Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 5 (2): 212-215, 2006
ISSN 1812-5387

Growth Activities of the Sugarbeet Pathogens *Sclerotium rolfsii* Sacc., *Rhizoctonia solani* Khun. and *Fusarium verticillioides* Sacc. under Cyanobacterial Filtrates Stress

Mohamed A. Rizk
Department of Botany, Faculty of Science, Cairo University,
Giza 12613, Egypt

Abstract: The present study was undertaken to explore the effect of cyanobacterial filtrates against three sugarbeet pathogens *Fusarium verticillioides*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Based on this study, it was concluded that *Phormidium fragile* and *Nostoc muscorum* filtrates have potential for the suppression of phytopathogenic fungi. *In vitro* and *in vivo* growth, sporulation and sclerotial production were significantly inhibited with the almost species of cyanobacteria.

Key words: Cyanobacteria, sugarbeet pathogens, growth, sporulation, scleratial production

INTRODUCTION

Microorganisms are proving to be rich sources of variety of bioactive natural products of scientific and commercial interest. Extensive screening programs of cyanobacteria have been conducted worldwide and led to the discovery of nevel compounds with antineoplastic, antimicrobial and antiviral properties (Caire *et al*., 1987; Patterson *et al*., 1991, 1993, 1994; Kulik, 1995; Falch *et al*., 1995; Moore, 1996; Jaki *et al*., 2001).

Fungi and bacteria are the chief biological agents that have been studied for the control of plant pathogens, particularly soil borne fungi. In addition viruses, amoebae, nematodes and arthropods have been mentioned as possible biocontrol agents (Whipps and McQuilken, 1993; Hanlon *et al*., 1994; Kulkarni *et al*., 1994).

A number of cyanobacteria and eukaryotic algae particularly macroalgae, produce various biologically active compounds, these include antibiotics which in laboratory tests inhibited bacteria and fungi that incite diseases of humans (Cano *et al*., 1990). Microalgae the best known for the production of toxins by certain species that live in fresh and saltwater. These toxins are associated with the mass growth (algal biomass) of these microorganisms and affect fish, birds and animals (Lawton and Cood, 1991). The marine algae *Acrosiphonia coalita* extract inhibited the growth of *Bacillus subtilis, Candida albicans* and *Streptococcus aureus* (Barnart *et al*., 1993).

Kulik (1995) reported that *Chaetomium globosum*, *Cunninghamella blakesleeana*, *Aspergillus oryzae* and the plant pathogen *Sclerotinia sclerotiorum* were inhibited *in vitro* by substances produced by various cyanobacteria.

The aim of this study was to investigate the influence of cyanobacterial filtrates as biocontrol agents against sugarbeet plant pathogens.

MATERIALS AND METHODS

Microorganisms and culture conditions: Fungi used in this investigation were isolated from saline solis or the rhizosphere of sugarbeet cultivars in Egypt; *Sclerotium ralfsii*, *Rhizoctonia solani* and *Fusarium verticillioides* (El-Abyad *et al*., 1988). These were maintained in culture on Czapek-Dox agar.

Cyanobacteria were grown on Bold basal medium for 21 days at room temperature. The cultures were homogenized and filtered through wathman No. 1 paper and the filtrate was filter-sterilized through 0.45 μm filter. Two concentrations of the filtrate (5 and 10% v/v) were prepared.

Agar diffusion method: This method was used to test the sensitivity of fungi against the cyanobacterial toxins (Odds, 1989; Hadacek and Greger, 2000). One milliliter of spore suspension of *F. verticillioides* or 10 sclerotia of each of *S. rolfsii* or *R. solani* poured into petridishes with Czapek-Dox agar medium and separate discs impregnated with algal filtrate were placed on the surface of the agar and incubated for 8 days at 27°C. The inhibition zone was recorded.

Growth activities of the pathogenic fungi were examined in the absence and presence of cyanobacterial filtrate concentrations (5 and 10%). Mycelial growth of fungi was determined by the soil tube method (El-Abyad and Saleh, 1971). The tubes were incubated at 27°C for

8 days. Growth was measured daily and compared with control. Three tubes were prepared for each treatment.

Dry weight yields were determined by mixing the filtrate of cyanobacteria with Czapek-Dox medium in 50 mL Erlenmeyer flask to produce the required concentration in a total vol 20 mL per flask. Control flasks without algal filtrate were also prepared. Each flask was inoculated with a 4 mm agar disk bearing mycelium of the fungus cut from young colonies and incubated at 27°C for 10 days. Three flasks were prepared for each treatment and filtered under suction, the mycelium produced was dried to constant weight at 80°C and the dry weight estimated.

