

**Performance of Algal Filtrates and Biocides  
for the Control of *Sclerotinia sclerotiorum*  
Causing Damping-off of Fennel**

**N.K. Soliman;\* M.S. Mansour\*; A.A. Hilal\*\* and  
Radwa M.S. Shafie\*\***

\* Plant Pathol. Dept., Fac. Agric., Cairo Univ., Giza, Egypt.

\*\* Plant Pathol. Res. Inst., ARC, Giza, Egypt.

**F**ennel, *Foeniculum vulgare* Mill., is one of the umbelliferous medicinal plants and is subjected to infection by *Sclerotinia sclerotiorum* (Lib.) de Bary, causing economic losses in plant stand and yield in Egypt. Biological control of *S. sclerotiorum* using algal filtrates of *Nostoc* spp., *Oseillatoria* spp., *Spirulina platensis*, *Chlorella* spp., *Anabaena oryzae*, *Wolleea* spp., *Anabaena flos-aquae* and *N. humifusum* as well as the commercial biocides namely; Rhizo-N, Plant guard, Bio-ARC and Bio-Zeid were evaluated under laboratory and greenhouse conditions. All the tested algal filtrates, except those of *Anabaena oryzae* and *Wolleea* spp., have significantly reduced the mycelial growth of *S. sclerotiorum*. However, filtrates of *Nostoc* spp., *Oseillatoria* spp. and *S. platensis* completely inhibited the radial growth of the fungus and significantly decreased damping-off percentages and increased length of shoot and root systems, plant fresh weight and number of branches/plant. All the used biocides have significantly reduced the radial growth of *S. sclerotiorum*. Rhizo-N gave the highest inhibition followed by Plant guard. The least inhibition of *S. sclerotiorum* growth was recorded by Bio-ARC and Bio-Zeid. Rhizo-N decreased damping-off percentages and increased the length of shoot and root systems, plant fresh weight and number of branches/plant.

**Keywords:** Algal filtrates, biological control, biocides, damping-off and *S. sclerotiorum*.

Fennel (*Foeniculum vulgare* Mill.) is one of the umbelliferous medicinal plants. In Egypt, the cultivated area of fennel reached 2209 feddans during 2007, yielding 3446 tons by average of 1.5 ton / feddan (Anonymous, 2007). Fennel is chiefly used medicinally with purgatives and to improve eyesight. Some people use fennel as an effective diuretic. Also, it is an aromatic stimulant, a carminative stomachic and used in improving the milk supply of a breastfeeding mother. Syrup prepared from fennel juice was formerly used for chronic coughs (El Bardai *et al.*, 2001). Fennel is infected by many diseases, *i.e.* damping-off (*Rhizoctonia solani*, *Pythium* spp. & *S. sclerotiorum*), root and crown rot (*R. solani*, *Pythium* spp., *Fusarium* spp., *Botrytis cinerea* & *S. sclerotiorum*) and stem rot (*S. sclerotiorum*) as reported by Boland & Hall (1994), Hilal *et al.* (1998) and Saharan & Mehta (2008). Damage resulted from *Sclerotinia* infection has greatly increased in the recent years.

Several antagonistic microorganisms including fungi, bacteria and algae have been reported to decrease the pathogenic activity of *S. sclerotiorum* (Boland, 1997; Zapata *et al.*, 1998; Aujla *et al.*, 2004 and Sharan & Mehta, 2008). However, algal filtrates have been recorded as active inhibitors to the *in vitro* fungal growth (Zaccro *et al.*, 2006) for *S. sclerotiorum*. This work aims to study the effect of some biocides and algal filtrates in the biological control of *S. sclerotiorum* on fennel plants.

### Materials and Methods

Fungal isolates were obtained from infected roots and stems of fennel seedlings and plants. Isolation was done on PDA plates incubated at 20°C for 7 days. In a preliminary pathogenicity test, *S. sclerotiorum* isolates (Nos. 3 and 5) were found to be virulent on fennel seedlings, thus these two isolates were chosen for the next studies.

Algal filtrates were kindly prepared and provided by Dr. Soha S.M. Mostafa. Microbiology Dept., Soils, Water & Environment Res. Institute, ARC, Giza, Egypt.

Algae samples were collected from lake "Manzala" and lake "Borollous" at Northern region of the Nile Delta, Egypt. The samples were diluted with sterilized distilled water to make 1/10 dilution/series. This first dilution was stirred for 30 minutes using a magnetic stirrer. Ten serial dilutions were then carried out with sterilized distilled water. One ml of 10<sup>-2</sup> to 10<sup>-6</sup> dilutions were poured on the surface of the agar medium and spread with glass spatula, then incubated in inverted position at room temperature for 3 weeks. Isolation was carried out using a dissociating microscope and the cyanobacterial colonies were picked out using inoculation needle for propagation, purification and identification.

