Egypt. J. Phytopathol., Vol. 39, No. 2, pp. 159-171 (2011)

Biological Control of Damping-Off, Root Rot and Wilt Diseases of Faba Bean by Cyanobacteria (Blue-Green Algal) Culture Filtrate

K.M.M. Morsy

Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.

The role of three strains of cyanobacterial (blue-green algal), *i.e. Nostoc muscorum, Spirulina platensis* and *Anabaena flos-aquae*, was evaluated as bioagents on controlling the infection of faba bean by *Rhizoctonia solani* and *Fusarium oxysporum*. The activity of indole acetic acid (IAA), protease enzyme and total phenols showed an increase in the filtrates of *Nostoc muscorum* and *Spirulina platensis* than the filtrate of *Anabaena flos-aqua*. Culture filtrate of the three cyanobacteria species belonging to three genera caused significant reduction to the mycelial growth of *R. solani* and *F. oxysporum*. Treating faba bean seeds with any of algal culture filtrates before sowing in artificially infested soil with any of *R. solani* and *F. oxysporum* (pot experiments) or in naturally infested soil (field experiments) resulted in significant reduction in both damping-off and dead plants (resulted from infection by root-rot and wilt diseases) compared with untreated seeds. Also, both *N. muscorum* and *S. platensis* were more efficient in reducing the infection by damping-off, root-rot and wilt at the high concentration (100%) than *A. flos-aquae*. On the other hand, treating faba bean seeds with any of the tested cyanobacteria filtrates significantly increased seed yield/plot compared with untreated seeds (control).

Keywords: *Anabaena flos-aquae*, biological control, cyanobacteria, faba bean, *Fusarium oxysporum*, *Nostoc muscorum*, *Rhizoctonia solani* and *Spirulina platensis*.

Faba bean (*Vicia faba* L.) is one of the most important legume crops in Egypt. It is grown mostly to fulfil food and feed requirements for human and animal consumption. Faba bean seeds are rich in protein and some other compounds (Morsy and Tarrad, 2005). Diseases are the major constrains to faba bean production in Egypt, as they cause enormous losses in the yield and its quality. The most important diseases are chocolate spot, damping-off, wilt, root rot, rust and viral diseases (Morsy, 1993 and Mazen, 2004). In this respect, El-Morsy *et al.* (1997), Mazen (2004) and El-Sayed (2006) isolated *Fusarium oxysporum* f.sp. *fabae*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani* and *Macrophomina phaseolina* from wilted and rotten roots of faba bean in different parts of Egypt and considered them the most important and wide spread fungal diseases observed at all locations.

Microalgal metabolites have attacked attention, because they are a resource for toxins and potential new drugs (Shimizu, 2003). Meanwhile, the nematicidal potential culture filtrates of the blue-green algae (cyanobacterium) *Microcoleus vaginatus* has been tested against *Meloidogyne incognita* on tomato in pots under

greenhouse conditions (Khan *et al.*, 2005). In addition, cyanobacterial strains belonging to the genera *Microcystis, Anabaena, Nostoc, Oscillatoria, Nodularia, Aphanizomenon* and *Cylindrospermum* are known to produce a number of cyclic peptide hepatotoxins and alkaloid neurotoxins exhibiting algicidal, fungicidal, pesticidal, cytotoxic immunosuppressive and enzyme–inhibiting activity, (Namikoshi and Rinehart, 1996 and Prasanna *et al.*, 2010). Certain free fatty acids produced by algae exert inhibitory effect on a variety of aquatic organisms (Mundt *et al.*, 2003). The inhibition is based on the fact that fatty acids primarily affect the plasma membranes (Wu *et al.*, 2006).

The aim of the present study was to evaluate the role of three biological control cyanobacterial strains, *i.e. Nostoc muscorum, Spirulina platensis* and *Anabaena flos-aquae* against faba bean damping-off, root–rot and wilt diseases caused by *Rhizoctonia solani* and *Fusarium oxysporum*.

Materials and Methods

Preparation of culture filtrate:

Pure algal cultures of the cyanobacteria *Anabaena flos-aquae, Nostoc muscorum* and *Spirulina platensis* were grown in a medium described by Watanabe (1951) at the Lab. of Phycol., Dept. of Botany, Fac. of Sci., Cairo Univ., Giza, Egypt, under continuous fluorescent white light. The light intensity was kept at 200 Lux and temperature 28±2°C, except for *Spirulina platensis*, which was grown on Zarrouk medium (Zarrouk, 1966) and temperature of 30±2°C. The cyanobacterial biomass was separated from the culture medium by centrifugation (40min, 8000g,10°C), under sterile condition. The supernatant containing extracellular products was sterilized by 0.22 µm Milipore membrane.

