

ALLELOPATHIC ACTIVITY OF ALGAL BLOOMS AGAINST SOME PLANT PATHOGENIC FUNGI IN EGYPT

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Abstract

Five algal species forming blooms at North Delta-Egypt, collected from diverse locations, were screened for their efficiency as antibiotic activity against some plant pathogenic fungi. The algal extracts (aqueous and alcoholic) as well as culture filtrate of *Spirulina maxima*, *Oscillatoria agardhii*, *Chlorella vulgaris*, *Ulva lactuca* and *Cladophora albida* were tested against a representative group of fungi. Growth inhibition of the fungal species was detected on exposure to each treatment of the algal species. The results show that *O. agardhii* was the most potent alga with an average growth inhibition of about 49.0 % over all treatments and all tested fungi. The most effective treatment for *S. maxima*, *O. agardhii* and *U. lactuca* was the culture filtrate; meanwhile, the ethanolic extract of both *C. vulgaris* and *C. albida* was the most pronounced treatment over all tested fungi. Complete inhibition was observed to *Sclerotium rolfsii*, *Sclerotinia sclerotiorum* and *Verticillium albo-atrum* on exposure to the culture filtrate of *O. agardhii*. The results are discussed in the light of the ability of these algae to produce bioactive secondary metabolites, secreted into the surrounded medium, which possess an inhibitory effect on the fungal growth.

Introduction

One of the main reasons for interest in allelopathy is that it could be one of the key factors that promote the dominance of marine and freshwater harmful algal bloom-forming species over other phytoplankton species (Legrand *et al.*, 2003). Bioactive secondary metabolites of the massive algal blooms at North Delta–Egypt might provide novel useful and structurally specific compounds with biological activity. Investigations of antibiotic activity of marine algae have focused on the effect of algal extracts on bacteria, with relatively little attention paid to their potential activity against fungi (Tariq, 1991). Microalgae produce a wide array of compounds with biological activity. These include antibiotics, algicides, toxins, pharmaceutically active compounds and plant growth regulators (Skulberg, 2000) as well as compounds active against mammalian cell tissue (Carmichael, 1992).

Blue green algae (cyanobacteria) have been recognized in the last decades as a source of novel cytotoxic and antifungal metabolites; some of these metabolites provide potential leads for the development of new pharmaceutical compounds (Frankmolle *et al.*, 1992a).

Rice (1984) defined allelopathy as any direct or indirect harmful or beneficial effects of one plant, including microbes, on other plants in its vicinity through chemicals that escape into the environment. Allelopathic activity among phytoplankton eutrophied system is usually detected after examining the sequence of the dominant algal species in an algal bloom.

Nevertheless, Amer (2002) claimed that in some cases the allelopathic algicidal activity inferred from dual culture could result from shifting environmental conditions, such as pH, and so appeared to be due to an allelopathic chemical. When these processes are better understood, microalgae might become economic sources of new drugs because production can be optimized in controlled culture.

Due to antifungal activity, Welch (1962) demonstrated that out of 35 marine algae, the red algae *Laurencia obtusa* and *Wrangelia argus* and the blue-green alga *Lyngbya majuscula* were the most effective against the pathogenic fungi. Shelat (1981) found that methanolic extract of *Gelidiella acerosa* was most effective against *Candida tropicalis* and that of *Gracilaria corticata* against *Candida albicans*. A broad-spectrum antifungal substance has been extracted from the culture medium of *Fischerella ambigua* (Ghasemi *et al.*, 2004).

Caccamese *et al.* (1981) demonstrated that the lipid extracts of *Cystoseria balearica* (Phaeophyceae) and *Codium effusum* (Chlorophyceae) showed an inhibitory effect against *Phoma tracheiphila* fungus.

The blue- green alga *Lyngbya majuscula* has been found to produce two antifungal compounds (Moore and Entzeroth, 1988). De Cano *et al.* (1990) demonstrated antifungal activity of the terrestrial cyanobacterium *Nostoc muscorum* against *Candida albicans* to be accomplished by phenolic compounds.

Bonjouklian *et al.* (1991) found that indolo-(2, 3-a)carbazoles (tjipanazoles) of *Tolypothrix tjipanasensis* extracts are responsible for the moderate fungicidal activity against *Candida albicans*, *Trichophyton mentagrophytes* and *Aspergillus flavus*. Also, laxaphycins extracted from the blue - green alga *Anabaena laxa*, exhibited marked antifungal activity against *Aspergillus oryzae*, *Candida albicans*, *Penicillium notatum*, *Saccharomyces cerevisiae* and *Trichopyton mentagrophytes* (Frankmolle *et al.*, 1992b).