Slant agar refrigerated (5°C) cyanobacteria were exposed to light (500 LUX) for 2 days then inoculated to a liquid medium and grown to reach the logarithmic phase of growth to each cyanobacterial strain. All cyanobacterial strains were occasionally purified using yeast extract agar medium. The purified cyanobacterial strains were then grown in 500 ml conical flasks containing 100ml sterilized BG11 medium (MgSO<sub>4</sub> · 7 H<sub>2</sub>O, K<sub>2</sub> HPO<sub>4</sub> · 3H<sub>2</sub>O, CaCl<sub>2</sub> · 2H<sub>2</sub>O, Na<sub>2</sub> CO<sub>3</sub>, citric acid) and incubated under illumination (3000 LUX) at temp. 28-32°C. Two weeks later, the developed cyanobacterial growth was filtered under aseptic conditions to obtain culture filtrates.

#### *Effect of some algal filtrates on mycelial growth of S. sclerotiorum:*

The crude filtrate of each alga, prepared as mentioned before, was individually mixed with PDA medium, at the rate of 1ml/9ml media (at 45°C) and poured in plates (Biondi *et al.*, 2006). Plate centres were inoculated with *S. sclerotiorum* and incubated at 20°C for 5 days. Algal filtrate-free PDA plates were used as control. The average radial growths were recorded when one plate was covered with fungal growth.

#### *Effect of some biocides on mycelial growth of S. sclerotiorum:*

Biocides (Table 1) were mixed with PDA medium (at 45°C). The plates centre were inoculated with *S. sclerotiorum* discs (5-mm-diam.) of PDA culture and

incubated at 20°C for 5 days. Biocides-free plates served as control. Average radial growth was recorded when one plate was covered with the fungal growth.

*Effect of seed soaking in some algal filtrates on damping-off of fennel:*

Apparently healthy seeds of fennel were soaked for one hour, in each filtrate of some algae (Table 1). The two isolates of *S. sclerotiorum* were grown on potato dextrose medium for 15 days at 20°C and macerated in the blender for 1 minute. Seeds were sown in soil infested with *S. sclerotiorum* isolates (30ml of fungal inoculum/ kg soil at the rate of 90 ml/pot), dispensed in plastic pots (20 cm in diam.) at the rate of 7 seeds/ pot. Seeds soaked in water served as control check. Four replicates were used for each treatment. Percentages of pre- and post-emergence damping-off were recorded after 15 and 45 days of sowing, respectively. Survival plants were also counted, 60 days after sowing. The average length of shoot and root system, fresh weight and number of branches were also recorded.

**Table 1. Biocides used as bioagents against *S. sclerotiorum***

Biocide (Trade name)	Producer name	Bioagent/(density/ml)	Dose	
			Per l medium	Per kg seed
Rhizo - N	El-Nasr Co.	<i>Bacillus subtilis</i> , $3 \times 10^7$ cfu/ml	4.0 g	4.0 g
Bio- ARC		<i>B. megaterium</i> , $2.5 \times 10^7$ cfu/ml	2.5 g	2.5 g
Bio-Zeid		<i>Trichoderma album</i> , $3 \times 10^7$ cfu/ml	2.5 g	2.5 g
Plant gaurd		<i>T. harzianum</i> , $3 \times 10^7$ cfu/ml	4.0 ml	4.0 ml

*Effect of soil treatment with some algal filtrates on damping-off of fennel:*

Crude algal filtrates were individually added to the soil infested with *S. sclerotiorum* isolates (30ml/kg soil) at the rate of 90 ml/pot (20-cm-diam.). Seeds of fennel were sown at the rate of 7 seeds / pot. Soil treated with water only was served as control. Four replicates were used for each treatment. Percentages of pre- and post-emergence damping-off were recorded as previously mentioned. Survival plants after 60 days of sowing were counted. Length of shoot and root system, fresh weight and number of branches were recorded.

*Effect of seeds treatment with some biocides on damping-off of fennel:*

Healthy-looking seeds of fennel were treated with biocides (Table 2). The seeds were sown in pots (20-cm-diam.) by mean of 7 seeds/pot. Soil of each pot, however, was previously infested with *S. sclerotiorum* isolates (30 ml/kg soil). Seeds treated with water only served as control treatment. Four replicates were used for each treatment. Percentages of pre-, and post-emergence damping-off were recorded as previously mentioned. Survival plants were counted. Length of shoot- and root-system, fresh weight and number of branches / plant were recorded.

