* R. Hesse, *Fusarium oxysporum* Schiecht. showed moderate frequency, being 10.1 and 8.1 %, respectively (Table ,1).

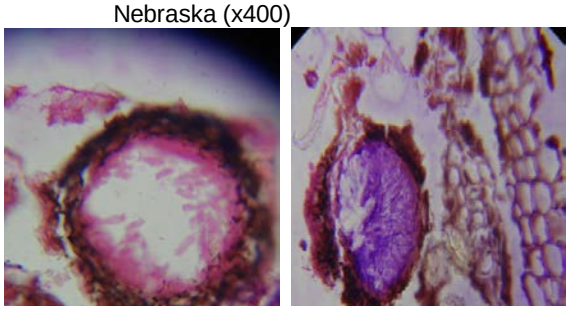
Table (1) .Occurrence and frequency (%) of the isolated fungi from diseased bean roots and stems collected from Giza and Qalyubia governorates.

Isolated Fungi	No. of isolates from Giza Gov.	No. of isolates from Qalyubia Gov.	Total No. of isolates	Frequency, %
Aspergillus niger (Tiegh.) Speg	1	3	4	4.0
Fusarium oxysporum Schiecht.	3	7	10	10.1
Fusarium solani (Mart.) Sacc.	13	10	23	23.2
Macrophomina phaseolina (Tassi.) Goid	7	9	16	16.2
Pythium debaryanum R. Hesse	3	5	8	8.1
Rhizoctonia solani Kuhn	17	21	38	38.4
Total	44	55	99	-----

Identification of *M. phaseolina:*

Proper identification of *M. phaseolina* was carried out at the Dep. of Plant Pathology, Fac.of Agric., Cairo Univ. Seeds of beans cv. Nebraska were planted in soil infested with the sclerotial state (*Sclerotium bataticola* Taub.) under greenhouse conditions. Infected bean hypocotyls were carefully examined for the presence of pycnidia .The morphology of pycnidia was studied in paraffin sections in bean hypocotyls bearing pycinidia as described under “Materials and Methods”. Pycnidia (Fig.1) are of dark colour, immersed at first but becoming more or less erumpent at maturity. They are globose with inconspicuous truncate ostiolum . No stroma was noticed, Conidia are hyaline, one celled, thin walled, variable in shape, elliptical or oval . Accordingly, it is suggested to name this pycnidial state as *Macrophomina phaseolina* (Tassi.) Goid synonym to *Sclerotium bataticola* Taub. When single spores were taken from the pycnidia and transferred to P.D.A. plates, they produced mycelium and sclerotia of *Sclerotium bataticola* Taub.

Figure 1, Pycnidia and pycnidiospores of *M.phaseolina* in a cross section in the hypocotyle of bean cv. Nebraska (x400)



Pathogenicity test :

All the isolated fungi Table (1) were used to test their pathogenic potentialities using Nebraska bean cv. as mentioned under Materials and Methods. Data presented in Table(2) indicate clear variations in respect to the ability of the tested fungi to attack bean. Severe symptoms were appeared on plants grown in soil infested with each of *R. solani, F.solani, M. phaseolina* .Each of these fungi was able to cause pre- and post-emergence damping-off of seedlings and severe root rot. *Fusarium oxysporum* caused the lowest percentage of pre- and post-emergence damping-off, in addition, it caused the typical symptoms of wilt. Meanwhile, *A. niger* was not able to infect bean seeds or bean plants.

Table(2) .Ability of fungi isolated from diseased bean plants to infect bean cv. Nebraska under greenhouse conditions.

Tested fungi	Damping-off (%)		Total	Root rot (%)	Wilt (%)	Survived plants (%)
	Pre-emerg,	Post-emerg.				
R. solani	26.67	13.33	40.0	33.33	0.00	26.67
F. solani	13.33	20.00	33.3	26.67	0.00	40.00
M. phaseolina	20.00	26.67	46.67	26.67	0.00	26.66
P. debaryanum	6.67	6.67	13.34	0.00	00.0	86.66
F. oxysporum	6.67	6.67	13.34	0.00	20.0	66.66
A. niger	0.00	0.00	0.00	0.00	0.0	100
Untreated Check	0.00	0.00	0.00	0.00	0.0	100

Effect of cyanobacterial filtrates on the linear growth of *M. phaseolina* :

It was observed that most of the concentrations of the three cynaobacterial filtrates resulted in significant inhibitory effect on the fungal growth of *M. phaseolina* . The reduction in linear growth was increased by increasing the concentration of the filtrate as shown in Figure (2) . The highest inhibition percentage was observed due to using the filtrate of *N. muscorum* at 75%conc., being 72.6 %, followed by *A. flos-aquae* at 75% conc. being 62.9 % respectively as shown in Table (3).