Konig and Wright (1997) suggested that the dichloromethane extract of the marine red alga *Laurencia obtusa* has antifungal activity especially towards *Ustilago violacea*. Both the aqueous and methanolic extracts of *Lyngbya majuscula* had antifungal activity against *Aspergillus flavus*, *Dictyosporium cocophilum*, *Westerdykella dispersa* in addition to some unknown species of Basidiomycetes, but *Botrydiplodia theobromae* was affected only by the methanolic extract (Zakaria *et al.*, 2002).

The aim of the present work is to test the harmful allelopathic effect of some algae known to form bloom at North Delta-Egypt against some plant pathogenic fungi namely, *Alternaria alternata*, *Fusarium oxysporum*, *Cephalosporium maydis*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Phoma* sp. and *Verticillium albo-atrum*.

Materials and Methods

The algal species namely *Spirulina maxima*, *Oscillatoria agardhii*, *Chlorella vulgaris*, *Cladophora albida* and *Ulva lactuca* are known to form blooms at North Delta-Egypt were chosen from their natural water habitat during their bloom occurrence to evaluate their harmful allelopathic effect against some plant pathogenic fungi.

Some of these algae were collected from their natural water habitat during their maximum growth period and used directly such as *Chlorella vulgaris*, *Cladophora albida* and *Ulva lactuca*. Fifty grams of the harvested algal materials were rinsed several times with tap water and then thoroughly washed with distilled water, to remove visible epiphytes and calcareous deposits and stored in freezer till extraction. In addition, the liquid left (culture filtrate) after algal harvesting was centrifuged to remove any insoluble materials and then sterilized by passing through filter membrane (0.2 µm) and then tested for antifungal activity.

On the other hand, both collected *Spirulina maxima* and *Oscillatoria agardhii* were thoroughly washed and artificially cultured in the laboratory for propagation. This task was done because of their severely polluted water habitat; hence pure cultures are necessary to avoid interference of the pollutants with algal action. Propagation of both algal species was done using the recommended synthetic media, Zarrouk (1966) for the former and Allen and Stanier (1968) for the latter, both cultured algae were incubated on a rotary shaking incubator at 150 rpm under light intensity of 4000 lux and temperature of 35°C. The propagation was continued up to their appropriate log phase stage (21 days and 15 days, respectively). Algal biomass was then separated from the culture medium and tested individually against some plant pathogenic fungi; the culture filtrate for each alga was also tested against the chosen plant pathogenic fungi.

Representative plant pathogenic fungi, their propagation and maintenance:

The plant pathogenic fungi namely, *Alternaria alternata*, *Fusarium oxysporum*, *Cephalosporium maydis*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Phoma* sp. and *Verticillium albo-atrum* were kindly supplied with the Plant Diseases Research Institute, Agricultural Research Center, Giza, Egypt, and used for experimentation during the present investigation.

Cezpaek's Dox medium (Stevens, 1981) was used to propagate and maintain these selected fungi. Fungi were inoculated on solidified Dox medium for 7-14 days at 28°C. Cultures were then kept in a refrigerator at 4 °C and were occasionally sub cultured every two months on the same medium.

Algal secondary metabolites extraction:

The aqueous extract was prepared by homogenizing 50 g of the frozen algal tissues in 50 mL distilled water for 20 min., followed by centrifugation at 4,000 rpm. The formed pellets were reextracted in 50 mL distilled water. Both formed supernatants were mixed together and kept at 4°C till work up. The ethanolic extract of algal tissues was prepared following the same procedure as in aqueous extraction, except that of 80 % ethanol was used as a solvent instead of distilled water. The supernatant was evaporated to dryness under vacuum at 40 °C and the residue was then dissolved in 50 mL of a mixture of polyethylene- glycol (PEG) 400 and physiological Tris-buffer (pH 7.4) at aratio of 4:6, respectively, and then subjected for testing their antifungal activity according to the procedure described by Tariq (1991).

Allelopathic assay technique:

The dilution plate method described by Tariq (1991) was used to assess the antibiotic activities of algal extracts and culture filtrate against the aforementioned pathogenic fungi. Aliquots (1mL) of the algal extract or culture filtrate, or Tris / PEG (as control) were mixed with 9 mL aliquots of Dox medium and poured into Petri dishes. Each dish was inoculated with a fungus mycelial disc (1.4 cm diameter) from the edge of 7-10 days-old culture of the tested fungus that was previously grown on Dox medium. Three dishes were used for each treatment and the mean radial extension of each culture was determined at regular time intervals, till the growth of each fungus has completely covered all the plate surface of the control treatment, after which the experiment has to be ceased.

All obtained data were statistically analyzed using the Least Significant Difference Comparison between means at the probability of 5% as described by Gomez and Gomez (1984).

Results and Discussion

1. *Spirulina maxima* Geitler:

Data in Table (1) present the biocontrol activity of *Spirulina maxima* against some pathogenic fungi. The various treatments of *S. maxima* led to significant reduction in growth of all the tested fungi, except for *Rhizoctonia solani* when treated with aqueous extract, where fungal growth exhibited a normal growth behavior.