*Statistical analysis:*

Data were analysed in a complete randomized block design according to Gomez and Gomez (1984).

## Results

### *Effect of some algal filtrates on mycelial growth of S. sclerotiorum:*

All the tested algal filtrates, except *Anabaena oryzae* and *Wollea* spp., have significantly reduced the mycelial growth of *S. sclerotiorum* as compared with the control check (Table 2). Algae filtrates of *Nostoc* spp., *Oseillatoria* spp. and *Spirulina plantensis* completely inhibited the mycelial growth of the fungal isolates followed by *Chlorella* spp. On the other hand, *Anabaena oryzae* and *Wollea* spp. did not inhibit the fungal growth at all.

**Table 2. Effect of some algal filtrates on mycelial growth of *S. sclerotiorum***

Tested algae	Radial growth (cm) of <i>S. sclerotiorum</i>	
	Isolate No. 3	Isolate No. 5
<i>Nostoc</i> spp.	0.0*	0.0
<i>Chlorella</i> spp.	3.5	3.5
<i>Oseillatoria</i> spp.	0.0	0.0
<i>Spirulina platensis</i>	0.0	0.0
<i>Anabaena oryzae</i>	9.0	9.0
<i>Anabaena flos-aquae</i>	4.5	9.0
<i>Wollea</i> spp.	9.0	7.7
<i>Nostoc humifusum</i>	0.0	3.9
Control (Algae filtrate-free medium)	9.0	9.0
L.S.D. at 0.05	0.3	0.3

\* Each figure represents the average of 4 replicated plates.

### *Effect of some biocides on mycelial growth of S. sclerotiorum:*

Data presented in Table (3) show that all the tested biocides have significantly reduced the radial growth of *S. sclerotiorum*. Rhizo-N gave the highest inhibition (0.5 cm), in case of isolate No. 3 followed by Plant guard. On the other hand, the least inhibition of the fungal growth was recorded on Bio-ARC (3.3 cm) and Bio-Zeid (4.3 cm) treatments in soil infested with isolate No. 3.

**Table 3. Effect of some biocides on mycelial growth of *S. sclerotiorum***

Tested biocide	Rate / l. medium	Radial growth (cm) of <i>S. sclerotiorum</i>	
		Isolate No.3	Isolate No.5
Rhizo - N	4.0 g	0.5 *	0.6
Bio-ARC	2.5 g	3.3	3.5
Bio-Zeid	2.5 g	4.3	4.5
Plant gaurd	4.0 ml	2.9	2.5
Control (biocide-free medium)	0	9.0	9.0
L.S.D. at 0.05		0.3	0.4

\* Average of 4 replicated plates.

*Effect of soaking seeds in some algal filtrates on damping-off of fennel seedlings, grown in soil infested with S. sclerotiorum:*

Data in Table (4) indicate that the tested algae significantly reduced pre- and post-emergence damping-off of fennel compared with the control check. *Spirulina platensis* gave the highest survivals (77.5%) in soil infested with isolate No. 3. On the other hand, the least effect was recorded with *Chlorella* spp. treatment (60.7%) in soil infested with isolate No. 3.

**Table 4. Effect of soaking seeds in some algal filtrates on pre- and post-emergence damping-off of fennel seedlings, grown in infested soil**

Tested algae	<i>S. sclerotiorum</i> isolates					
	Isolate No. 3			Isolate No. 5		
	Pre-emergence (%)	Post-emergence (%)	Survival (%)	Pre-emergence (%)	Post-emergence (%)	Survival (%)
<i>Nostoc</i> spp.	12.2*	14.2	73.6	12.0	15.0	73.0
<i>Spirulina platensis</i>	10.3	12.2	77.5	10.9	13.5	75.6
<i>Oseillatoria</i> spp.	11.0	15.4	73.6	12.1	16.1	71.8
<i>Chlorella</i> spp.	15.3	24.0	60.7	16.0	25.4	58.6
Control (algae-free)	21.3	65.1	13.6	24.5	66.0	9.5
Control (fungus-free)	0.0	0.0	100.0	0.0	0.0	100.0
L.S.D. at 0.05	0.4	0.5	0.6	0.3	0.6	0.7

\* Average of 4 replicates.

*Effect of soaking fennel seeds in algal filtrates on length of shoot and root systems, fresh weight and number of branches of fennel plants, grown in soil infested with S. sclerotiorum:*

Data in Table (5) show that the tested algae filtrates have significantly increased the length of shoots and roots systems, fresh weight and number of branches per plant as compared with the control check. Filtrate of *S. platensis* gave the highest increases in shoot system (103.1 cm), root system (41.4cm) and fresh weight (72.3g) in soil infested with isolate No. 3. On the other hand, *Chlorella* spp. gave the lowest increases in shoot system (93.2 cm), root system (37.6 cm) and fresh weight (59.3 g) in soil infested with isolate No. 3.

