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Biological control of soil borne cucumber diseases using green marine macroalgae

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Abstract

Background: In this study, the potential of extracts and powders of green seaweeds, *Ulva fasciata*, and *Enteromorpha flexuosa* was evaluated as biocontrol against the pathogenic soil-borne fungi, *Macrophomina phaseolina* and *Fusarium solani*, infecting cucumber plants in Egypt. The antifungal activity of the algal extracts was evaluated in vitro against the pathogens mycelial fungal growth using five organic solvents.

Results: Obtained results indicated that mycelial growth inhibition was noticed with *F. solani* in all algal extracts tested. In *M. phaseolina*, all algal extracts did not inhibit the fungal growth but affected microsclerotia formation (the main source of the second infection). In the case of *F. solani*, the highest reduction (68.6%) was noticed, while the chloroform extract of *U. fasciata* inhibited the radial growth of *F. solani* to 2.5 cm when *E. flexuosa* inhibited the radial growth to 4.3 cm as compared with the control (8.0 cm). The gas chromatography-mass spectrometry and the infrared spectroscopy analyses revealed that iron-monocarbonyl, cyclononasiloxane, and their functional groups, including amine, ether, etc., might play a core role in the anti-fungal activity of the seaweed extracts used.

Conclusion: This work concluded that the macroalgae species with many unique antifungal properties components had an inhibitory effect against soil-borne cucumber diseases. The antimicrobial activity might be explored in the future in numerous diverse applications in agriculture and plant disease control, revealing their actions to control some plant fungal pathogens.

Keywords: Biocontrol, *Ulva fasciata*, *Enteromorpha flexuosa*, Cucumber, Soil-borne fungi

Background

Plant pathogenic fungi are mainly one of the main factors for decreasing food production worldwide (Saharan et al. 2015). Fungicides are widely used for long periods to control plant diseases, but they cause health problems by excessive use of those fungicides presented their use (Kim et al. 2009). Human beings need more safe fertilizers to satisfy for higher growing yield food. Unlike chemical fertilizer, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting, and non-hazardous to humans and animals (Dhargalkar and Pereira 2005). The advantage of using marine algal extracts as a fertilizer due to its fast absorption by the plant within several hours after application and safe to

humans, animals, and the environment (El-Sheekh 2000). Therefore, seaweeds have a vital role in agriculture, reducing the use of pesticides and chemical fertilizer. So, the utilization of seaweeds and their extracts will help achieving higher agricultural production. The seaweed extracts showed antagonistic activity against *Fusarium oxysporum*. The methanolic extract of the marine macroalga, *Cystoseira myrica*, showed higher antifungal activity than *Sargassum cinereum*. Therefore, it is a long time to strengthen an essential substitute for chemical fertilizer not only to maximize production but also to make the environment sustainable. Algal extracts showed antifungal activity against different pathogens, especially horticultural plants (Righini and Roberti 2019). A wide range of results showed antifungal extracts of green algae and diatoms (Mostafa et al. 2014).

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Seaweeds are the multicellular marine algae that inhabit the coastal regions of oceans and seas. Seaweed extracts have been used as bio-fertilizers or anti-pathogenic agents. Extracts have been used as a foliar spray for many crops, including various pulses, cereals, and horticultural crops (El Shafay et al. 2016). The seaweed extracts contain many active components such as vitamins, amino acids, cytokinins, abscisic acid, and auxin-like growth-promoting substances (El-Sheekh et al. 2020). The reduction effect of aqueous extracts of *Ulva fasciata* and *Enteromorpha flexuosa* on the mycelial growth of *Rhizoctonia solani*, *F. solani*, and *Macrophomina phaseolina* revealed that all the macroalgae decreased the mycelial radial growth of *R. solani* and *F. solani*, but with *M. phaseolina*, there was no noticeable reduction in mycelial growth for all the tested macroalgae (El-Sheekh et al. 2018). Seaweed extracts proved to develop tolerance to environmental stress, increase uptake nutrients from the soil (Turan and Köse 2004), and enhances antioxidant properties (Cornish and Garbary 2010). The uses of marine algal extracts have gained popularity because of their potential use in sustainable and organic agriculture, especially in rain-fed crops, to improve mineral absorption and avoid excessive fertilizer application (Harman et al. 2004). Further work is required to find new control strategies more effective and less toxic, like marine algae explored in different regions of the world, and stimulate the growth of different crops.

The present work aimed to determine two major Egyptian green seaweeds antifungal potentiality against two phytopathogenic fungi causing significant economic losses on cucumber plants in addition to the characterization of the algae extracts.