Table (1): Antifungal activity of *Spirulina maxima* against different species of pathogenic fungi.

Fungal species		Treatment				
		Control	Aqueous extract	Ethanollic extract	Culture filtrate	L.S.D. (5%)
<i>Alternaria alternata</i>	L. G.	7.5	4.9	6.0	1.6	0.15
	Red. %	0.0	34.7	20.2	78.7	
<i>Fusarium oxysporum</i>	L. G.	7.5	7.0	6.6	5.9	0.33
	Red. %	0.0	6.7	12.0	21.3	
<i>Cephalosporium maydis</i>	L. G.	7.5	3.7	4.8	1.3	0.43
	Red. %	0.0	50.7	36.0	82.7	
<i>Macrophomina phaseolina</i>	L. G.	7.5	5.5	5.9	4.8	0.35
	Red. %	0.0	26.7	21.3	36.0	
<i>Sclerotium rolfsii</i>	L. G.	7.5	3.8	4.6	2.8	0.28
	Red. %	0.0	49.3	38.7	62.7	
<i>Sclerotinia sclerotiorum</i>	L. G.	7.5	2.4	2.9	1.6	0.19
	Red. %	0.0	68.0	61.3	78.7	
<i>Rhizoctonia solani</i>	L. G.	7.5	7.5	6.1	4.4	0.40
	Red. %	0.0	0.0	18.7	41.3	
<i>Verticillium alboatrum</i>	L. G.	7.5	3.8	5.7	0.9	0.37
	Red. %	0.0	49.3	24.0	88.0	

L.G.= Linear Growth (cm); Red. %= Reduction percentage

Among the various algal treatments used, algal culture filtrate recorded the most drastic effect on the fungal growth (61.2 % average reduction), followed by the aqueous extract (35.7 % average reduction), and the ethanolic extract was the least effective treatment (29.0 % average reduction) due to the tested fungi.

The effect of various treatments on the tested fungi growth was differed according to the fungal species. The highest effect of the aqueous extract treatment was attained against the growth of *Sclerotinia sclerotiorum* (68.0% reduction), compared to other fungi. While, *R. solani* was the only fungal species, which had not affected by the exposure to this treatment.

Likewise, the ethanolic extract was more effective against *S. sclerotiorum* in reducing its growth by 61.3 %, compared to the other tested fungi.

Due to the most adverse effect of the culture filtrate against the tested fungi, *Verticillium alboatrum* and *Cephalosporium maydis* showed the lowest growth in response to this treatment (88.0 % and 82.7 % reduction), respectively.

In conclusion, the present data reveal that the most effective antifungal agent against the tested fungi was the culture filtrate of *S. maxima*. The broad activity might point out to the ability of the alga to produce bioactive secondary metabolites, secreted into the surrounded medium. These bioactive substances seem to hinder the growth of tested fungi with different extents. *S. maxima*, like the other Cyanophytes has the ability to produce a variety of lethal toxins. These

results are in accordance with Hayashi *et al.* (1996) who found that a natural sulfated polysaccharide, calcium spirulan, produced by *S. platensis*, had an antiviral effect. In this respect, Ozdemir *et al.* (2004) found that volatile components and various extracts of *S. platensis* had potent antimicrobial activities against several strains of both Gram-positive and Gram-negative bacteria and *Candida albicans*.

2. *Oscillatoria agardhii* Gomont:

All treatments of *Oscillatoria agardhii* (Table 2) exhibited positive significant effects against the growth of the tested fungal species, except for both *Fusarium oxysporum* and *Phoma* sp. when treated with any of aqueous and ethanolic extracts.

Among the different treatments used, culture filtrate was the most potent in reducing fungal growth (86.6 % average reduction), followed by aqueous extract (39.2 % average reduction) and then ethanolic extract (22.7 % average reduction). Aqueous extract was more active against *S. sclerotiorum* (93.30 % growth reduction). While, *Macrophomina phaseolina* was the most affected fungus among the tested organisms due to the effect of ethanolic extract (50.1 % growth reduction). Culture filtrate led to complete inhibition (no growth) in *Sclerotium rolfsii*, *S. sclerotiorum* and *V. alboatrum*, while, the growth of *Alternaria alternata*, *C. maydis*, *M. phaseolina* and *Phoma* sp. were reduced by 90.1, 98.7, and 89.3 %.

The present results pointed out to a strong drastic effect of culture filtrate of *O. agardhii* against all the tested fungi, leading to complete inhibition of some fungi, surpassing the effect of both aqueous and ethanolic extracts. This broad activity of culture filtrate might be reflecting the presence of bioactive substances produced by the alga and secreted into the bathing medium. These results are concurred with those obtained by (Carmichael, 1992 and Brittain *et al.*, 2000) who found that *Oscillatoria*, a member of cyanobacteria, is capable of producing lethal neurotoxins and hepatotoxins. Moreover, Marwah *et al.* (1995) found that the effect of culture filtrate of the alga *Oscillatoria* sp. was in duplicate and resulted in growth inhibition of the tested organisms. These findings are coincided with the current data, which, revealed that the *Oscillatoria* culture filtrate had the greatest antifungal effect.