*Effect of soil treated with some algal filtrates on damping-off of fennel seedlings, grown in soil infested with S. sclerotiorum:*

All algal filtrates tested significantly reduced pre- and post-emergence damping-off of fennel compared with the control (Table 6). Algae filtrate of *S. platensis*, however, gave the highest increase in healthy survivals (80.5%) in soil infested with isolate No. 3. On the other hand, *Chlorella* spp. gave the lowest increase (72.5%) in soil infested with isolate No. 5.

**Table 5. Effect of soaking seeds in algal filtrates on length of shoot and root systems, fresh weight and number of branches of fennel plants, grown in infested soil**

Tested algae	<i>S. sclerotiorum</i> isolates							
	Isolate No.3				Isolate No.5			
	Length (cm)		Fresh weight g/plant	No. branches /plant	Length (cm)		Fresh weight g/plant	No. branches /plant
	shoot	root			shoot	root		
<i>Nostoc</i> spp.	97.8*	40.0	70.3	16	95.2	39.5	69.0	14
<i>Spirulina platensis</i>	103.1	41.4	72.3	16	101.5	42.3	70.2	15
<i>Oseillatoria</i> spp.	96.2	40.5	68.8	13	94.3	39.8	67.0	14
<i>Chlorella</i> spp.	93.2	37.6	59.3	11	91.5	38.2	57.2	13
Control (algae-free)	23.0	6.5	17.2	5	20.5	7.2	20.2	6
Control (fungus-free)	101.0	41.0	70.3	16	100.0	40.3	70.2	16
L.S.D. at 0.05	3.0	2.5	2.1	2.2	2.9	3.0	2.7	0.3

\*Average of 4 replicates.

**Table 6. Effect of soil treated with some algal filtrates on damping-off of fennel seedlings, grown in infested soil**

Tested algae	<i>S. sclerotiorum</i> isolates					
	Isolate No.3			Isolate No.5		
	Pre-emergence (%)	Post-emergence (%)	Survival (%)	Pre-emergence (%)	Post-emergence (%)	Survival (%)
<i>Nostoc</i> spp.	8.7*	10.3	81.0	8.3	11.2	80.5
<i>Spirulina platensis</i>	7.2	12.3	80.5	8.0	10.1	81.8
<i>Oseillatoria</i> spp.	9.9	10.1	80.0	10.1	11.6	78.3
<i>Chlorella</i> spp.	12.3	16.9	70.8	13.5	15.0	71.5
Control (algae-free)	21.3	63.0	15.7	28.5	60.3	11.2
Control (fungus-free)	0.0	0.0	100.0	0.0	0.0	100.0
L.S.D. at 0.05	0.3	0.5	0.7	0.4	0.5	0.8

\* Average of 4 replicates.

*Effect of soil treated with algal filtrates on length of shoot and root system, fresh weight and number of branches of fennel plants, grown in soil infested with S. sclerotiorum:*

Data presented in Table (7) show that all algal filtrates tested significantly increased length of shoot-and root-systems, fresh weight and number of branches of fennel plants as it compared with the control. *S. platensis* gave the highest increases in shoot system (105.3 cm), root system (45.3 cm) and fresh weight (73.1g) in soil infested with isolate No.3. On the other hand, *Chlorella* spp. gave the lowest increases in shoot system (94.3 cm), root system (40.0 cm) and fresh weight (58.3 g) in soil infested with isolate No.5.

**Table 7. Effect of soil treated with algal filtrates on length of shoot and root systems, fresh weight and number of branches of fennel plants, grown in infested soil**

Tested algae	<i>S. sclerotiorum</i> isolates							
	Isolate No.3				Isolate No.5			
	Length (cm)		Fresh weight g/plant	No. branches /plant	Length (cm)		Fresh weight g/plant	No. branches /plant
	shoot	root			shoot	root		
<i>Nostoc</i> spp.	100.2*	44.0	71.0	16	102.5	43.2	70.0	15
<i>Spirulina platensis</i>	105.3	45.3	73.1	15	103.8	43.8	71.3	14
<i>Oseillatoria</i> spp.	97.3	42.6	69.1	13	96.2	43.0	68.3	14
<i>Chlorella</i> spp.	96.0	41.0	60.5	11	94.3	40.0	58.3	13
Control (algae-free)	23.0	10.0	16.8	5	21.3	8.1	20.6	6
Control (fungus-free)	103.2	45.0	72.3	16	104.0	44.2	71.2	16
L.S.D. at 0.05	2.6	1.7	1.2	1.3	0.7	2.1	1.9	2.0

\* Average of 4 replicates.