Effect of culture filtrate on the linear growth of the two pathogenic fungi:

The filtrate of the three tested cyanobacterial strains was taken under sterilized conditions and added to the autoclaved PDA medium just before pouring the medium to give 25, 50, 75 and 100% (v/v) algal filtrate concentrations then poured in sterilized Petri-dishes (9 cm. in diameter). Four Petri dishes were prepared for each concentration. The dishes were inoculated with 4mm discs taken from 7-day-old PDA fungal cultures.

Plates containing PDA medium without algal filtrates were used as control. The plate were incubated at 25±2°C for 5 days .The linear fungal growth of each of *R. solani* and *F. oxysporum* was measured when the pathogenic fungi completely covered control treatment by determining the mean of colony growth diameters (Cobb *et al* ., 1968).

Estimation the activity of indole acetic acid:

Colorimetric estimation of indole acetic acid (IAA) was carried out by gas liquid chromatography in the Principal Central Lab., Fac. of Agric., Cairo Univ. (Du and Xu, 2000).

Estimation the activity of protease enzyme:

Protease activity was determined by modified procedure (Tsuchidia *et al.*, 1986) using 2% casein in 0.2 M carbonate buffer (pH 10) as a substrate. Casein solution (0.5ml) with an equal volume of suitable diluted enzyme solution was incubated at 40°C. After 10 min the reaction was terminated by addition of 1 ml of 10% trichloroacetic acid. The mixture was centrifuged, supernatant was taken to 5 ml of 0.44 M Na_2CO_3 and 1 ml of two-fold diluted Folin–Ciocalteau reagent was added. After 30 min the colour developed was read at 660 nm against reagent blank prepared in the same manner. Tyrosine served as the reference standard. The optical density of these solutions was measured.

Estimation of phenolic compounds:

The total phenolic compounds was determined by Folin–Ciocalteau method (Meda *et al.*, 2005) in the Principal Central Lab., Fac. of Agric., Cairo Univ.

Greenhouse experiment:

Pathogenic isolates of *F. oxysporum* and *R. solani* were grown on barley sand medium for 15 days at 25±2°C. Clay pots (30 cm in diameter) were sterilized by dipping in 5% formalin for 5 min and then left in open air till dryness. Also, the soil was sterilized by 5% formalin. Soil infestation with each fungus was carried out at the rate of 3% of soil weight, then distributed into the pots, irrigated twice at 3 days intervals before sowing to enhance fungal growth. Faba bean seeds (cv. Giza 40) previously soaked for 12 hours in any of the tested concentrations of the three cyanobacterial filtrates were sown in the pots at the rate of 10 seed/pot. Untreated seeds (12 h soaking in water only), were sown as control (check) treatment. Three pots were used as replicates for ach particular treatment. Percentages of pre– and post–emergence damping–off were recorded 15 and 30 days after sowing, respectively. Survived plants were also counted 60 days after sowing.

Field experiment:

The experiments were carried out in a field naturally infested with the causal organisms of damping-off, root-rot and wilt diseases of faba bean located at the experimental farm of Ety El-Baroud, Agricultural Research Station (Behera governorate) during two successive growing seasons, *i.e.* 2008/2009 and 2009/2010. Faba bean seeds (cv. Giza 40) previously soaked for 12 hours in any of the tested concentrations of the three cyanobacterial filtrates or untreated ones, were used for sowing. Field plots contained 5 ridges occupying an area of 10.5 m^2 (3.5x3m). Two seeds / hill were sown with 25 cm apart between hills. Percentages of damping-off (the average of pre-, post-emergence) and dead plants (resulted from root-rot and/or wilt) were recorded 30 and 60 days after sowing. At the end of the growing season, seed yield was harvested and weighed as kg/plot and the average was recorded.

Statistical analysis:

Data obtained were statistically analyzed using complete randomized and split design blocks 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).

Results

Effect of cyanobacterial filtrates on mycelial growth of R. solani and F. oxysporum:

It is clear (Table 1) that all concentrations of the three cyanobacterial filtrates resulted in significant inhibitory effect to the fungal growth of the two tested fungi. The reduction in the linear growth was increased with the increasing in the concentration of the cyanobacterial filtrates. The filtrate of *N. muscorum* and *S. platensis* completely inhibited the mycelial growth of both fungi at concentration of 100% followed by *A. flos-aquae,* which recorded intermediate effect. At the concentration of 75%, *S. platensis* completely inhibited the linear growth of *F. oxysporum*, while in the case of *R. solani* the reduction reached to 95.0%. In addition, *N. muscorum* resulted in 89.4 and 82.8% inhibition for the growth of both fungi at the concentration of 75%, respectively.