Sporulation counts: Czapek-Dox agar was mixed aseptically with cyanobacterial filtrate to produce the required concentration and poured into petridishes. The petridishes were inoculated with a 4-mm disk of myceliym of *F. verticillioides*, incubated for 10 days at 27°C and the sporulation was counted by a haemocytometer.

Production of sclerotia by *S. rolfsii* was studied on Czapek-Dox agar medium. This was supplemented with two various amounts of algal filtrate as described earlier, inoculated with agar disk bearing mycelium of the fungus and incubated for 10 days at 27°C. The number of sclerotia was visually counted. Method of Manning *et al.* (1971) was used for production of sclorotia of *R. solani.*

Statistical analysis: The obtained data were carried out according to Snedecor and Cochran (1980), using LSD to compare the significance of the results.

RESULTS AND DISCUSSION

All selected cyanobacterial genera *Anabaena*, *Nostoc* and *Phormidium* were tested for their capability to exert inhibition effect.

The inhibition zone test revealed as shown in Table 1. The culture filtrates of cyanobacterial species have a remarkable inhibition affect on the tested pathogenic fungi of sugarbeet.

Mycelial dry weight yields were significantly suppressed by all selected cyanobacterial sp. (Table 2). In case of *Fusarium verticillioides*, the lowest growth appeared only with *Phormidium fragile* and *Nostoc muscorum* filtrates. It was noticed that, with increasing the concentration of filtrate, the fungal growth decreased. The maximum inhibition was 52% at *Phormidium* filtrate. The same trend was observed in case of *S. rolfsii*. Also, the greatest inhibition in the biomas yields of *R. solani* was 38 and 31% with *Nostoc muscorum* and *Phormidium fragile*, respectively.

Table 1: Inhibition zone of fungi (mm) *F. verticilloides*, *S. rolfsii* and *R. solani* under the effect of cyanobacterial filtrates

Cyanobacterial sp.	*F. verticillioides*	*S. rolfsii*	*R. solani*
Anabaena flos-aquae	21	33	15
Anabaena sp.	30	27	17
Nostoc muscorum	17	25	18
Nostoc calcicola	15	28	30
Phormidium fragile	30	30	25

Table 2: Dry weight yields of fungi (mg) in Czapek-Dox under the effect of two different concentrations of cyanobacterial filtrates (5 and 10%), incubation period 10 days at 27°C

		Fungal sp.		
Cyanobacterial sp.	Conc. (%)	*F. verticillioides*	*S. rolfsii*	*R. solani*
Control	0	175.0	205.0	353.0
Anabaena flos-aquae	5	168.0	180.0	331.0
	10	155.0	175.0	310.0
LSD 5%		4.8	10.4	11.7
1%		7.8	17.3	19.3
Anabaena sp.	5	171.0	186.0	282.0
	10	162.0	170.0	275.0
LSD 5%		6.1	4.8	37.0
1%		10.0	7.8	61.1
Nostoc muscorum	5	172.0	175.0	235.0
	10	150.0	161.0	220.0
LSD 5%		8.0	1.6	11.9
1%		13.3	2.7	19.3
Nostoc calcicola	5	155.0	193.0	301.0
	10	153.0	180.0	295.0
LSD 5%		8.2	10.5	11.3
1%		13.6	16.5	18.4
Phormidium fragile	5	95.0	154.0	305.0
	10	83.0	142.0	306.0
LSD 5%		17.3	5.6	11.2
1%		28.5	9.2	18.4

Table 3: Growth of *Fusarium verticillioides, Selerotium rolfsii* and *Rhizoctonia solani* in soil (mm) under the effect of two different concentrations of cyanobacterial filtrates (%), incubation period 8 days at 27°C

		Fungal sp.		
Cyanobacterial sp.	Conc. (%)	*F. verticillioides*	*S. rolfsii*	*R. solani*
Control	0	96.0	105.0	111.0
Anabaena flos-aquae	5	25.0	77.0	108.0
	10	18.0	72.0	106.0
LSD 5%		7.4	27.5	11.4
1%		12.0	45.5	18.8
Anabaena sp.	5	30.0	61.0	109.0
	10	30.0	56.0	107.0
LSD 5%		5.9	9.8	11.1
1%		9.6	16.1	18.4
Nostoc muscorum	5	45.0	65.0	105.0
	10	40.0	59.0	103.0
LSD 5%		6.7	11.6	12.3
1%		11.1	22.5	20.7
Nostoc calcicola	5	56.0	66.0	85.0
	10	52.0	57.0	83.0
LSD 5%		16.8	10.4	12.7
1%		27.6	17.2	21.1
Phormidium fragile	5	22.0	50.0	57.0
	10	20.0	41.0	45.0
LSD 5%		9.4	6.0	21.5
1%		15.5	9.8	35.6