While, Mudassir (1995) found that ethanolic extracts of *Chara vulgaris*, *Cladophora fracta* and *Oscillatoria subbrevis* exhibited strong antifungal activity against ten fungal strains. Oxygenated fatty acids (Coriolic and α -dimorphecolic acids) have been isolated from *Oscillatoria redekei*. These compounds possess antifungal activity against the rice blast fungus *Piricularia oryzae* (Kato *et al.*, 1993).

Table (2): Antifungal activity of *Oscillatoria agardhii* against different species of pathogenic fungi.

Fungal species		Treatment				
		Control	Aqueous extract	Ethanollic extract	Culture filtrate	L.S.D. (5%)
<i>Alternaria alternata</i>	L. G.	7.5	4.3	6.0	0.7	0.31
	Red. %	0.0	42.7	20.0	90.1	
<i>Fusarium oxysporum</i>	L. G.	7.5	6.7	6.5	4.9	0.57
	Red. %	0.0	10.1	13.3	34.7	
<i>Cephalosporium maydis</i>	L. G.	7.5	2.6	6.2	0.1	0.28
	Red. %	0.0	65.3	17.3	98.7	
<i>Macrophomina phaseolina</i>	L. G.	7.5	2.8	3.7	0.6	0.31
	Red. %	0.0	62.7	50.1	92.0	
<i>Sclerotium rolfii</i>	L. G.	7.5	6.6	7.5	0.0	0.17
	Red. %	0.0	12.0	0.0	100.0	
<i>Sclerotinia sclerotiorum</i>	L. G.	7.5	0.5	4.9	0.0	0.52
	Red. %	0.0	93.3	34.7	100.0	
<i>Rhizoctonia solani</i>	L. G.	7.5	7.5	4.7	1.9	0.69
	Red. %	0.0	0.0	37.3	74.7	
<i>Phoma</i> sp.	L. G.	7.5	5.7	5.5	0.8	0.58
	Red. %	0.0	24.0	26.7	89.3	
<i>Verticillium alboatrum</i>	L. G.	7.5	4.3	6.9	0.0	0.57
	Red. %	0.0	42.7	8.0	100.0	

L.G.= Linear Growth (cm); Red. %= Reduction percentage

3. *Chlorella vulgaris* Beijerinck:

All *C. vulgaris* treatments (Table 3) led to significant decrease in the linear growth of the testing fungi except for *F. oxysprum*, *M. phaseolina* and *R. solani* when treated with both aqueous extract and culture filtrate, and *V. alboatrum* when treated with aqueous extract only.

Among the three treatments used, ethanolic extract was the most effective leading to an average growth inhibition of about 66.4 % below the control, followed by culture filtrate and aqueous extract, which led to comparable growth reduction of about 18.36 and 16.6 %, respectively.

Nevertheless, the most severe inhibition effect of aqueous extract was against *C. maydis* (61.3 % below the control). While, a strong drastic effect of ethanolic extract was recorded against *R. solani*, leading to complete growth inhibition. *S. rolfii* was the most affected fungus among the tested organisms due to the effect of culture filtrate (61.3 % growth reduction).

The present results suggest that, among the three investigated treatments, ethanolic extract was the most effective against the tested fungi compared to the

aqueous extract and the culture filtrate. However, the inhibitory effect due to the ethanolic extract of *C. vulgaris* may be explained by that *C. vulgaris* produce active substances, soluble in alcohol but insoluble in water, which, lead to selective inhibition of the growth of the tested fungi. These findings are concurred with Pratt *et al.* (1944) who demonstrated that chlorellin, a mixture of fatty acids with antibacterial activity accumulated in the culture of *Chlorella*. Ostensvik *et al.* (1998) and Skulberg (2000) who revealed that methanolic extract of natural blooms of microalgae possessed more pronounced growth inhibition against several strains of bacteria than the aqueous extract. More recently, Abdel-Baky *et al.* (2002) isolated volatile metabolites from the green microalga, *C. vulgaris*, which had a strong phytotoxic effect. In addition, Matusiak and Krzywicka (1975) reported that an ethanolic extract from the terrestrial Chlorophyte, *Chlorella vulgaris* Beij. inhibited the growth of *Aspergillus oryzae*.

Table (3): Antifungal activity of *Chlorella vulgaris* against different species of pathogenic fungi.