Table 1. Effect of cyanobacterial filtrates on the linear growth of *R. solani* and *F. oxysporum*, 5 days after incubation at 25±2°C.

Tested cyanobacteria	Conc. (%)	*Rhizoctonia solani*		*Fusarium oxysporum*	
		Linear growth (mm)	Reduction (%)	Linear growth (mm)	Reduction (%)
Nostoc muscorum	0.00	90.00*	0.00	90.00	0.00
	25	55.5	38.6	49.3	45.2
	50	35.0	61.1	27.5	69.4
	75	15.5	82.8	9.5	89.4
	100	0.00	100	0.00	100
Spirulina platensis	0.00	90.00	00.0	90.00	0.00
	25	20.0	77.8	16.3	81.9
	50	13.3	85.2	5.7	93.7
	75	4.5	95.0	0.00	100
	100	0.00	100	0.00	100
Anabaena flos-aquae	0.00	90.00	0.00	90.00	0.00
	25	73.5	18.3	79.3	11.9
	50	61.3	31.9	67.7	24.8
	75	57.7	35.8	58.3	35.2
	100	44.3	50.8	50.5	43.8
L.S .D. at 5%		2.87	----	0.99	----

* Average of 4 replicates.

Pot experiment :

It is evident (Table 2) that the three cyanobacterial filtrates caused significant reduction to pre-and post-emergence damping-off caused by the two tested fungi compared to the control treatment. In this respect, the filtrate of *S. platensis* when used at 100% resulted the highest reduction to both pre-and post-emergence damping-off. The respective averages for pre-emergence damping-off were 6.7 and 0.0% and for post-emergence damping-off were 13.3 and 6.7%, for infection by

Table 2. Effect of soaking faba bean seeds (cv. Giza 40) in each of the three cyanobacterial filtrates on the incidence of pre-and post-emergence damping-off sown in infested soil with *R. solani* and *F. oxysporum* under greenhouse conditions

Tested cyanobacteria	Conc. (%)	*Rhizoctonia solani*			*Fusarium oxysporum*		
		Pre-emergence (%)	Post-emergence (%)	Plant survival (%)	Pre-emergence (%)	Post-emergence (%)	Plant survival (%)
Nostoc muscorum	25	20.0	20.0	60.0	20.0	16.7	63.3
	50	20.0	16.7	63.3	16.7	13.3	70.0
	75	16.7	13.3	70.0	16.7	10.0	73.3
	100	10.0	16.7	73.3	13.3	10.0	76.7
Spirulina platensis	25	13.3	20.0	66.7	16.7	10.0	73.3
	50	10.0	20.0	70.0	13.3	10.0	76.7
	75	10.0	16.7	73.3	13.3	6.7	80.0
	100	6.7	13.3	80.0	10.0	6.7	83.3
Anabaena flos-aquae	25	33.3	16.7	50.0	30.0	13.3	56.7
	50	33.3	10.0	56.7	26.7	13.3	60.0
	75	26.7	13.3	60.0	20.0	16.7	63.3
	100	26.7	10.0	63.3	20.0	13.3	66.7
Control	0.00	40.0	36.7	23.3	33.3	20.0	46.7
L. S. D at 5%	-	2.72	3.05	6.11	5.96	2.28	6.13

R. solani and *F. oxysporum*, respectively. Meanwhile, *N. muscorum* resulted in 10.0 and 13.3% for pre-emergence damping-off and 16.7 and 10.0% for post-emergence damping-off, respectively. On the other hand, the filtrate of *A. flos-aquae* was of low efficiency compared with *S. platensis* and *N. muscorum* in this regard.

Activity of indole acetic acid, protease enzyme and total phenols in cyanobacterial filtrates:

The production of indole acetic acid (IAA), protease enzyme and total phenols in the culture filtrates of *N. muscorum*, *S. platensis* and *A. flos-aquae* is shown in Table (3). Obtained data indicate that both *S. platensis* and *N. muscorum* were more effective than *A. flos-aquae* in increasing the activity of indole acetic acid, protease enzyme and total phenols.