*VII. Effect of seeds treated with some biocides on damping-off of fennel seedlings, grown in soil infested with S. sclerotiorum:*

Data presented in Table (8) demonstrate that all the biocides tested have significantly lowered pre- and post-emergence damping-off percentages than the control and increased survivals compared with that of control treatment. Rhizo-N gave the highest survivals (79.7%) in soil infested with isolate No.3. On the other hand, the least effect was recorded for Bio-Zeid treatment (62.7%) in soil infested with isolate No.5.

**Table 8. Effect of seeds treated with some biocides on damping-off of fennel seedlings, grown in infested soil**

Treatment	Rate/kg Seed *	<i>S. sclerotiorum</i> isolates					
		Isolate No.3			Isolate No.5		
		Pre-emergence (%)	Post-emergence (%)	Survival (%)	Pre-emergence (%)	Post-emergence (%)	Survival (%)
Rhizo-N	4.0 g	9.3**	11.0	79.7	10.8	12.1	77.1
Bio-ARC	2.5 g	12.0	13.9	74.1	12.9	15.8	71.3
Bio-Zeid	2.5 g	16.5	17.6	65.9	17.0	20.3	62.7
Plant guard	4.0 ml	12.0	14.8	73.2	14.8	16.3	68.9
Control (biocide-free)	0.0	21.3	63.1	15.6	23.8	65.3	10.9
Control (fungus-free)	0.0	0.0	0.0	100	0.0	0.0	100
L.S.D. at 0.05		1.3	1.2	1.6	1.5	1.7	1.6

\* According to Youssef (2008).

\*\* Average of 4 replicates.

VIII. *Effect of fennel seeds dressing with some biocides on length of shoot and root systems, fresh weight and number of branches of plants, grown in soil infested with S. sclerotiorum:*

All tested treatments (Table 9) have significantly increased length of shoot- and root systems, fresh weight and number of branches per plant as it compared with control. Rhizo-N gave the highest increases in shoot system (100.0 cm), root system (43.2 cm) and fresh weight (68.5 g) in soil infested with isolate (No.3). On the other hand, Bio-Zeid gave the lowest increases in shoot system (90.8 cm), root system (35.3 cm) and fresh weight (53.2 g) in soil infested with isolate (No.5).

**Table 9. Effect of fennel seeds treated with some biocides on shoot and root systems, fresh weight and number of branches of plants, grown in infested soil**

Treatment	Rate Conc./ kg seed	<i>S. sclerotiorum</i> isolate							
		Isolate No.3				Isolate No.5			
		Length (cm) of:		Fresh weight (g / plant)	Number of branches /plant	Length (cm) of:		Fresh weight (g / plant)	Number of branches /plant
		Shoot	Root			Shoot	Root		
Rhizo- N	4.0 g	100.0*	43.2	68.5	15	99.8	42.0	67.0	15
Bio-ARC	2.5 g	98.3	41.3	66.3	15	96.2	40.5	64.5	14
Bio-Zeid	2.5 g	91.3	37.2	57.4	12	90.8	35.3	53.2	11
Plant guard	4.0 ml	95.3	40.8	63.2	14	95.3	40.8	63.2	14
Control (biocide-free seeds)	0.0	23.5	6.5	17.2	5	20.3	7.2	20.6	6
Control (fungus free)	0.0	103.0	44.1	69.8	16	102.3	44.2	70.3	16
L.S.D. at 0.05	-	2.3	2.1	2.0	1.9	0.9	2.2	2.5	2.1

\* Average of 4 replicates.

### Discussion

Testing the inhibitory activities of algal filtrates on mycelial growth of *S. sclerotiorum* demonstrated that *Spirulina plantensis* filtrate was the most effective antifungal agent against *S. sclerotiorum*. This activity might point to the ability of the algae to produce bioactive secondary compounds, secreted into the surrounding medium. These bioactive substances seem to hinder growth of the isolates of the tested fungus. *S. plantensis*, like the other cyanophytes *Nostoc* spp., *Nodularia* spp. and *Anabaena* spp. has the ability to produce a variety of lethal toxins (Surakka *et al.*, 2000). These results are in harmony with Caccamese *et al.* (1981) who reported that cyanobacteria are probably best known for production of toxins by certain species that live in both fresh and salt water. Moreover, Moore and Entzeroth (1988) found that filtrate of *S. plantensis* resulted in growth inhibition of the tested microorganisms. These findings coincided with the current data, which revealed that