Fungal species		Treatment				
		Control	Aqueous extract	Ethanolic extract	Culture filtrate	L.S.D. (5%)
<i>Alternaria alternata</i>	L. G.	7.5	5.9	4.2	6.2	0.33
	Red. %	0.0	21.3	44.0	17.3	
<i>Fusarium oxysporum</i>	L. G.	7.5	7.5	4.5	7.5	0.26
	Red. %	0.0	0.0	40.0	0.0	
<i>Cephalosporium maydis</i>	L. G.	7.5	2.9	1.0	6.3	0.28
	Red. %	0.0	61.3	86.7	16.0	
<i>Macrophomina phaseolina</i>	L. G.	7.5	7.5	3.7	7.5	0.24
	Red. %	0.0	0.0	50.1	0.0	
<i>Sclerotium rolfsii</i>	L. G.	7.5	6.3	1.2	2.9	0.76
	Red. %	0.0	16.0	84.0	61.3	
<i>Sclerotinia sclerotiorum</i>	L. G.	7.5	5.5	2.0	5.3	0.60
	Red. %	0.0	26.7	73.3	29.3	
<i>Rhizoctonia solani</i>	L. G.	7.5	7.5	0.0	7.5	0.00
	Red. %	0.0	0.0	100.0	0.0	
<i>Phoma</i> sp.	L. G.	7.5	5.7	2.5	6.0	0.40
	Red. %	0.0	24.0	66.7	20.0	
<i>Verticillium albo-atrum</i>	L. G.	7.5	7.5	3.5	5.9	0.25
	Red. %	0.0	0.0	53.3	21.3	

L.G.= Linear Growth (cm); Red. %= Reduction percentage

4. *Cladophora albida* (Hudson) Kützing:

The different treatments of *Cladophora albida* (Table 4) exhibited more or less comparable antifungal activity. Nevertheless, ethanolic extract was the

most effective with an average inhibitory effect of about 33.0 % growth reduction below the control, followed by aqueous extract and culture filtrate with an average growth reduction of about 25.2 and 22.8 % below the control, respectively.

Table (4): Antifungal activity of *Cladophora albida* against different species of pathogenic fungi.

Fungal species		Treatment				
		Control	Aqueous extract	Ethanollic extract	Culture filtrate	L.S.D. (5%)
<i>Alternaria alternata</i>	L. G.	7.5	5.7	6.4	7.5	0.23
	Red. %	0.0	24.0	14.7	0.0	
<i>Fusarium oxysporum</i>	L. G.	7.5	4.8	3.3	3.8	0.37
	Red. %	0.0	36.0	56.0	49.3	
<i>Cephalosporium maydis</i>	L. G.	7.5	5.6	4.5	6.4	0.32
	Red. %	0.0	25.3	40.0	14.7	
<i>Macrophomina phaseolina</i>	L. G.	7.5	3.9	3.2	5.7	0.30
	Red. %	0.0	48.0	57.3	24.0	
<i>Sclerotium rolfsii</i>	L. G.	7.5	4.1	2.9	5.6	0.40
	Red. %	0.0	45.3	61.3	25.3	
<i>Sclerotinia sclerotiorum</i>	L. G.	7.5	7.5	7.5	6.9	0.10
	Red. %	0.0	0.0	0.0	8.0	
<i>Rhizoctonia solani</i>	L. G.	7.5	5.9	5.8	5.9	0.49
	Red. %	0.0	21.3	22.7	21.3	
<i>Phoma</i> sp.	L. G.	7.5	6.3	4.8	5.2	0.34
	Red. %	0.0	16.0	36.0	30.7	
<i>Verticillium alboatrum</i>	L. G.	7.5	6.7	6.8	5.1	0.37
	Red. %	0.0	10.7	9.3	32.0	

L.G.= Linear Growth (cm); Red. %= Reduction percentage

With respect to the response of individual fungi to the different treatments, *M. phaseolina*, *F. oxysporum* and *S. rolfsii* were subjected to the most drastic inhibition in growth (48.0, 49.3 and 61.3 %, respectively) under the impact of aqueous, ethanolic extracts and culture filtrate, respectively. By contrast, the least affected fungus was *S. sclerotiorum*, with mild growth inhibition 8.0 % only under the effect of culture filtrate and no growth reductions (0.0 %) under the effect of aqueous and ethanolic extracts.

The marked effectiveness of ethanolic extract against the tested fungi may due to the ability of the organic solvent to extract the bioactive substances produced by the macroalga *Cladophora albida*. This result is in accordance with those obtained by Mudassir (1995) who found that ethanolic extracts of *Chara*

vulgaris, *Cladophora fracta* and *Oscillatoria subbrevis* exhibited strong antifungal activity against ten fungal strains.

Recently, Kamenarska *et al.* (2004) found that the chloroform-n-butanol extract and the volatiles isolated from *Cladophora rivularis* (L.) Hoek, were found to possess marked activity against the Gram - positive *Staphylococcus aureus* with no activity neither against the Gram - negative *Escherichia coli* nor the fungus *Candida albicans*.