Table 3. Activity of indole acetic acid (IAA), protease enzyme and total phenols in the culture filtrates of three cyanobacteria

Tested cyanobacteria	Protease (IU ml^{-1})	Total phenols (%)	IAA µg/g F. wt
Nostoc muscorum	0.217	0.145	1.370
Spirulina platensis	0.245	0.298	2.826
Anabaena flos-aquae	0.012	0.090	1.150

Field experiments :

Data in Table (4) show that both damping-off and dead plants were significantly reduced due to soaking the seeds in any of the tested cyanobacteria filtrates before sowing compared to control treatment (untreated seeds). In this regard, the filtrate of *A. flos-aquae* was the lowest efficient in reducing damping-off than filtrates of *S. platensis* and *N. muscorum* at the high concentration, being 16.4, 10.9 and 11.9%, on the average, respectively. On the other hand, the effect of the three filtrates on the incidence of dead plants was significantly differed, being 7.2, 4.6 and 4.9%, on the average, respectively. Control treatment recorded 27.4 and 14.9% for damping-off and dead plants percentage, on the average, respectively.

The reduction in both damping-off and dead plants (due infection by root-rots and wilt diseases) was significantly reflected on the produced seed yield. In this respect, seed previously soaked in *S. platensis* filtrate produced the highest seed yield than that treated with *N. muscorum* and *A. flos-aquae*, being 6.0, 5.3 and 4.8 kg/plot, on the average, respectively. Control treatment produced 4.1 Kg / plot.

Table 4. Effect of soaking faba bean seeds (cv. Giza 40) in each of the three cyanobacterial filtrates on damping-off, dead plants and seed yield during 2008/2009 and 2009/2010 growing seasons under field conditions at Ety El-Baroud Experimental Station

Tested cyanobacteria	Conc. (%)	Damping-off (%)*		Mean	Dead plants (%)**		Mean	Seed yield (kg/plot)		Mean
		2008 / 09	2009 / 10		2008 / 09	2009 /10		2008 / 09	2009 / 10	
Nostoc muscorum	75	12.8	13.5	13.2	5.3	6.8	6.1	5.3	4.9	5.1
	100	11.3	12.4	11.9	4.5	5.3	4.9	5.5	5.0	5.3
Spirulina platensis	75	10.2	12.8	11.5	4.5	5.3	4.9	5.7	5.2	5.5
	100	9.5	12.2	10.9	4.3	4.8	4.6	6.2	5.8	6.0
Anabaena flos-aquae	75	17.8	19.3	18.6	8.3	9.5	8.9	4.8	4.6	4.7
	100	15.5	17.3	16.4	6.5	7.8	7.2	4.9	4.7	4.8
Control	0.00	26.3	28.5	27.4	14.5	15.3	14.9	4.2	3.9	4.1
Mean	0.0	14.8	16.6	-	6.8	7.8	-	5.2	4.9	-
L.S.D at 5% for:										
Treatment (T)	=	0.67			0.22			0.11		
Concentration (C)	=	1.11			0.26			0.13		
Growing season (G)	=	0.56			0.86			0.23		
T × C	=	1.92			0.46			0.25		
T x G	=	n.s			n.s			n.s		
C x G	=	n.s			n.s			n.s		
T x C x G	=	n.s			n.s			n.s		

* included pre-, and post-emergence damping-off.