*Spirulina* culture filtrate had the greatest antifungal effect. Calvo (2007) found that a natural sulphated polysaccharide, calcium spirulin produced by *S. platensis*, had an antifungal effect against *S. sclerotiorum*. Also, the present data pointed out the ability of *Nostoc* spp. to produce secondary compounds, which possessed an inhibitory effect on the fungal growth. These findings concur with Moore *et al.*, (1989) who reported that *Nostoc* spp. is already well documented as possessing commonly antifungal activity. The marked effectiveness of filtrate of *Nostoc* spp. may be due to its antifungal substances. Surakka *et al.* (2000) reported that *Nostoc* spp. was highly cytotoxic to fungal cells. Burja *et al.* (2001) mentioned that *N. commune* produces cryptophycin, nostophycin, nostocylamide, nostocyclin, nostodione and diterpenoid. Also, Zaccro *et al.* (2006) found that the growth of *S. sclerotiorum* was inhibited by extracellular products of *Nostoc* spp. The present results point to a strong effect of culture filtrate of *Oseillatoria* spp. against the tested isolates. These results are in agreement with those reported by Moore and Entzeroth (1988) who found that *Oscillatoria*, a member of cyanobacteria is capable of producing lethal toxins oscillapeptin and oscillamide. El-Gamal (2006) mentioned that culture filtrate of *S. maxima* and *O. agrdhii* caused complete inhibition to *S. sclerotiorum* growth. On the other hand, the antifungal activity of *Chlorella* spp. might be due to active substances, which lead to selective inhibition of the isolates tested. These results are in harmony with those reported by Pratt *et al.* (1994) who demonstrated that chlorellin, a mixture of fatty acids with antifungal activity accumulated in the culture filtrate of *Chlorella* spp.

Greenhouse experiments revealed that reduction in pre-and-post emergence damping-off caused by *S. sclerotiorum* occurred when fennel seeds were either soaked in culture filtrate or directly sown in soil drenched with filtrates of *S. platensis*, *Nostoc* spp., *Oseillatoria* spp. and *Chlorella* spp. These results are somewhat similar to those obtained by Biondi *et al.* (2006) who mentioned that adding culture filtrate of *Nostoc* strain to soil resulted in complete inhibition to *S. sclerotiorum* the known ability of *Nostoc* strain to produce cryptophycins, that has a wide spectrum as antifungal substance and the potency of its filtrates found in our experiments. The effectiveness of soaking seed in algae filtrates might be due to the seed absorption of active substances into seeding tissues, which prevented the infection and disease development. As for soil treatment, Biondi *et al.* (2006) stated that the efficacy of irrigated soil with culture filtrates may be due to the capability of antifungal like substances to penetrate into the fungal cell, consequently causing alterations in fungal metabolism. Cyanobacteria have a promotive effect on the growth of plant. These results concur with Gupta and Lata (1964) who found that cyanobacteria accelerated seed germination and promoted seeding growth.

Furthermore, antifungal activity of four bioproducts were evaluated against mycelial growth of *S. sclerotiorum* isolates. Maximum reduction in mycelial growth of the fungal isolates was achieved by using Rhizo-N followed by Plant guard. *Bacillus* sp. Which is known to produce bacillomycin and fengycin (Koumoutsi *et al.*, 2004). As for the genus *Trichoderma*, Chet (1984) stated that it has a substantial ability to suppress a wide range of plant pathogenic fungi by various mechanisms including the production of cell-wall degrading enzymes. Several possible mechanisms have been suggested to be involved in *Trichoderma*'s

antagonism such as (a) production of volatile or nonvolatile antibiotics by the fungus (Baker and Griffin, 1995), (b) space-or nutrient-carbon, nitrogen, iron limiting factors that compete with the host (Sivan and Chet, 1989) and (c) direct mycoparasitism, where by the host-fungus cell wall is degraded by the lytic enzymes secreted by *Trichoderma* (Zapata *et al.*, 1998). The obtained findings are also in agreement with those of Gzaczyk *et al.* (2002) and Aujla *et al.* (2004). While, Rhizo-N, Plant guard and Bio-ARC reduced seedlings damping-off caused by *S. sclerotiorum* isolates under greenhouse conditions. These findings are in harmony with those obtained by Boland (1997), Gzaczyk *et al.* (2002) and Handoro *et al.* (2003) who found that *Trichoderma album* and *Trichoderma viride* reduced the disease intensity of white rot of pea caused by *S. sclerotiorum* when applied as seed or soil treatment.

#### Acknowledgement

The authors would like to thank Dr. Soha S.M. Mostafa, Soils, Water & Environment Res. Institute, ARC, Giza, Egypt, for her valuable help during the research processes.