Table (3). Effect of three Cynobacteria cultural filtrates on the linear growth of *M.phaseolina* , 4 days after incubation at 30 ± 1ºC.

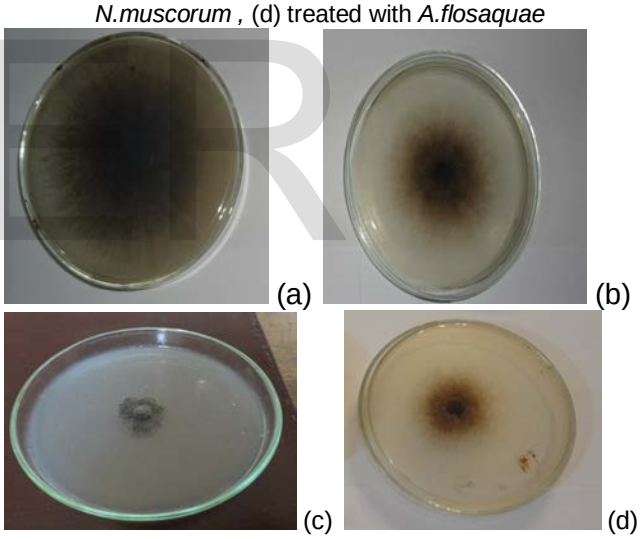
The tested Cyanobacteria	Average linear growth (mm) at conc. (%)			Mean
	25	50	75	
S.platensis	69.0	60.0	49.3	59.4
N.muscorum	39.3	30.3	24.7	31.4
A. flos-aquae	39.3	30.3	24.7	31.4
Control	90	90	90	90
Mean	59.5	52.7	47.2	----

L.S.D. at 5 % for : Cyanobacteria (CB) = 2.8
Conc. (C)= 3.0
CB x C = 4.2

Effect of cyanobacteria filtrates on the dry weight :

It was observed that most of the concentrations of the three cynaobacterial filtrate resulted in significant inhibitory effect on the fungal growth of *M. phaseolina* . The reduction in the growth was increased by increasing the concentration of the filtrate. It was observed that the highest reduction in the dry weight of *M. phaseolina* was obtained by using *N. muscorum* cultural filtrate at 75% conc., being 9.7 g as shown in Table(4) .

Figure(2) .Effect of cultural filtrates of three cyanobacteria on linear growth of *M.phaseolina.(a)-ve check* ,(b) treated with *S. platensis*, (c) treated with *N.muscorum* , (d) treated with *A.flosaquae*



Table(4) .Effect of three Cynobacteria cultural filtrates on dry weight of *M.phaseolina* , 15 days after incubation at 30 ± 1ºC.

The tested cyanobacteria	Average dry weight (mg) at conc. (%)			Mean
	25	50	75	
S.platensis	10.8	10.2	10.0	10.3
N.muscorum	9.8	9.6	9.6	9.7
A. flos-aquae	10.7	10.5	10.3	10.5
Control	14.2	14.2	14.2	14.2
Mean	11.4	11.1	11.0	-----

L.S.D. at 5 % for : Cyanobacteria (CB) = 2.0
Conc. (C = n.s.
CB x C = 3.1

Effect of seed treatment with cyanobacteria on disease incidence and root rot severity:

Data of planting the treated seeds with cyanobacterial filtrates in infested soil with *M. phaseolina* are presented in Table (5).

Table (5). Effect of three cyanobacteria on the incidence of damping- off and root-rot severity caused by *M.phaseolina* , greenhouse experiment.

The tested Cyanob-acteria	% Damping-off at conc.(%)			Mean	Root-rot severity at conc.			Mean
	25	50	75		25	50	75	
S. platensis	66.7	53.3	40.0	53.3	4.3	3.0	2.6	3.3
N. muscorum	60.0	40.0	33.3	44.4	5.0	4.0	1.6	3.5
A. flosaquae	60.0	33.3	26.7	40.0	1.3	1.0	1.0	1.1
*Control	90	90	90	90	5.0	5.0	5.0	5..0
Mean	69.2	54.2	47.5	---	3.9	3.3	2.6	-----

* Bean seeds planted in infested soil

L.S.D. at 5 % for :

Cyanobacteria (CB) =	2.9	1.4
Conc. (C) =	3.7	n.s.
CB x C =	4.0	1.5

It was observed that treated seeds with cyanobacteria filtrates decreased the incidence of damping-off and increase the number of survived plants , as presented in Table(5). Also treating seeds with the filtrates increased the values of crop parameters of bean plants, *i.e.* plant height , number of leaves , number of pods/ plant and fresh and dry weight / plant as shown in Tables(7 and 8) .