** included dead plants caused by root-rot and wilt diseases.

Discussion

Acquired resistance by using biotic-agents as biological control seems to be one of alternatives to substitute for, or at least to decrease the use of fungicides in plant disease control. Excessive and improper use of pesticides including fungicides presents a menace to the health of human, animal and environment (Guzzo *et al.*, 1993). The present study was planned mainly to investigate the possibility of minimizing the infection by damping-off, root rot and wilt diseases of faba bean using the filtrates of three cyanobacteria as a biological control agents. The obtained data revealed that the culture filtrate of any of the three cyanobacteria, *i.e. Nostoc muscorum, Spirulina platensis* and *Anabaena flos-aquae* contained a wide variety of compounds of biological activities such as antibiotics and toxins (Carmichael, 1992 and Kulik, 1995). However, the growth of the isolated fungi from roots of faba bean was seriously affected when subjected to the filtrates. The reduction in the growth of *Rhizoctonia solani* and *Fusarium oxysporum* by increasing the concentration of the three cyanobacterial filtrates was significant. The most effective filtrates were *S. platensis* and *N. muscorum*, which completely inhibited the growth of both fungi at concentration 100% (crude filtrate). Meanwhile, *A. flos-aquae* recorded intermediate effect. In addition, *S. platensis* filtrate completely inhibited the linear growth of *F. oxysporum* at concentration 75%. Similar results were reported by De Caire *et al.* (1987) and De Caire *et al.* (1990) who found that the growth of the plant pathogens, *S. sclerotiorum* and *R. solani*, was inhibited by cells extract or extra cellular products of *N. muscorum*. De Cano *et al.* (1990) found that phenolic compound in extracts from cells of *N. muscorum* significantly inhibited the growth of *Candida albicans* and *Staphylococcus aureus*. Also, Frankmölle *et al.* (1992a) reported that crude ethanolic extracts from *Anabaena laxa* inhibited the growth of *Aspergillus oryzae, Candida albicans, Penicillium notatum, Saccharomyces cerevisiae* and *Trichophyton mentagrophytes*. The fungicidal compounds were isolated and purified and given the name laxaphycins A, B, C, D and E and their structures were determined by Frankmölle *et al.* (1992b). In addition, Abo-Shady *et al.* (2007) reported that cyanobacteria filtrates strongly inhibited the pathogenic fungi isolated from leaves, stems and roots of faba bean. Moreover, mycelial growth of several pathogenic fungi such as *F. oxysporum, Penicillium expansum, Phytophthora cinnamomi, R. solani, S. sclerotiorum* and *Verticillium albo-atrum* was inhibited by the methanol extracts of cyanobacterium *Nostoc* strain ATCC 53789 (Biondi *et al.*, 2004).

Control of damping-off, root rot and wilt diseases of faba bean using cyanobacteria filtrates of *N. muscorum, S. platensis* and *A. flos-aquae* caused significant reduction to both damping-off, root-rot and wilt diseases, either in pot or field experiments, compared to control treatments.

The obtained data showed that the filtrate of cyanobacteria was able to reduce pre-and post-emergence damping-off mortality caused by *R. solani* and *F. oxysporum* and increased the survived plants compared with the control under greenhouse conditions. In addition, the efficiency of the three cyanobacterial filtrates was positively correlated with the activity of the protease enzyme, total phenols

compounds and indole acetic acid, where the treated seeds with the culture filtrate of the former two cyanobacteria were more efficient in decreasing the infection by damping-off and increasing survived plants compared with the third cyanobacterium. These results are in conformity with the obtained data by De Caire *et al.* (1990) who reported that extracellular products from *N. muscorum* are promising as a biological control of soybean seedlings damping-off. Kulik (1995) mentioned that filtrates or cell extracts from cyanobacteria were applied to seeds as protectants against damping-off fungi such as *Fusarium* sp., *Pythium* sp. and *R. solani*. Moore (1996) showed that *Nostoc* sp. (GSV 224) has a potent fungicidal activity and can be used in the treatment of plants and agricultural crops for disease control.

In general, it has been found that the filtrate of each of *N. muscorum* and *S. platensis* was more efficient in reducing disease incidence and increased the seed yield / plot in field experiments. Meanwhile, filtrate of *A. flos-aquae* was the lowest one. However, the potential activity of cyanobacteria to inhibit certain soil borne diseases could be attributed to produce a wide range of plant growth regulators such as abscisic acid, ethylene, jasmonic acid, auxin and cytokinin-like substances as well as the cytokinin isopentenyl adenine (Ördög and Pulz, 1996 and Strik *et al.*, 1999) and these substances can also influence fungal growth (Zulpa *et al.*, 2003). On other hand, some species of cyanobacteria can fix nitrogen and thus add significant amount of this element to the soil in temperate and tropical regions (Metting, 1981). Shtina (1991) reported the methods for stimulating the growth of cyanobacteria in agricultural soils include proper fertilization and irrigation regimes, and adding life cyanobacteria and their metabolites to soil (algalization). In addition, many cyanobacteria produce many varieties of secondary metabolites particular antibiotics and biotoxins (Carmichael, 1992).

The antifungal activity has been due to the presence of plant bioactive compounds, *i.e.* total phenolic compounds, total saponins and alkaloids in the cyanobacteria culture filtrates employed as natural defence mechanisms against pathogenic bacteria, fungi, viruses and pests (El-Mahmood and Ameh, 2007). There are a number of reports on the antifungal activity of phenolic substance extracted from cyanobacteria. De Cano *et al.* (1990) found that phenolic compounds in extracts from cells of *N. muscorum* significantly inhibited the growth of *Candida albicans* and *Staphylococcus aureus*. Furthermore, Samapundo *et al.* (2007) observed that the phenolic compounds such as, vanillic and caffeic acid treatments caused reduction in *F. verticillioides* and *F. proliferatum* growth. Sekine *et al.* (2009) detected that phenolic hydroxyl compounds have antifungal activity against white-and brown-rot fungi.