5. *Ulva lactuca* Linnaeus:

The three tested treatments of *U. lactuca* (Table 5) had significant positive effects against the different tested fungi and that culture filtrate was the most effective, leading to an average growth inhibition of 38.70 %, followed by aqueous extract (33.2 % growth reduction) and then ethanolic extract (17.9 % reduction).

Table (5): Antifungal activity of *Ulva lactuca* against different species of pathogenic fungi.

Fungal species		Treatment				
		Control	Aqueous extract	Ethanolic extract	Culture filtrate	L.S.D. (5%)
<i>Alternaria alternata</i>	L. G.	7.5	4.9	6.8	3.2	0.34
	Red. %	0.0	34.7	9.3	57.3	
<i>Fusarium oxysporum</i>	L. G.	7.5	1.5	6.8	3.6	0.23
	Red. %	0.0	80.0	9.3	52.0	
<i>Cephalosporium maydis</i>	L. G.	7.5	6.8	6.7	6.6	0.47
	Red. %	0.0	9.3	10.7	12.0	
<i>Macrophomina phaseolina</i>	L. G.	7.5	7.0	5.1	2.9	0.40
	Red. %	0.0	6.7	32.0	61.3	
<i>Sclerotium rolfsii</i>	L. G.	7.5	4.5	1.2	1.1	0.31
	Red. %	0.0	40.0	84.0	85.3	
<i>Sclerotinia sclerotiorum</i>	L. G.	7.5	7.5	7.5	7.5	0.00
	Red. %	0.0	0.0	0.0	0.0	
<i>Rhizoctonia solani</i>	L. G.	7.5	7.5	7.5	7.5	0.00
	Red. %	0.0	0.0	0.0	0.0	
<i>Phoma</i> sp.	L. G.	7.5	0.7	6.3	5.6	0.40
	Red. %	0.0	90.7	16.0	25.3	
<i>Verticillium alboatrum</i>	L. G.	7.5	4.70	7.50	3.40	0.20
	Red. %	0.0	37.3	0.0	54.7	

L.G.= Linear Growth (cm); Red. %= Reduction percentage

Growth of *Phoma* sp. and *S. rolfsii* were subjected to the maximum inhibition under the effect of aqueous, ethanolic extracts and culture filtrate treatments. While, *S.sclerotiorum* and *R. solani* were the only fungal species, which had not affected by the exposure to all algal tested treatments, both species

recorded 0.0 % growth inhibition with both aqueous ethanolic extracts and culture filtrate.

In conclusion, the obtained results indicated that *U. lactuca* can act as a potential source for the bioactive compounds against some pathogenic fungi. Marine macroalgae are well - documented to possess antibacterial and antifungal activities (Vlachos *et al.*, 1996).

Based on fungal response to the different treatments, it can be concluded that the same fungus may respond differentially to the different extracts of the same algal species. Tariq (1991) suggested that different substances in the same seaweed might cause the algal inhibitory effect against the tested fungi.

In agreement with the present results, Lima-Filhol *et al.* (2002) found that water extract of *Ulva fasciata* was effective against *Proteus vulgaris*, but organic solvent extract was not.

Kumar and Rengasamy (2000) observed that *Ulva lactuca* was one of eleven seaweeds that had antibacterial activity against the plant pathogenic bacterium, *Xanthomonas oryzae* pv. *Oryzae*. Also, Awad (2000) reported that *Ulva lactuca* contains steroid compounds, which have antimicrobial activity against various microorganisms.

Recently, Cluzet *et al.* (2004) found that prior treatment of *Medicago truncatula* with *Ulva* extract protected the plants against subsequent infection by the pathogenic fungus *Colletotrichum trifolii*.

In conclusion, the algal species tested in this study against some plant pathogenic fungi revealed the potential effect in affecting the growth of these fungi. The priority was for the culture filtrate of *Oscillatoria agaredhii* against all the tested fungi (86.6 % reduction in the growth). Moreover, this treatment has completely adverse the growth of *S. rolfsii*, *S. sclerotiorum* and *V. alboatrum* (100 % growth reduction).

References

- Abdel-Baky, H. H.; Shallan, M. A.; El-Baroty, G. and El-Baz, F. K.** (2002). Volatile compounds of the microalga *Chlorella vulgaris* and their phytotoxic effect. *Pakistan J. Biol. Sci.*, **5:61-65**.
- Allen, M. M. and Stanier, R. Y.** (1968). Selective isolation of blue-green algae from Water and Soil. *J. Gen. Microbiol.*, **51:203-209**.
- Amer, M. S.** (2002). Antibiotic production by some species of blue-green algae (Cyanobacteria). M. Sc. thesis, Tanta Univ., Egypt, **pp.121**
- Awad, N. E.** (2000). Biologically active steroid from the green alga *Ulva lactuca*. *Phytotherapy Res.*, **14:641-643**.
- Bonjouklian, R.; Smitka, T. A.; Doolin, L. E.; Molloy, R. M.; Debono, M.; Shaffer, S. A.; Moore, R. E.; Stewart, J. B. and Patterson, G. M. L.** (1991). Tjipanazoles, new antifungal agents from the blue-green alga *Tolypothrix tjipanasensis*. *Tetrahedron*, **47:7739-7750**.