Table 4: Numbers of spores produced ($\times 10^4$) of *Fusarium verticillioides*, Numbers of sclerotia/plate produced by *Sclerotium rolfsii* and *Rhizoctonia solani* under the effect of two different concentrations of cyanobacterial filtrates (%) on Czapek-Dox agar, incubation period 10 days at 27°C

		Fungal sp.		
Cyanobacterial sp.	Conc. (%)	*F. verticillioides*	*S. rolfsi*	*R. solani*
Control	0	225	433	172
Anabaena flos-aquae	5	202	390	175
	10	186	375	173
LSD 5%		4.9	30.1	9.2
1%		8.1	49.6	15.1
Anabaena sp.	5	195	425	163
	10	197	421	158
LSD 5%		14.4	28	3.5
1%		23.8	47.3	5.5
Nostoc muscorum	5	210	427	166
	10	205	415	162
LSD 5%		4.4	21.5	14
1%		7.2	35.4	22
Nostoc calcicola	5	198	375	176
	10	196	352	168
LSD 5%		5.6	31.6	8.3
1%		9.2	52.4	13.8
Phormidium fragile	5	173	353	115
	10	155	205	95
LSD 5%		12.9	17.1	23
1%		21.2	28	38.1

These results are in agreement with the results reported by Cano *et al.* (1990), Smitka *et al.* (1992), Caire *et al.* (1993), Fish and Cood (1994) and Borowitzka (1995), who reported that the extracts of *Nostoc muscorum* and *Phormidium* sp. significantly inhibited the growth of *Candida albicans*, *Sclerotinia sclerotiorum* and *Staphylococcus aureus*.

In connection, Adam (1999) studied the effect of nostoc muscorum on the growth of some crop plants. Growth parameters of wheats, sorghum, maize and lentil were significantly increased with addition of algal filtrate.

In vivo studies (Table 3) showed that *F. verticillioides* and *S. rolfsii* were very sensitive to almost cyanobacterial species. The maximum inhibition of *Fusarium* growth in soil was 81% with *Anabaena flos-aquae* and 60% incase of *Sclerotium* with *Phormidium* filtrate.

Most species of cyanobacteria studied, have no significant affect on growth of *R. solani* in soil. Kulik (1995) reported that the growth of *R. solani* on *PDA* was significantly inhibited by using *Nostoc muscorum* extract.

A new bioactive compound known as tanikolide was isolated from the marine cyanobacterium *Lyngbya majuscula*. This compound exhibited activity against *Artemia salina*, *Biomphalaria glabrata* and *Candida albicans* (Singh *et al.*, 1999). The growth activities of *Fusarium oxysporum betae*, *F. oxysporum lycopersici* and *F. oxysporum* vasinfectum were inhibited with increasing the concentration of cyanobacterial extracts (Moussa and Shanab, 2001).

Number of spores produced by *F. verticillioides* was highly significantly decreased with all different filtrates of cyanobacteria. The lowest number of the fungal spores was (155×10^4) compared to control by using *Phormidium* filtrate (Table 4).

Production of sclerotia by *S. relfsii* and *R. solani* was highly significantly suppressed with almost cyanobacteria (Table 4). The lowest number of sclerotia was appeared with *Phormidium fragile*. *Nostoc muscorum* has no affect on the production of sclerotia in both fungi. These results are in agreement with that obtained by Benjamin *et al.* (1999), Begum *et al.* (1999) and Moussa and Shanab (2001).

Finally, it may be worth noting that the sensitivity to cyanobacteria metabolites not only depend on the fungal genus but also its species and/or mode of growth.

REFERENCES

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

Barnart, M.W., G.G. Whatley and W.H. Gerwick, 1993. Unprecedented oxylipins from the marine green alga *Acrosiphonia coalita*. J. Natl. Prod., 56: 245-259.

Begum, Z.N.T., M.A. Bashar and R.M. Dilruba, 1999. Antifungal activity of freshwater blue green algae in laboratory culture. Bangaladesh J. Bot., 28: 125-131.

Benjamin, P., E. Pelosi, F. Juttner and B. Pushparaj, 1999. Toxicological analysis of the marine cyanobacterium *Nodularia harveyana*. J. Applied Phycol., 10: 527-530.

Borowitzka, M.A., 1995. Microalgae as sources of pharmaceuticals and other biologically active compounds. J. Applied Physol., 7: 3-15.

Caire, G.Z.de., M.S. de Cano, M.C.Z. de Mule, D.R. de Halperin and M. Galvagno, 1987. Action of cell-free extracts and extracellular products of *Nostoc muscorum* on growth of *Sclerotinia sclerotiorum*. Phyton., 47: 43-46.

Caire, G.Z.de., M.S. de Cano, M.C.Z. de Mule and D.R. de Halperin, 1993. Screeing of cyanobacterial bioactive compounds against human pathogens. Phyton. Buenos. Aires., 54: 59-65.