Indole acetic acid was produced in the cyanobacterial culture filtrates and the capacity of indole acetic acid biosynthesis has been found in free-living and symbiotic cyanobacteria of the genera *Nostoc, Chlorogloeopsis, Calothrix, Plectonema, Gloeothece, Anabaena, Cylindrospermum* and *Anabaenopsis* (Sergeeva *et al.*, 2002).

The activity of protease enzymes in the cyanobacterial filtrates revealed that hydrolytic enzymes may contribute to the fungicidal activity of the cyanobacterial strains, besides other bioactive compounds, including indole acetic acid (Tantawy, 2011).

Conclusion

For a number of reasons, cyanobacteria are suitable candidates for exploitation as biocontrol agents of plant pathogenic bacteria and fungi. Cyanobacteria produce a large number of antibacterial and antifungal products, many can be grown in quantity in mass culture, and they are not a threat to the environment (except for the production of toxic blooms in freshwater and marine habitats and slimy areas on turf by a relatively small number of cyanobacteria). Nevertheless, there are two important considerations that have to be taken into account: (1) Although it has been reported in many studies that cyanobacteria is capable of producing substances *in vitro* that can inhibit the growth of bacteria and fungi, it still remains to be proven that these substances are produced in nature. However, even if it is shown that cyanobacteria do not produce antibacterial and antifungal substances *in vivo*, the substances produced by them *in vitro* may prove useful in controlling bacterial and fungal plant pathogens. (2) Since the majority of species of cyanobacteria are obligate photoautotrophs, they will not be able to grow blew the surface of the soil in the vicinity of germinating seeds or plant roots. In addition, many cyanobacteria are not terrestrial but are found in freshwater and marine habitats.

Formulations of intact cyanobacteria and microalgae could be applied to the above-ground portions of plants and it is possible that they might offer protection against pathogenic baoteria and fungi. However, it a greater chance of success might be attained with formulations of culture filtrates or cell extracts from cyanobacteria applied to seeds as protectants against damping-off fungi such as *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani* or sprayed on leaves to protect them from the pathogenic bacteria and fungi.

Acknowledgments

The researcher is deeply grateful to Dr. Sekina T. Tantawy, Professor of Phycology, Dept. of Botany, Fac. of Sci., Cairo Univ., Giza, Egypt, for her useful suggestions and providing all the needed facilities.

References

Abo-Shady, A.M.; Al-Ghetto, B.A.; Rahhal, M.H. and Abd-Elmonem, H.A. 2007. Biological control of faba bean pathogenic fungi by three cyanobacterial filtrates. *Pakist. J. Biol. Sci.*, **10**: 3029-3038.

Biondi, N.; Piccardi, R.; Margheri, M.C.; Rodolfi, L.; Smith, G.D. and Tredici, M.R. 2004. Evaluations of *Nostoc* strain ATCC 53789 as a potential source of natural pesticides. *Appl. Environ. Microbiol.*, 70: 3313-3320.

Carmichael, W.W. 1992. Cyanobacteria secondary metabolites–the cyanotoxins. *J. Appl. Bact.*, 72: 445-459.

Cobb, F.W.; Krstic, M.; Zavarin, E. and Barber, W. 1968. Inhibitory effect of volatile oleoresin components on *Phoma annosus* and four *Ceratocystis* species. *Phytopathology*, 58: 1327-1335.

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 extracts and extracellular production of *Nostoc muscorum* on growth of *Sclerotinia sclerotiorum*. *Phyton*, 47: 43-46.

De Caire, G.Z.; De Cano, M.S.; De Mule, M.C.Z. and De Halperin, D.R. 1990. Antimycotic products from the cyanobacterium *Nostoc muscorum* against *Rhizoctonia solani*. *Phyton-Buenos-Aires*, 51: 1-4.

De Cano, M.S. ; De Mule, 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 cyanobacterium *Nostoc muscorum*. *J. Appl. Phycol.*, 2: 79- 81.

Du, L.M. and Xu, Q.Q. 2000. Study on the direct determination of auxins by gas chromatography. *Se Pu*, 18(1): 160-161. (In Chinese).

El-Mahmood, A.M. and Ameh, G.M. 2007. *In vitro* antibacterial activity of *Parkia biglobosa* (Jacq.) root bark extract against some microorganisms associated with urinary tract infections. *African J. Biotechnol.*, 6 (11): 1272-1275.