- Brittain, S.; Mohamed, Z. A.; Wang, J.; Lehmann, V. K. B.; Carmicheal, W. W. and Rinehart, K. L.** (2000). Isolation and characterization of microcystins from a River Nile strain of *Oscillatoria tenuis* Agardh ex Gomont. *Toxicon*, **38:1759-1771**.
- Caccamese, S.; Azzolina, R.; Furnari, G.; Cormaci, M. and Grasso, S.** (1981). Antimicrobial and antiviral activities of some marine algae from eastern Sicily. *Bot. Mar.*, **24:365-367**.
- Carmichael, W. W.** (1992). Cyanobacteria secondary metabolites-the cyanotoxins. *J. Appl. Bacteriol.*, **72:445-459**.
- Cluzet, S.; Torregrosa, C.; Jacquet, C.; Lafitte, C.; Fournier, J.; Mercier, L.; Salamagne, S.; Briand, X.; Esquerre-Yugaye, M. T. and Dumas, B.** (2004). Gene expression profiling and protection of *Medicago truncatula* against a fungal infection in response to an elicitor from green algae *Ulva* spp. *Plant Cell and Environ.*, **27:917-928**.
- De Cano, M. M. S.; De Mule, M. C. Z.; De Caire, C. 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.*, **1:79-82**.
- Frankmole, W. P.; Knubel, G.; Moore, R. E. and Patterson, G. M. L.** (1992a). Antifungal cyclic peptides from the terrestrial blue-green alga *Anabeana laxa*. II. Structure of laxaphycins A, B, D and E. *J. Antibiotic*, **45:1458-1466**.
- Frankmole, W. P.; Larsen, L. K.; Caplan, F. R.; Patterson, G. M. L.; Knubel, G.; Levine, I. A. and Moore, R. E.** (1992b). Antifungal cyclic peptides from the terrestrial blue-green alga *Anabeana laxa*. I. Isolation and biological properties. *J. Antibiotic*, **45:1451-1457**.
- Ghasemi, Y.; Yazdi, M. T.; Shafiee, A.; Amini, M; Shadman, S. and Zarrini, G.** (2004). Parsigguine, a novel antimicrobial substance from *Fischerella ambigua*. *Pharmaceutical Biology*, **42:318-322**.
- Gomez, K. A. and Gomez, A. A.** (1984). Statistical procedures for Agricultural research, (2nd ed), International Rice Res. Inst. publications. Los Banos, Manila, Philippines. pp. **20-29** and **359-387**.
- Hayashi, T.; Hayashi, K.; Maeda, M. and Kojima, I.** (1996). Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. *J. Natural Products*, **59:83-87**.
- Kamenarska, Z.; Stefanov, K.; Konaklieva, S. D.; Najdenski, H.; Tsvetkova, I. and Popov, S.** (2004). Chemical composition and biological activity of the brackish water green alga *Cladophora rivularis* (L.) Hoek. *Bot. Mar.*, **47:215-221**.
- Kato, T.; Yamaguchi, Y.; Namai, T. and Hirukawa, T.** (1993). Oxygenated fatty acids with anti-rice blast fungus activity in rice plants. *Biosci. Biotechnol. Biochem.*, **57:283-287**.