Methods

Macroalgae collection

Green seaweeds were collected from Port Said and Alexandria coasts, Egypt. The algae collected immediately were washed carefully under running fresh water to remove the sand and the other extraneous matter. After that, algae were drained and wiped by blotting sheet, then air-dried at 45 °C for 5 days. Algae were dried entirely and ground in a mechanical grinder, according to Soliman et al. (2018).

Pathogenic fungi

The two plant pathogenic fungi caused significant economic losses on the Cucumber crop, namely, *F. solani* (Mart.) Sacc., and *M. phaseolina* (Tassi) Goid. were obtained from the culture collection of the Mycology Research and Diseases Survey Department.

Macroalgae identification

The collected green macroalgae, *U. fasciata* Delile, and *E. flexuosa* (Wulfen) were identified according to (Papenfuss 1968; Gribb 1983; Womersley 1984; Aleem 1993; Madkour and El-Shoubaky 2007).

Preparation of algal extracts

Five solvents were experimented by ethyl acetate, methanol, benzene, acetone, and chloroform in addition to water by adding 200 ml of each solvent to 50 g of algal powder (V/W). After that, mixtures were shaken for 10 days on an orbital shaker at room temperature (25 °C), then extracts were filtered using cheesecloth, followed by Whatman paper No. 2 (Kumar et al. 2008).

In vitro assay of fungal growth reduction

Dual cultural plates with potato dextrose agar (PDA) medium were used to study the reduction effect of the 2 algal extracts against *M. phaseolina* and *F. solani*, as described by (Kumar et al. 2008). In each plate, 2 wells (5 mm in diameter) were made 4 cm apart. One well was inoculated with (100 µl) each of the tested algal extract. The opposite well was inoculated by a disk (5 mm) of each pathogen (4 days old culture). For each treatment, 3 plates were used. The control plates were inoculated only with each of the pathogenic fungi. All plates were incubated at 25 ± 2 °C for 6–10 days. When the control mycelial fungal growth covered the entire medium surface in plates, all plates were then examined, and the linear growth of the pathogens was measured. The growing cultures were observed visually and microscopically for evidence of a reduction. The percentage of reduction in mycelial growth (X) of the fungal pathogens was calculated using the following the formula of (Nikam et al. 2007):

$$X = [G1-G2/G1] \times 100$$

Where X , % of reduction in growth $G1$, linear growth of pathogenic fungus in control plates

$G2$, linear growth of pathogenic fungus in dual plates with algae extract.

Analysis of crude extracts (chloroform and ethyl acetate) by GC-mass and infra-red (IR)

The gas chromatography-mass spectrometry analysis (GC) for derivatives of chloroform and ethyl acetate extracted filtrates of *U. fasciata* and *E. flexuosa* was chosen in function of the percentage of reduction in mycelial growth from *in vivo* experiment by using an Agilent 6890 series II gas chromatograph. An Agilent 5973 mass spectrometer with electron ionization, mode (EI) generated at 70 eV (ion source at 230 °C and transfer line at 280 °C). The GC was performed using a HP5-MS

capillary column (30 m × 0.25 mm, the film thickness of 0.25 μm). Operating conditions were as follows: carrier gas, helium with a flow rate of (1 ml min⁻¹). The initial temperature was programmed from 80 to 280 °C (at 8 °C min⁻¹) and maintained at 280 °C for 5 min. all compounds were identified by comparison of both the mass spectra (Wiley and Nist library) (Liu et al. 2008; Soliman et al. 2018). The infrared (IR) absorption spectrum of the purified fraction was evaluated to determine the possible functional groups in the algal extracts responsible for bio-reduction for fungal pathogens. This work was estimated at the National Center for Research (He et al. 2016).

In vivo assay of greenhouse experiment

Two algal powders were used in the present study and tested for their potentiality as biocontrol agents against the 2 tested pathogenic cucumber seeds fungi, *M. phaseolina*, and *F. solani*, in greenhouse experiments. Preparations of the algal powders were used in this investigation to evaluate their effect on disease incidence of the 2 fungal pathogens as follows: autoclaved sterilized sandy-loam soil and was infested with sorghum-grain inoculums of each pathogen *M. phaseolina* and *F. solani* at the rate 3% (w/w)/kg soil. Infested pots were irrigated and kept for 7 days to ensure fungi dispersal in the soil before seed sowing. The infested soil was amended with dry algae powders in the ratio (1 g powder of each algal powder: kg soil) w/w at seed sowing. Three seeds were sown in each pot, 4 replicates were used for each treatment. The control treatment was carried out by the chemical fungicide vitavax as seed coating 3 g vitavax/kg seeds before seed sowing. Pots were kept in the greenhouse until the end of the experiment (60 days), and the cucumber's yield was weighted for all treatments. Seeds of cucumber were obtained from the commercial sector in Egypt. Seeds were surface-sterilized in a 2% sodium hypochlorite solution for 2 min before sowing (Sultana et al. 2011).