El-Morsy ,G.A. ; Abou-Zeid, N.M. and Hassanein, A.M. 1997. Identification of Fusarium wilt caused by *Fusarium oxysporum* and pathogen variability in faba bean, lentil and chickpea crop in Egypt. *Egypt. J. Agric. Res.*, 75: 551-564.

El-Sayed, Sahar A. 2006. Use of intercropping and other treatment for controlling faba bean diseases. Ph.D. Thesis, Fac. Agric., Benha Univ., Egypt.

Fisher, R.A. 1948. *Statistical Methods for Research Workers*. Oliver and Boyd, London, UK.

Frankmölle, W.P.; Larsen, L.K.; Caplan, F.R.; Patterson, G.M.I.; Knubal, G.; Levine, I.A. and Moorc, R.E. 1992a. Antifungal cyclic peptides from the terrestrial blue-green alga *Anabaena laxa*. I. Isolations and biological properties. *J. Antibiotics*, 45: 1451-1457.

Frankmölle, W.P.; Knubal, G.; Moorc, R.I. and Patterson, G.M.I. 1992b. Antifungal cyclic peptides from the terrestrial blue-green alga *Anabaena Laxa*.II Structures of Laxaphycins A.B.C.D. and E. *J. Antibiotics*, 45: 1458-1466.

Guzzo, S.D.; Back, E.A.; Martins, E.M. and Moraes, W.B. 1993. Crude expolysaccarides (EPS) from *Xanthomonas campestris* pv. *maniohtis*, *Xanthomonas campestris* pv. *campestris* and commercial xanthan gum as inducers of protection in coffee plants against *Hemileia vastatrix*. *J. Phytopathol.*, 139: 119-128.

Khan, Z.; Park, S.D.; Shin, S.Y.; Bae, S.G.; Yeon, I.K. and Seo, T.J. 2005. Management of *Meloidogyne incognita* on tomato by root–dip treatment in culture filtrate of the blue-green algae, *Microcoleus vaginatus*. *Bioresource Technology*, **96**: 1338-1341.

Kulik, M.M. 1995. The potential for using cyanobacteria and algae in the biological control of plant pathogenic bacteria and fungi .Eur. *J. Plant Pathol.*, **101**: 585-599.

Mazen, M.M. 2004. Resistance induction against diseases of faba bean crop. Ph.D. Thesis, Fac. Agric., Suez Canal Univ., Egypt.

Meda, A.; Lamien, C.E.; Romito, M.; Millogo, J. and Nacoulma, O.G. 2005. Determination of total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, **91**: 571-577.

Metting, B. 1981. The systematic and ecology of soil algae. *Bot. Rev.*, 47: 195- 311.

Moore, R.E. 1996. Cyclic peptides and depsipeptides from cyanobacteria. *J. Ind. Microbiol.*, **16**: 134-143.

Morsy, K.M.1993. Pathological and genetical studies for improving resistance of faba bean to chocolate spot disease. M.Sc. Thesis, Fac. Agric:, Cairo Univ., Egypt.

Morsy, K.M. and Tarrad, A.M. 2005. Effect of infection with *Botrytis fabae* Sard. and mechanical leaf defoliation on yield loss in faba bean. Egypt. *J. Appl. Sci.*, **20** (11B): 443-445.

Mundt, S.; Kreitlow, S. and Jansen, R. 2003. Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei* HUB 051. *J. Appl. Phycol.*, **15**: 263-267.

Namikoshi, M. and Rinehart, K.L. 1996. Bioactive compound produced by cyanobacteria . *J. Indus. Microbiol. Biotechnol.*, **17**: 373-384.

Ördög, V. and Pulz, O. 1996. Diurnal changes of cytokinin-like activity in a strain of *Arthronema africanum* (cyanobacteria), determined by bioassays. *Algolog. Stud.*, **82**: 57-67.

Prasanna, R.; Sood, A.; Jaiswal, P.; Nayak, S.; Gupta, V.; Chaudhary, V.; Joshi, M. and Natarajian, C. 2010. Rediscovering cyanobacteria as valuable sources of bioactive compounds . *Appl. Biochem. Microbiol.*, **46**: 133-147.

Samapundo, S.; De Meulenaer, B.; Osei-Nimoh, D.; Lamboni, Y.; Debevere, J. and Devlieghere, F. 2007. Can phenolic compounds be used for the protection of corn from fungal invasion and mycotoxin contamination during storage. *Food Microbiol.*, **24**: 465-473.