Activity of oxidative-reductive enzymes :

The results show that soaking bean seeds in the cultural filtrates of the three cyanobacteria increased the activity of the oxidative reductive enzymes where, the highest enzymatic activity was observed with *N.muscorum* with 0.56 mg/min as shown in Table (6) .

Table (6). Effect of cyanobaccerial filtrates on the activity of oxidative reductive enzymes.

Treatment	Peroxidase (mg/min)	Polyphenoloxidase (mg/min)
-ve check (untreated with *M. phaseolina*)	0.46	0.09
+ve check (treated with *M. phaseolina*)	0.16	0.039
S.platensis	0.35	0.11
N. muscorum	0.56	0.08
A. flos-aquaea	0.53	0.08

4 Discussion

It is obvious that there is a growing need to find new alternative methods to fungicides ,biological control using cyanobacteria is one of these promising alternatives. This study was carried out to investigate the possibility of minimizing the infection by charcoal rot and damping-off diseases of bean, The obtained results showed that the culture filtrate of the three cyanobacteria ; *N. muscorum* , S. *platensis*, *A. flos-aquae* exhibited inhibitory effect on the growth of *M. phaseolina* in P.D.A. Petri dishes. These data are in accordance with Carmichael, (1992) and Kulik, (1995) who proved that the cyanobacteria cultural filtrates contained a wide variety of biologically active compounds such as antibiotics and toxins

Also De Cano *et al.*(1990) found that phenolic compounds in extracts from cells of *N. muscorum* significantly inhibited the growth of *candida albicans* . Also, Frankmolle *et al* (1992a) reported that crude ethanolic extracts from *A. flos-aquaea* inhibited the growth of *Aspergillus orazae, Penicillium notatum, Saccharomyces cerevisiae*. These fungicidal compounds were isolated and purified and given the name laxaphycinsA, B, C, D and E and their structures were determined by Frankmolle *et al* (1992b).

Morsy, (2011) indicated that cyanobacterial filtrates reduced the infection of faba bean with fungi causing root rots and improved plant growth parameters.

Also , Abdel-Hafez *et al.*(2015) proved *in vitro* and under greenhouse conditions that extracellular metabolites of *N. muscorum* and *Oscillatoria* sp. reduced the linear growth of *Alternaria porri*, which causes onion purple blotch disease.

The obtained data proved that the filtrate of cyanobacteria was able to reduce pre- and post-emergence damping-off caused by *M. phaseolina* and increased the number of survived plants compared with the check under green house conditions. Also the results showed that the treated plants with culture filtrates of the three cyanobacteria exhibited tolerance reaction to infection with charcoal rot.

Table (7). Effect of three cyanobacteria on plant height , number of leaves and pods/ plant , greenhouse experiment

The tested Cyanobacteria	Plant height (cm) at conc.(%)			Mean	No. of leaves / plant at conc.(%)			Mean	No. of pods/ plant at conc.(%)			Mean
	25	50	75		25	50	75		25	50	75	
S. platensis	30.0	30.3	34.0	31.4	5.3	5.3	5.6	5.4	2.6	2.0	2.7	2.4

N.muscorum	17.3	28.7	30.0	25.3	3.7	5.7	5.7	5.0	0.6	1.7	1.7	1.3
A. flos-aquae	30.3	33.7	34.0	32.7	6.7	6.7	6.7	6.7	3.0	2.1	2.1	2.4
Control	24.3	24.3	24.3	24.3	4.0	4.0	4.0	4.0	1.0	1.0	1.0	1.0
Mean	25.5	29.3	30.6	------	4.9	5.4	5.5	------	1.8	1.7	1.8	----

L.S.D. at 5 % for :

Cyanobacteria (CB) = 3.2 1.3 0.9

Conc. (C) = 3.5 n.s. n.s.

CB x C = 4.2 1.9 1.3

Table (8). Effect of three Cyanobacteria on fresh and dry weight /plant of bean plants grown in soil infested with *M.phaseolina*, greenhouse experiment.