Statistical analysis

The results of all experiments were statistically analyzed using one-way analysis of variance (ANOVA) to test for significance, and the Fisher test was used for mean separations by the GENSTAT computer package system. Means were made following Fishers LSD (LSD at 0.01 for in vitro experiment, while it was at 0.05 for in vivo experiment) (Gajardo et al. 2017).

Results

In vitro effect of green algal extracts on fungal growth reduction

The antifungal activity of crude extracts, chloroform, methanol, ethyl acetate, acetone, benzene, and aqueous

of *U. fasciata* and *E. flexuosa* against two pathogenic fungi were determined by dual culture plates with PDA. As shown in Table 1, the linear growth of the pathogenic fungi was significantly reduced at different levels. The extracts of *U. fasciata* inhibited the radial growth of *F. solani* to 2.5, 5.6, 6.4, 6.5, 7.5, and 7.7 cm for chloroform, ethyl acetate, water, benzene, acetone, and methanol, respectively. *E. flexuosa* inhibited the radial growth to 4.3, 6.0, 6.6, 6.7, 7.8, and 7.8 cm for chloroform, benzene, ethyl acetate, water, acetone, and methanol, respectively, as compared with the control (8.0 cm). On the other hand, *M. phaseolina* was noticed in all treatments areal mycelia of the fungus (malformation) and no formation of microsclerotia (the main source of the second infection). Figure 1 showed that the effect of different secondary metabolites extracts has great biological activities for cucumber pathogenic fungi.

Analysis of green macroalgae extracts

IR spectroscopy indicates the chemical groups obtained by solvents, which revealed a significant number of chemical groups. Data in Table 2 showed 4 extracts from 2 macroalgae for both solvents, chloroform, and ethyl acetate. The extracted contents revealed various chemical groups, and it was noticed some similarity in these groups obtained by the two macroalgae such as C–O (Ether), N–H (Amine), C=O (Carbonl), N–O (Nitro), C=C (Aromatic), O–H (Alcohol), and others, on the other hand, there were not similar groups obtained by the two macroalgae such as C=C (Aromatic) and N–O (Nitro) with chloroform extracts only.

The chemical analyses of the chloroform and ethyl acetate extracts were conducted by GC-mass. Cyclononasilxane and iron-monocarbonyl compounds were fractions observed in both extracts with different ratios and recorded as an antifungal, antibacterial, and antiviral effect.

In vivo effect of green algal powders on disease incidence

The reduction effect of macroalgae powders showed that all of them not only decreased the damping-off caused by the pathogens under study but also increased the plants survival (dry weight and yield) than the control treated by *F. solani* and *M. phaseolina*, under greenhouse condition (Table 3).

The overall treatments showed different significant degrees in reducing the percentage of dead plants and increased dry weight and yield than the controls. When the plants treated with each of *U. fasciata* and *E. flexuosa* or in a combination, it was noticed that the lowest percentage of dead plants (8.3%) was with those infected with *M. phaseolina*, similarly to that of vitavax fungicidal treatment as compared with the control (66.7%) (Table 3). These results agree with other reports demonstrating

Table 1 Effect of *Ulva fasciata* and *Enteromorpha flexuosa* extracts by using different solvents on the mycelial fungal growth reduction of *Fusarium solani* and *Macrophomina phaseolina* of cucumber on PDA medium

Extraction from green algae by different solvents	<i>Ulva fasciata</i>				<i>Enteromorpha flexuosa</i>			
	<i>Fusarium solani</i>		<i>Macrophomina phaseolina</i>		<i>Fusarium solani</i>		<i>Macrophomina phaseolina</i>	
	Reduction of hyphal growth		Reduction of hyphal growth		Reduction of hyphal growth		Reduction of hyphal growth	
	Cm	%	Cm	%	Cm	%	Cm	%
Ethyl acetate	5.6	26.8	8	0	6.6	17.2	8	0
Methanol	7.7	4	8	0	7.8	1.9	8	0
Acetone	7.5	5.8	8	0	7.8	1.9	8	0
Benzen	6.5	18.9	8	0	6.0	24.6	8	0
Chloroform	2.5	68.7	8	0	4.3	45.8	8	0
Water	6.4	20	8	0	6.7	16.2	8	0
Control	8	0	8	0	8	0	8	0
L.S.D. at 0.01	S = 0.023		F = 0.021		S × F = 0.051			

S solvent, F fungi, S×F solvent × fungi

the efficient green algae as a unique antiprotozoal and anti-mycobacterial agent plus increasing plant yield production (Sultana et al. 2011).