Sekine, N.; Ashitani, T.; Murayama, T.; hibutani, S.; Hattori, S. and Takahashi, K. 2009. Bioactivity of latifolin and its derivatives against termites and funi. *J. Agric. Food Chem.*, **57**: 5707-5712.

Sergeeva, E.; Liaimer , A. and Bergman, B. 2002. Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. *Planta,* **215**: 229-238.

Shimizu, Y. 2003. Microalgal metabolites. *Current Opinion in Microbiol,* **6**: 236-242.

Shtina, E.A. 1991. Regulations of the development of algae in soil. *Pochvovedeniye,* **8**: 57-65.

Snedecor, G.W. and Cochran, G. 1967. *Statistical Methods*. 6th Ed. State Univ. Press, Ames, IA. 213pp.

Strik, W.A.; Ördöge, V. and Saden, J. 1999. Identification of cytokinin isopentenyladenine in a strain of *Arthronema africanum* (cyanobacteria). *J. Phycol.,* **35**: 89-92.

Tantawy, Sekina T. 2011. Biological potential of cyanobacterial metabolites against some soil pathogenic fungi. *J. Food, Agric. and Environ.* **9**(1): 663-666.

Tsuchidia, O.; Yamagota, Y.; Ishizuka, J.; Aria Yamada, J. and Takcuchi, M. 1986. An alkaline proteinase of an alkalophilic *Bacillus* sp. *Curr. Microbiol.,* **14**: 7-12.

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.

Wu, J.T.; Chiang, Y.R.; Huang, W.Y. and Jane, W.N. 2006. Cytotoxic effect of free fatty acids on phytoplankton algae and cyanobacteria. *Aquatic Toxicol.,* **80**: 338-345.

Zarrouk, C. 1966. Contribution a i' etude d' une cyanophycee. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch. et Gardner) Geitler. Ph.D. Thesis, Univ. of Paris, France.

Zulpa, G.; Zaccaro, M.C.; Boccazzi, F.; Parada, J.L. and Storm, M. 2003. Bioactivity of intra and extracellular substances from cyanobacteria and lactic acid bacteria on wood blue stain fungi. *Biol. Control,* **27**: 345-348.

(Received 10/11/2011;
in revised form 12/12/2011)

المكافحه الحيوية لأمراض سقوط البادرات وعفن الجذور والذبول فى الفول بأستخدام رواشح الطحالب الخضراء المزرقة

قدرى مصطفى محمد مرسى
معهد بحوث أمراض النباتات ، مركز البحوث الزراعية ، الجيزة ، مصر.

تناول هذا البحث دراسة تأثير ثلاثة رواشح من مزارع الطحالب الخضراء المزرقة وهى نوستوك مسكوريم ، سبيريولينا بلاتنسس ، أنابينا فلوس أكوا فى المكافحه الحيوية لكل من الفطرين ريزوكتونيا سولانى والفيوزاريم اوكسيسبورم. أحدثت رواشح الطحالب انخفاضا معنويا فى النمو الميسليومى للفطرين المختبرين فى المعمل ، وقد لوحظ زيادة نشاط الإندول اسيتك أسيد وانزيم البروتييز والفينولات الكلية فى راشح كل من نوستوك مسكوريم ، سبيريولينا بلاتنسس عن راشح أنابينا فلوس أكوا.

وجد أن معاملة بذور الفول نقعا بأى من رواشح هذه الطحالب قبل الزراعة فى تربة معدية صناعيا بأى من الفطرين ريزوكتونيا سولانى والفيوزاريم اوكسيسبورم (تجربه أصص) أو تربة ملوثة طبيعيا بالفطريات الممرضة الكامنة في التربة (تجربه الحقل) أدى الى حدوث نقص معنوى فى الاصابة بكل من سقوط البادرات وموت النباتات (الناتج عن الاصابه بأعفان الجذور والذبول) مقارنة بالبذور غير المعاملة. كما وجد أن البذور المعاملة براشح كل من نوستوك مسكوريم ، سبيريولينا بلاتنسس بالتركيز العالى (100%) كانت أكثر فاعلية فى خفض الإصابة بكل من سقوط البادرات وموت النباتات. (الناتج عن الاصابه بالذبول وعفن الجذور) ، وكان راشح انابينا فلوس أكوا أقلهم تأثيرا فى خفض الإصابة فى الحقل. وبصفة عامة أدت معاملة البذوربأى من الرواشح الثلاثة إلى زيادة معنوية فى محصول البذور.