The tested cyanobacteria	Fresh weight (g) / plant at conc.(%)			Mean	Dry weight (g) / plant at conc.(%)			Mean
	25	50	75		25	50	75	
S. platensis	19.6	17.7	18.5	18.6	3.8	2.5	4.8	3.7
N.muscorum	3.7	9.2	11.6	8.2	0.9	2.5	2.5	1.9
A. flos-aquae	21.0	19.7	21.3	20.6	4.0	4.7	4.3	4.3
Control	8.0	8.0	8.0	8.0	2.1	2.1	2.1	2.1
Mean	8.4	13.5	14.9	------	2.7	3.0	4.4	------

L.S.D. at 5 % for :

Cyanobacteria (CB) = 2.8 1.6

Conc. (C) = 2.7 n.s.

CB x C = 3.2 1.5

5 References

[1] Abdel-Hafez S.I.I. ; Abo-El yousr, K.A. M. and Abdel-Rahim, I.R.(2015). Fungicidal activity of extracellular products of cyanobacteria against *Alternaria porri* , Euro. J. of Phyco., 50: 239-245.

[2] Abdel-Kader, M.M. (1997). Field application of *Trichoderma harzianum* as biocide for control bean root-rot disease. Egypt. J. Phytopathol., 25: 19-25.

[3] Attia, M.F. (1966). Pathological and Physiological Studies on *Sclerotium bataticola* Taub., The Incitent of Charcoal Rot Disease of Sweet potato in U.A.R. M. Sc. Thesis., Fac. of Agric., Cairo Univ. , pp.154

[4] Barnett, H.L. and Hunter, B.B.(1972). Illustrated Genera of Imperfect Fungi. Minnesota Burgess Pub. Co. pp. 241.

[5] Bonjouklian, R.; Smitka, T.A.; Doolin L.E.; Molloy R.M.; Debono, M.; Shaffer S.A.; Moore, R.E.; Stewart, J.B.; Patterson, G.M. and Tjipanazoles, L. (1991). New antifungal agents from the blue-green alga *Tolypothrix tjipanas-ensis*. Tetrahedron, 47:7739-7750.

[6] Booth, C. (1971) The genus *Fusarium*. Kew. Commonwealth Mycological Institute.

[7] Booth , C. and Waterson, J.M. (1964). Descriptions of Pathogenic Fungi and Bacteria. Commonwealth Mycological Institute, Ferry lane, Kew, Surrey, England.

[8] Carmichael, W.W.(1992). Cyanobacteria secondary metabolites- the cyanotoxines. J. Appl. Bact., 72:445-459

[9] Cobb, F. W. ; Krstic, Jr. M. ; Zavarin, E. and Barber, H. W. Jr.(1968). Inhibitory effects of volatile oleoresin components on *Fomes annosus* and four

Ceratocystis species. Phytopathology, 58: 1327:1335.

[10] Davidson, S. (1975). Human Nutrition Dietetics, 6th ed., London, Churchill Livingstone (c.f. Snap beans in the developing world, by Williams Jansen) CIAT Bean Program.

[11] De Caire, G.Z.; De Cano, M.S.; De Mule, M.C.Z.; De Halperin, D.R and Galvagno,M. (1987). Action of cell-free extract and extracellular production of *Nostoc muscorum* on growth of *Sclerotinia sclerotiorum* . Phyton., 47:43-46.

[12] De Caire, G.Z.; De Cano, M.S.; De Mule, M.C.Z. and De Halperin, D.R.

[13] (1990). Antimycotic products from the cyanobacterium *Nostoc muscorum* against *Rhizoctonia solani* . Phyton Buenos Aires, 51:1-4.

[14] De Cano, M.s.; de Mulé, M.C.Z.; de Caire, G.Z.and de Halperin,D.R. (1990). Inhibition of *Candida albicans* and *Staphylococcus aureus* by phenolic compounds from the terrestrial cyano-bacterium *Nostoc muscorum* . J. of Appl. Phyco., 2:79–81

[15] Dhingra, O.D. and Sinclair, J.B.(1985). Basic Plant Pathology, 2nd Ed. Lewis Pub. CRC Press, USA. 434p.

[16] Fisher, R.A. (1948). Statistical Methods For Research Workers. Oliver and Boyd. London.