- Konig, M. G. and Wright, D. A.** (1997). Sesquiterpene content of the antibacterial dichloromethane extract of the marine red alga *Laurencia obtusa*. *Planta Med.*, **63:168-187**.
- Kumar, K. A. and Rengasamy, R.** (2000). Evaluation of antibacterial potential of seaweeds occurring along the coast of Tamil Nadu, India against the plant pathogenic bacterium *Xanthomonas oryzae pv. Oryzae* (Ishiyama) dye. *Bot. Mar.*, **43:409-415**.
- Legrand, C.; Renefors, K.; Fistarol, G. O. and Graneli, E.** (2003). Allelopathy in phytoplankton-biochemical, ecological and evolutionary aspects. *Phycologia*, **42:406-419**.
- Lima-Filhol, J. V. M.; Carvalho, A. F. F. U.; Freitas, S. M. and Melo, V. M. M.** (2002). Antimicrobial activity of extracts of six macroalgae from the Northeastern Brazilian coast. *Braz. J. Microbiol.*, **33(4):311-314**.
- Marwah, J. B.; Shakila, T.M.; Rao, N. S. and Bagchi, S. N.** (1995). Detoxification of a local *Microcystis* bloom by an algicidal antibiotic from *Oscillatoria late-virens*. *Indian J. Exp. Biol.*, **33:97-100**.
- Matusiak, K. and Krzywicka, A.** (1975). Influence of the extract of *Chlorella vulgaris* on growth of fungi. *Acta Microbiol. Polon Ser. B*, **51-54**.
- Moore, R. E. and Entzeroth, M.** (1988). Majusculamide D and deoxymajusculamide D, two cytotoxins from *Lyngbya majuscula*. *Phytochem.*, **27:3101**.
- Mudassir, I.** (1995). Biochemical studies of algae from inland waters of Balochistan. Ph. D. thesis, Balochistan, Quetta Univ., Pakistan.
- Ostensvik, O.; Skulberg, O. M.; Underdal, B. and Hormazabal, V.** (1998). Antibacterial properties of extracts from selected planktonic freshwater Cyanobacteria a comparative study of bacterial bioassays. *J. Appl. Microbiol.*, **84:117-1124**.
- Ozdemir, G.; Karabay, N. U.; Dalay, M. C.; Pazarbasi, B.** (2004). Antibacterial activity of volatile components and various extracts of *Spiurulina platensis*. *Phytotherapy Res.*, **18:754-757**.
- Pratt, R.; Daniels, T. C.; Eiler, J. J.; Gunnison, J. B.; Kumler, W. D.; Oneto, J. F. and Strait, L. A.** (1944). Chlorellin, an antibacterial substance from *Chlorella*. *Science*, **99:351-352**.
- Rice, E. L.** (1984). Allelopathy. Academic Press, Orlando, Florida.
- Shelat, Y. A.** (1981). Screening of Rhodophyceae for antifungal activity. *J. Geobios (Jodhpur)*, **8:51-54**.
- Skulberg, O. M.** (2000). Microalgae as a source of bioactive molecules-experience from Cyanophyta research. *J. Appl. Phycol.*, **12:341-348**.
- Stevens, R. B.** (1981). In: Mycology guidebook. Mycological Society of America, Univ. of Washington Press, USA. **pp. 683**.
- Tariq, V. N.** (1991). Antifungal activity in crude extracts of marine red algae. *Mycol. Res.*, **95:1433-1440**.

- Vlachos, V.; Critchley, A. T. and Von Holy, A.** (1996). Establishment of a protocol for testing antimicrobial activity in southern African macroalgae. *Microbios*, **88:115-123**.
- Welch, A. M.** (1962). Preliminary survey of fungistatic properties of marine algae. *J. Bacteriol.*, **83:97-99**.
- Zakaria, A. M.; Abdel-Wahab, M. A. and El-Sharouny, H. M.** (2002). Antimicrobial activity of Egyptian marine cyanobacterium *Lyngbya majuscula gomont*. *Egypt. J. Phycol.*, **3:84-90**.
- Zarrouk, C.** (1966). Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch. Et Gardner) Geitler. Ph. D.Thesis. University of Paris, France.

النشاط الأليوباثيك لتجمعات الطحالب ضد بعض الفطريات الممرضة للنبات

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لإختبار قدرة الطحالب على إنتاج مواد حيوية مضادة لنمو بعض الفطريات الممرضة للنبات فإنه قد تم اختيار خمسة أنواع من الطحالب المتجمعه بكثافة فى مواقع مختلفة بمنطقة شمال الدلتا, واختبار فاعلية أى من المستخلص المائى والكحولى بالإضافة إلى راشح البيئة لكل من

Spirulina maxima, *Oscillatoria agardhii*, *Chlorella vulgaris*, *Ulva lactuca* and *Cladophora albida*

ضد مجموعة من الفطريات الممرضة. وقد تم تقدير نسبة الانخفاض فى نمو الفطريات عند تعرضها لكل معاملة من معاملات الطحالب على حدة. وقد أظهرت النتائج ان طحلب *O. agardhii* هو أقوى الطحالب تأثيراً, مسجلاً نسبة إنخفاض فى نمو الفطريات قدرها 49.00% فى المتوسط بالنسبة إلى كل المعاملات والفطريات معاً بالمقارنة مع تأثير هذه المعاملات للطحالب الأخرى وأيضاً بالمقارنة مع نمو هذه الفطريات بدون معاملات الطحالب. كما أظهرت النتائج تفوق معاملة راشح البيئة لكل من طحلب *Ulva lactuca*, *S. maxima* and *O. agardhii* بينما سجلت معاملة المستخلص الكحولى (الإيثيلى) لكل من طحلب *C. albida* and *vulgaris* أقوى تأثير ضد مجموعة الفطريات المختبرة, كما لوحظ ان هناك تثبيطاً كلياً لنمو الفطريات التالية

Sclerotium rolfsii, *Sclerotinia sclerotiorum* and *Verticillium alboatrum*
وذلك عند تعرضها لراشح البيئة لطحلب *O. agardhii*. كما أوضحت النتائج قدرة تلك الطحالب على إفراز نواتج ثانوية نشطة يمكنها تثبيط نمو تلك الفطريات.