#### References

- Anonymous, 2007. Study of the Indicators Agriculture Statistics. *Ministry of Agriculture*, ARC, 256 pp.
- Aujla, I.S.; Sandhu, K.S.; Singh, P.P. and Handoro, F. 2004. The effect of soil amendments and treatment with *Trichoderma* spp. on the survival of sclerotia of *S. sclerotiorum*. *J. Res. Punjab Agric. Univ.*, **39** (4): 521-527. (C.f. CAB Abstr., 2004).
- Baker, R. and Griffin, G.J. 1995. Molecular strategies for biological control of fungal plant pathogen. Pages: 153-182. In: "*Novel Approaches to Integrated Pest Management*". Reuveni, R. (ed.). Lewis Publishers Inc., Florida, USA.
- Biondi, N.; Piccardi, R.; Margheri, M.C.; Rodolfi, L.; Smith, G.D. and Tredici, M.R. 2006. Evaluation of *Nostoc* strain ATCC 53789 as a potential source of natural pesticides. *Appl. and Environ. Microbiol.*, **70**(6): 1-15.
- Boland, G. J. 1997. Stability analysis for evaluating the influence of environment on chemical and biological control of white mould (*S. sclerotiorum*) of bean. *Biological Control*, **9** (1): 7-14. (C.f. CAB Abstr., 1997).
- Boland, G. J. and Hall, M.R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. *Can. J. Plant Pathol.*, **16**: 93-108.
- Burja, A. M.; Burgess, J.G. and Wright, P.C. 2001. Marine cyanobacteria a prolific source of natural products. *Tetrahedron*, **57**: 9347-9377.
- Caccamese, S.; Azzonlina, R.A.; Furnari, G. and Grasso, S. 1981. Antimicrobial and antiviral activities of some marine algae from Eastern Sicily. *Bot. Mar.*, **24**: 365-367.

- Calvo, M.A. 2007. Antifungal activity of some Mediterranean algae. *Mycopathologia*, **93** (1): 1.
- Chet, I. 1984. Application of *Trichoderma* as biocontrol agent. Proc. 6<sup>th</sup> Cong. *Mediterr. Phytopathol. Un.*, Cairo, Egypt, pp. 110-111.
- El Bardai, S.; Lyoussi, B.; Wibo, B. and Morel, N. 2001. Pharmacological evidence of hypotensive activity of *Foeniculum vulgare* in spontaneously hypertensive rat. *Clin. Exp. Hypertens.*, **23** (4): 329-343.
- El-Gamal, Manal A.A.H. 2006. Study on algal blooms at North Delta Egypt. Ph.D. Thesis, Fac. Sci., El Mansora Univ., Egypt, 161 pp.
- Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedure for Agricultural Research*. 2<sup>nd</sup> Ed. John Wiley, 680 pp.
- Gupta, A.B. and Lata, K. 1964. Effect of algal growth hormones on the germination of paddy seeds. *Hidrobiologia*, **24** (1-3): 430-434.
- Gzaczyk, N.B.; Trojanowska, K. and Stachowiak, B. 2002. Inhibition of ergosterol biosynthesis in fungal plant pathogens by *Bacillus* sp. *Polish J. Environ. Studies*, **11**(5): 593-597. (C.f. CAB Abstr., 2002).
- Handoro, F.; Sandhu, K.S. and Singh, P.P. 2003. Biological control of white rot (*S. sclerotiorum*) of pea (*Pisum sativum* L.). *J. Res. Punjab Agric. Univ.*, **39** (3): 382-390. (C.f. CAB Abstr., 2003).
- Hilal, A.A.; Abo-El-Ala, I.M.; Baiuomy, A.M. and El-Morsy, S.A. 1998. Studies on commonly and newly occurring diseases of seven medicinal and aromatic plants and yield losses in relation to some agricultural practices in Egypt. *Egypt. J. Appl. Sci.*, **13** (7): 41-60.
- Koumoutsi, A.; Chen, X.H.; Henne, A. and Borris, R. 2004. Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive lipopeptide in *Bacillus amyloliquefaciens* strain Fz B42. *J. Bacteriol.*, **186**: 1084-1096.
- Moore, R.E. and Entzeroth, M. 1988. Majusculamide D and deoxymajusclamide D, two cytotoxins from *Lyngbia majuscula*. *Phytochem.*, **27**: 3101-3115.
- Moore, R.E.; Banarjee S.; Bornemann, V.; Larsen, L.K. and Williams, D.E. 1989. Novel cytotoxins and fungicides from blue-green algae. *Pure and Appl. Chem.*, **61**(3): 521-524.
- Pratt, R.; Daniels, T.C.; Eiler, J.J. and Strait, L.A. 1994. Chlorellin and antifungal substance from *Chlorella*. *Science*, **99**: 351-352.
- Saharan, G.S. and Mehta, N. 2008. *Sclerotinia* Diseases of Crop Plants: Biology, Ecology and Disease Management. Springer Science & Business Media B.V. Publishing, India, 485pp.