[17] FrankmÖlle, W.P.; Larsen L.K.; Caplan F.R.; Patterson, G.M.I. ; Knuabal, G.; Levine, I.A. and Moore, R.E. (1992a). Antifungal cyclic peptides from the terrestrial blue-green algae *Anabaena laxa*. Isolations and biological properties . J. Antibiotics, 45: 1451-1457.

[18] FrankmÖlle, W.P.; Knubal, G., Moorc, R.I. and Patterson, G.M.I. (1992 b) . Antifungal cyclic peptides from the terrestrial blue-green alga *Anabaena laxa*. Structures of laxaphy-cins A.B. C. D. and E. J. Antibiotics, 45 : 1458:1466.

[19] Gillman, J.C. (1957). A Manual of Soil Fungi . The Iowa State College Press, Iowa, USA.450 pp.

[20] Hewedy, M.A.; Rahhal, M.M.H. and Ismail, I.A. (2000) . Pathological studies on soybean damping-off disease. Egypt. J. Applied Sci., 15: 88-102.

[21] Johanson, O.A.(1940). Plant Microtechn-ique. MC Graw-Hill Bock Co., Inc New York.

[22] Khan, Z.; Purk, S.D.; Shin, S.Y.; Boe,S.G.; Yeon,I.K. and Seo, J.J. (2005). Management of *Meloidogyne incognita* on tomato by root-dip treatment in culture filtrate of the blue-green algae, *Microcoleus vaginatus* . Bio-resource Technology, 96 : 1338-1341.

[23] Kiviranta, J.; Abdel-Hameed, A.; Sivonen, K.; Niemelä, S.I. and Carlberg, G. (2006). Toxicity of cyanobacteria to mosquito larvae-screening of active compounds. Envrion. Toxicol. Water Qual.8:63–71.

[24] Kochba, J.; Lavee, S.; Spiegel-Roy, P. (1977) . Differences in peroxidase activity and isoenzymes in embryogenic and non-embryogenic 'Shamouti' orange ovular callus lines. Plant Cell Physiol. ,18:463–467.

[25] Kulik, M.M. (1995). The potential for using cyanobacteria (blue- green algae) and algae in the biological control of plant pathogenic bacteria and fungi. Eur. J. Plant Pathol., 101(6) : 585-599.

[26] Lisker, N.; Cohen, L.; Chalutz, E. and Fuchs, Y. (1983). Fungal infections suppress ethylene-induced phenylalanine ammonia-lyase activity in Grapefruits. Physiol. Plant Pathol., 22, 331-338.

[27] Liu L.; Kloepper, J.W. and Tuzun, S., (1995). Introduction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. Phytopa-thology, 85: 695-698.

[28] Morsy, K.M.M.(2011). Biological control of damping-off, root rot and wilt diseases of faba bean by cyanobacteria (blue-green algal) culture filtrate. Egypt.J. Phytopathol., 39 (2):159-171.

[29] Papavizas, G.C. and Lumsden, R. D.(1980). Biological control of soil- borne fungal propagules. Ann. Rev. of Phyto-pathol., 18:389–413.

[30] Purkayastha, S.; Kaur, B.; Dilbaghi, N.and Chaudthury, A.(2006). Characterization of *Macrophomina phaseolina*, the charcoal rot pathogen of cluster bean, using conventional techniques and PCR -RAPD based molecular markers. Plant Pathol., 55:106-116.

[31] Sass, J.E. (1940). Elements of Botanical Microtechnique . Mc Graw –Hill Book co. , Inc. New York.

[32] Schlegel, I.; Doan, N.T.; De Chazol, N. and Smith,G.D. (1999). Antibiotic activity of new cyano-bacterial isolates from Australia and Asiaagainst green algae and cyanobacteria. J. Appl. Phycol., 10 : 471 - 479.

[33] Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods.8th Ed. , Iowa State Univ.Press , Ames, Iowa , U.S.A.
[34] Snyder, W.C. and Hansen, H.N. (1940). The species concept in *Fusarium* . Amer. J. Bot. 27 : 64-67
[35] Steven, C. and Russell, J. (1993). Bioactive natural products: detection, isolation and structural determination. CRC Press.
[36] Watanabe , A.(1951). Production in cultural solution of some amino acids by the atmospheric nitrogen-fixing blue-green algae . Arch. Biochem. Biophys., 43 : 50-55.
[37] Zarrouk. C. (1966). Contribution a l'etude d'une cyanobacterie: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (Setchell et Gardner) Geitler. Ph.D. Thesis, Univ. of Paris, France.