Discussion

The in vitro effect of the green algae, *U. fasciata*, and *E. flexuosa* extracts on fungal growth reduction showed a reduction in the two pathogenic fungi. This effect was determined by dual cultural plates with PDA. This result was in agreement with that of Reis et al. (2018), who found that the extracted compounds produced by *U. fasciata* showed antagonistic effects against fungal pathogens, while Selvin and Lipton (2004) found that *U. fasciata* contained more biological effects as antibacterial than another alga. Also, *E. flexuosa* inhibited the radial

growth of the two pathogenic fungi under investigation. The intensity of the disease on a crop is related to the population of viable sclerotia in abiotic factors)Lodha and Mawar 2020(. All treatments showed different degrees of increase for dry and total weights of cucumber yield than the control, these findings were also in accordance with Sultana et al. (2011).

The results were consistent with Senthilkumar et al. (2014), who showed that the extracts of *Enteromorpha flexuosa* recorded maximum activity against *Streptococcus pyogenes*. On the other side, the effect of aqueous extracts of the same two macroalgae on the mycelial growth of the same two fungi showed less reduction than organic solvents (El-Sheekh et al. 2018). Senthilkumar et al. (2014) emphasized that the presence of bio-active metabolites in green algae, which can be soluble

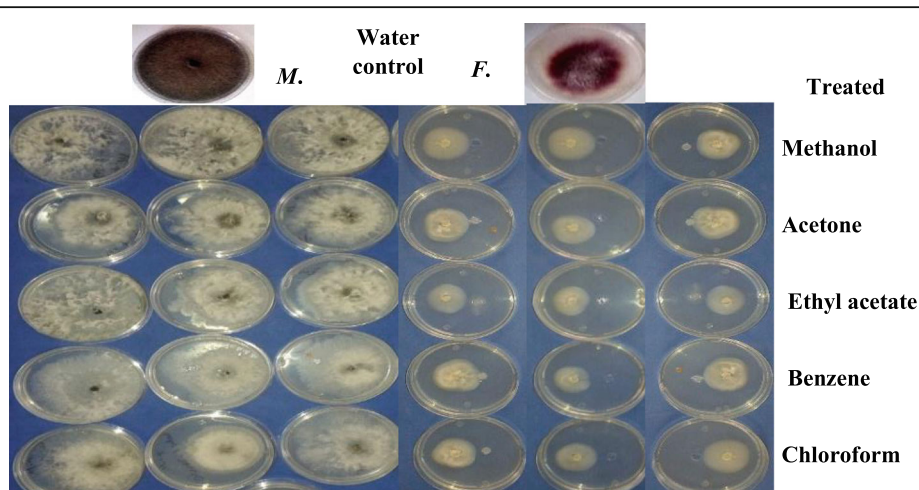


Fig. 1 Effect of different solvent extracts from green macroalgae on mycelial fungal growth of *Macrophomina phaseolina* and *Fusarium solani* on PDA medium in 3 replicates

Table 2 Determination of chemical compounds of partially purified extract filtrate of chloroform and ethyl acetate from *Ulva fasciata* and *Enteromorpha flexuosa* using infrared (IR)

Macroalgae	No.	Chloroform		Ethyl acetate	
		Wave number (cm ⁻¹)	Functional group	Wave number (cm ⁻¹)	Functional group
<i>Ulva fasciata</i>	1	3422.06	N-H (Amine)	3428.81	N-H (Amine)
	2	2960.2	C-H (Alkan)	2990.09	C-H (Alkan)
	3	2920.66	C-H (Alkan)	1763.58	C=O (Carbonyl)
	4	2852.2	C-H (Alkan)	1642.09	C-Br (Alkyl halide)
	5	1708.62	C=O (Carbonyl)	1377.89	C-H (Alkan)
	6	1647.88	C=C (Alkene)	1243.86	C-F (Alkyl halide)
	7	1458.89	C=C (Aromatic)	1054.87	C-O (Ether)
	8	1259.29	C-F (Alkyl halide)	799.35	C-Cl (Alkyl halide)
	9	1026.91	C-O (Ether)	629.644	C=C (Alkene)
	10	861.06	=C-H (Alkene)	442.583	(Alkyl halide)
	11	779.35	C-Cl (Alkyl halide)		
	12	700.034	C-Cl (Alkyl halide)		
	<i>Enteromorpha flexuosa</i>	1	3417.24	N-H (Amine)	3430.74
2		2958.27	C-H (Alkan)	2990.09	C-H (Alkan)
3		2928.38	C-H (Alkan)	1763.58	C=O (Carbonyl)
4		2871.49	C-H (Alkan)	1633.41	C-Br (Alkyl halide)
5		1727.91	C=O (Carbonyl)	1378.85	C-H (Alkan)
6		1646.91	C=C (Alkene)	1243.86	C-F (Alkyl halide)
7		1462.74	C=C (Aromatic)	1053.91	C-O (Ether)
8		1385.6	N-O (Nitro)	799.35	C-Cl (Alkyl halide)
9		1260.25	C-F (Alkyl halide)	621.931	C=C (Alkene)
10		1152.26	C-F (Alkyl halide)	417.513	(Alkyl halide)
11		1027.87	C-O (Ether)		
12		862.989	=C-H (Alkene)		
13		802.242	C-Br (Alkyl halide)		
14		695.212	=C-H (Alkene)		
15		452.225	(Alkyl halide)		