- Sivan, A. and Chet, I. 1989. The possible role of competition between *T. harzianum* and *Fusarium oxysporum* on rhizosphere colonization. *Phytopathology*, **79**: 198-203.
- Surakka, A.; Sihvonen, L.M.; Vuorela, P. and Sivonen, K. 2000. Benthic cyanobacteria from the Baltic sea contain cytotoxic *Anabaena*, *Nodularia* and *Nostoc* strains and an apoptosis-inducing *Phormidium* strain. *J. Antibiotic*, **45**: 1145-1457.
- Youssef, L.M.G. 2008. Studies on faba bean (*Vicia faba*) root rot diseases. M.Sc. Fac. Agric. (Saba-Bacha), Alexandria Univ., 115pp.
- Zaccro, G.Z.; De Cano, M.S. and Galvagno, M. 2006. Action of cell free extracts and extracellular products of *Nostoc muscorum* on growth of *S. sclerotiorum*, *Phyton*, **47**:43-46.
- Zapata, R.; Spivak, S.S.; Filippini, O.S. and Fabrizio, M.D.C. 1998. Control of watery soft rot of witloof chicory (*S. sclerotiorum*) by *Trichoderma album*. *Seed. Sci. Technol.*, **17** (2): 151-155.

(Received 20/10/2009;  
in revised form 21/12/2009)

### فعالية روائح مزارع الطحالب والمبيدات الحيوية فى مقاومة

الفطر سكليروتينيا سكليروشيورم المسبب لموت البادرات الشمر

نور الدين كامل سليمان\* ، مصطفى سيد منصور مبروك\* ،

عرفه عبد الجليل هلال\*\* ، رضوى محمود صبرى أحمد شفيق\*\*

\* قسم أمراض النبات- كلية الزراعة- جامعة القاهرة- الجيزة.

\*\* معهد بحوث أمراض النباتات- مركز البحوث الزراعية- الجيزة.

يُعتبر نبات الشمر من أهم نباتات العائلة الخيمية الطبية ويُصاب باللفط سكليروتينيا سكليروشيورم الذى يُسبب للنبات العديد من المظاهر المرضية منها موت البادرات و يحدث المرض خسائر اقتصادية كبيرة فى المحصول. تم الحصول على الطحالب عن طريق قسم الميكروبيولوجى معهد بحوث الاراضى والمياه حيث تم عزلها من عينات التربة التى تم جمعها من منطقة شمال السحلنا. تم تقدير فعالية المقاومة البيولوجية للفطر المسبب بواسطة روائح مزارع الطحالب: الأوشلاتوريا ، السبيروليونا بلاتنيس ، نوستوك ، كلوريل ، أنابينا أوريزا ، أنابينا فلوس أكوا ، وولا ، نوستوك هامفيوسام وذلك كل على حده كما تم استخدام أربعة من المبيدات الحيوية تحت ظروف المعمل والصوبة.

أثبتت النتائج المتحصل عليها أن جميع روائح مزارع الطحالب المستخدمة تقلل النمو الميسليومي ماعدا أنابينا أوريزا. كما وجد أن روائح مزارع طحالب النوستوك ، الأوشلاتوريا ، الاسبيروليونا تعطى تثبيطاً تاماً للنمو الميسليومي للفطر سكليروتينيا سكليروشيورم وقللت نسبة موت البادرات كما أدت إلى زيادة طول كل من الجذر والساق والوزن الرطب وعدد أفرع نباتات الشمر، وقد أعطى روائح مزرعة الطحلب المبيرويلونا بلاتنيس أقل نسبة لموت البادرات وتلاه روائح مزرعة كل من الطحلب نوستوك و أوشلاتوريا.

لوحظ أيضاً من النتائج أن جميع المبيدات الحيوية المستخدمة قللت النمو الميسليومي للفطر سكليروتينيا سكليروشيورم وأعطى المبيد الحيوى ريزو- إن أعلى تثبيط للنمو وتلاه البلانت جارد وكان أقلهم فعالية المبيد الحيوى البيوزيد كما أعطى ريزو- إن أقل نسبة موت للبادرات وأدى إلى زيادة طول كل من الجذر والساق والوزن الرطب وعدد أفرع نباتات الشمر.