Cano, M.M.S., M.C.Z. de Mule, G.Z. de Caire and D.R. de Halperin, 1990. Inhibition of *Candida albicans* and *Staphylococcus aureus* by phenolic compounds from the terrestrial cyanobacterium *Nostoc muscorum*. J. Applied Phycol., 2: 79-81.

El-Abyad, M.S. and Y.E. Saleh, 1971. Studies with *Fusarium oxysporum* f.sp. *vasinfectum*, the cause of cotton wilt in Egypt; germination, sporulation and growth. Trans. Brit. Mycol. Soc., 57: 427-437.

El-Abyad, M.S., H. Hindorf and M.A. Rizk, 1988. Impact of salinity stress on soil borne fungi of sugarbeet. I. Pathogenicity implications. Plant and Soil, 110: 27-32.

Falch, B.S., G.M. Konig, A.D. Wright, O. Sticher, C.K. Angerhofer, J.M. Pezzuto and H. Bachmann, 1995. Biological activities of cyanobacteria: Evaluation of extracts and pure compounds. Planta Med., 61: 321-328.

Fish, S.A. and G.A. Codd, 1994. Bioactive compound production by thermophilic and thermotolerant cyanobacteria (blue-green algae). World J. Microbiol. Biotechnol., 10: 338-341.

Hadacek, F. and H. Greger, 2000. Testing of antifungal natural products: Methodologies, comparability of results and essay choose. Phytochem. Annals., 1: 137-147.

Hanlon, D., M.L. Rosario, G.W.G. Venema, D. Van Sinderen, 1994. Identification of TipC, a noval 62 kDa McP-like protein from *Bacillus subtilis*. Microbiology, 140: 1847.

Jaki, B., O. Zerbe, J. Heilmann and O. Sticher, 2001. Two novel cyclic peptides with antifungal activity from the cyanobacterium *Tolypothrix byssoidea* (EAWAG 95). J. Nat. Prod., 64: 154-158.

Kulkarni, S.A., K. Srikant and S. Kulkarni, 1994. Biological control of *Sclerotium rolfsii* Sacc., a causal agent of stem rot of ground nut. Karnataka Agric. Sci., 7: 365.

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: 585-599.

Lawton, L.A. and G.A. Cood, 1991. Cyanobacterial (blue-green algae) toxins and their significance in UK and European waters. J. Inst. Water Environ. Manage., 5: 460-465.

Manning, W.J., D.F. Grossan, A.L. Adams, 1971. Method for production of sclerotia of *Rhizoctonia solani*. Phytopathologia, 60: 179-180.

Moussa, T.A.A. and S.M.M. Shanab, 2001. Impact of cyanobacterial toxicity stress on the growth activities of some phytopathogenic *Fusarium* sp., Az. J. Microbiol., 53: 267-281.

Moore, R.E., 1996. Cyclic peptides and depsipetides from cyanobacteria: A review. J. Ind. Microbiol., 16: 134-143.

Odds, F.C., 1989. Antifungal activity of saperconazole (R. 66905) *in vitro*. J. Antimicrobial Chemother., 24: 533-537.

Patterson, G.M.L., C.L. Baldwin, F.R. Caplan, H. Karuso and L.K. Larsen *et al*., 1991. Antineoplastic activity of cultured blue-green algae (cyanophyta). J. Phycol., 27: 530-536.

Patterson, G.M.L., K.L. Baker, C.L. Baldwin, C.M. Bolis and F.R. Caplan *et al*., 1993. Antiviral activity of cultured blue-green algae (cyanophyta). J. Phycol., 29: 125-130.

Patterson, G.M.L., L.K. Larsen and R.E. Moore, 1994. Bioactive natural products from blue-green algae. J. Applied Phycol., 6: 151-157.

Singh, I.P., K.E. Milligan and W.H. Gerwick, 1999. Tanikolide, a toxic and antifungal lactone from the marine cyanobacterium *Lyngbya majuscule*. J. Natl. Prod., 62: 1333-1335.

Smitka, T.A., R. Bonjouklian, L. Doolin, N.D. Jones and J.B. Deeter *et al*., 1992. Ambiguine isonitriles, fungicidal haplindole-type alkaloids from three genera of blue-green algae belonging to the stigonemataceae. J. Org. Chem., 57: 587-861.

Snedecor, G.W. and W.G. Cochran, 1980. Statistical Methods. 7th Edn., Iowa State Univ. Press, USA., pp: 255-269.

Whipps, J.M. and M.P. McQuilken, 1993. Aspects of Biocontrol of Fungal Plant Pathogens. In: Jones, D.G. (Ed.) Exploitation of Microorganisms Chapman and Hall, London, UK., pp: 45-79.