Table 3 Effect of green macroalgal powder treatments on the percentage of dead plants, total dry weight (gm) and total yield (gm) infested with the two pathogens compared with vitavax fungicide after 60 days of planting

Fungi treatment	<i>Fusarium solani</i>			<i>Macrophomina phaseolina</i>		
	% of dead plants	Total dry weight (gm)	Total yield (gm)	% of dead plants	Total dry weight (gm)	Total yield (gm)
<i>Ulva fasciata</i>	50	22.5	57	33.3	34.6	122.4
<i>Enteromorpha flexuosa</i>	41.7	31.5	78.4	50	43.7	149.4
<i>U. fasciata</i> + <i>E. flexuosa</i>	16.7	37.9	136	16.7	31.4	100
vitavax	16.7	11.6	43.2	8.3	16.2	66.9
Control fungus only	50	19.5	25.2	66.7	15	25.2
L.S.D. at 0.05	$F = 0.35$ $T = 0.43$	$F = 1.9$ $T = 2.3$	$F = 0.3$ $T = 0.7$	$F = 0.35$ $T = 0.43$	$F = 1.9$ $T = 2.3$	$F = 0.3$ $T = 0.7$

T treatment, F fungi

in solvents, could be related to the high and low efficiency of organic extracts against microorganisms (Omar et al. 2012). These findings are in accordance with several reports which demonstrated that the extracts from green algae show antiprotozoal and anti-mycobacterial activities (Jimenez et al. 2011). Obtained results also agree with other reports demonstrating the efficient green algae as a unique antiprotozoal and anti-mycobacterial agent plus increasing plant yield production (Sultana et al. 2011).

Gunasena and Senarath (2017) concluded that all components from algal extracts recorded a suppressive effect against different microorganisms like fungi, bacteria, and viruses, which explained the ability of macroalgae *U. fasciata* and *E. flexuosa* extracts and powders to decrease the fungal growth of the 2 pathogens infecting cucumber plants.

Further studies are needed to explore the functional mechanisms of these green macroalgae and their vehicles to reduce fungal growth and factors, which affect *M. phaseolina* as malformation of growth, as mentioned earlier. It can be concluded that green macroalgae contained various compounds with different antimicrobial characterization might be responsible for the interesting results in controlling pathogens and promoting plant growth. Finally, macroalgae containing compounds with different antimicrobial properties might be responsible for obtained interesting results regarding the control of the pathogen. However, more investigations are needed to explore and identify the chemical and biochemical compounds involved in the presented macroalgae extracts. Those further studies will not only help to understand how those natural compounds are able to inhibit the fungal pathogens but also how they participate in enhancing the cucumber plant growth.

Conclusion

The green macroalgae, *U. fasciata* and *E. flexuosa* can be used as an excellent alternative bioagents to control pathogenic soil-borne fungi, *M. phaseolina*, and *F. solani*, which may limit the extensive use of chemical fungicides as the plant disease management. Depending on the obtained results, it was found that the green macroalgae also can act as a potential source of nutrients for plant growth and improving its yield. The results also proposed that the macroalgae species with unique antimicrobial properties might be explored in the future in numerous diverse applications in agriculture and plant disease control, revealing their actions to control some plant fungal pathogens.

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Authors' contributions

AYA carried out the experimental work. MME, ASS, SEA-G, and HMS supervised and suggested the problem. MME helped in the experimental work. All authors contributed writing draft and MME finalized and the manuscript in the final form. All authors have read and approved the manuscript.

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Consent for publication

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Competing interests

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