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Antifungal Activity of Some Mediterranean Seaweed Against *Macrophomina phaseolina* and *Fusarium Oxysporum in Vitro*

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ABSTRACT

Seaweeds are naturally rich with biological active metabolites that could be promising to be used in the biological control. The present study was conducted to evaluate the antifungal potentiality of metabolites extracted from *Ulva fasciata*, *Ulva lactuca* and *Cladophora sericea*; against *Macrophomina phaseolina* (Tassi) Goid. and *Fusarium oxysporum* Schltdl. Four organic solvents hexane, chloroform, acetone and methanol were used for extraction of seaweeds metabolites to assay their biological efficiency on mycelium growth reduction of two candidate soil borne fungal pathogens on potato dextrose agar medium. The highest percentage of *F. oxysporum* and *M. phaseolina* growth reduction was observed by acetone extract of *U. fasciata* and methanol extract of *C. sericea*. The highest inhibition against *M. phaseolina* reached up to 28.97% by *U. fasciata* acetone extract and 24.77% by *C. sericea* methanol extract. The highest percentage of mycelial growth inhibition of *F. oxysporum* was observed by *U. fasciata* acetone extract (23.58%) and *C. sericea* methanol extract (17.01%). Analysis of acetone extract of *U. fasciata* by gas chromatography mass spectrophotometer GC/MS revealed the presence of some organic compounds with antifungal properties such as Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-, Di-n-octyl phthalate, 1,2-Benzenedicarboxylic acid, diisodecyl ester, Didecyl phthalate, Phthalic acid, bis (7-methyloctyl) ester, gamma Sitosterol, Cholest-5-en-3-ol, 24-propylidene-, (3. beta.) and Cyclononasiloxane, octadecamethyl-. The results suggest that the studied algal species can be used as potential bio-agent source in biological control of soil borne phytopathogenic fungi.

Keywords

Cladophora sericea, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Ulva fasciata*, *Ulva lactuca*.

1. INTRODUCTION

Seaweeds produce a wide range of promising bioactive metabolites that have broad spectrum of biological activities such as antifungal, antibacterial and antioxidant activity. In recent years, there is an urgent need to explore novel natural products to overcome the problem of increasing the resistance of microorganisms [1]. Many researches are focused on crude extracts of seaweeds collected from Mediterranean seashores because of their potent antimicrobial action [2]. Bioactive secondary metabolites

that produced by marine macroalgae are several like, Alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, polyphenols, sterols, quinones, lipids and glycerols, most of them have antimicrobial activities [3]. Chemical composition differs between seaweeds according to species, maturity, habitats and environmental conditions [4].

Fusarium oxysporum and *Macrophomina phaseolina* are ubiquitous soil-borne pathogenic fungi that affect the root system of plants or the stem, in some cases developing on upper parts of the plant through transport or growth in the vessels, leading to vascular diseases. Such pathogens can cause economic loss because of plant yield loss [5]. Seaweeds extracts have antifungal activity against soil borne plant pathogenic fungi in addition it can stimulate plant growth [6]. Shobier *et al*., [7] reported that ethyl acetate and methanol extracts of *U. lactuca* collected from Alexandria Mediterranean coast showed high antifungal activities against *Fusarium solani, Fusarium oxysporum, Tricoderma hamatum, Aspergillus flavipes* and *Candida albicans*. Red alga like *Gracilaria confervoides* extracts reduced the radial growth of some pathogenic fungi such as *Rhizoctonia solani* and *Fusarium solani* [8]. Furthermore, Galal *et al*., [9] recorded positive antifungal activities of *Codium fragile* methanol extract against *Alternaria alternate, Fusarium oxysporum, Alternaria brassicicola, Ulocladium botrytis* and *Botryotricum piluliferum*.

This research aimed to: 1) assess the antifungal activity of three Mediterranean seaweeds taxa collected from Port Said governorate against two soil borne phytopathogenic fungi namely *M. phaseolina* and *F. oxysporum* under laboratory conditions and 2) detect bioactive compounds in algal crude extract by GC/MS analysis.

2. MATERIALS AND METHODS

2.1 Seaweed collection and processing

Seaweeds were harvested at morning during October and November 2017 from rocky substrate at the Mediterranean Egyptian coast at Port Said (31.276929 N, 32.2680327 E) (Fig. 1.).

Algal samples were hand-picked, washed with sea water to remove sand particles, invertebrate and epiphytes and were transported to the laboratory immediately in plastic bags half filled with sea water to avoid dryness. Samples were washed thoroughly with tap water and then with distilled water to remove adhering substances and salts and were dried under the shade at room temperature for 7 days. Air dried samples were ground with electric blender to a fine powder and transferred to labeled glass bottles for screening their antifungal activity [10]. The algal species were identified based on their morphology and anatomy using Seaweeds-a field manual [11], Seaweeds of India: the diversity and distribution of seaweeds of Gujarat coast [12] and A Field Guide to the British Seaweeds: As required for assistance in the classification of water bodies under the Water Framework Directive [13].

Fig. 1. (A) Map showing the collection site of seaweeds and (B) The area in where seaweeds were collected.

2.2 Extract preparation for antifungal assay

Two gram of each dried algal powder was successively extracted by maceration with continuous shaking for 24 hours using different organic solvents; hexane, chloroform, acetone and methanol (1:10; w/v). The crude extract was obtained through evaporation of the organic solvent and then re-weighted. The percentage of each extract was determined as according to equation [14]:

$$\text{Extract \%} = (\text{Weight of extract (g)} / \text{Weight of sample (g)}) \times 100$$

The dried crude extracts were dissolved in dimethyl sulfoxide (DMSO), then sterilized by Millipore filtration method (0.22µm) [6] and stored in refrigerator for further use. The concentration of each extract was calculated according to the following equation (Concentration= weight of extract (mg) / volume of solvent (ml)) [15].

2.3 Isolation of phytopathogenic fungi

Fusarium isolation was prepared from collecting the tomato root naturally infested tissues. Small pieces (*ca.*≤ 9 mm^2) from individual root samples were cut and placed on potato dextrose agar (PDA) medium in a Petri dish and incubated at 28°C in the dark for 7 days. The identification as *F. oxysporum* is based on morphological characteristics [16].

Potato tuber symptomatic plants were surface sterilized by 0.5% NaOCl for 3 min. Cross sections were made in tuber to observe the affected area. It was disinfected with70% ethanol for 30 s. 5 mm necrotic tissue were cultivated on (PDA) amended with 500µL-1ml chloramphenicol. Plates were incubated at 25°C for 6 days. Macrophomina identification was based on the growth characteristics, agar pigmentation and the presence of microsclerotia [17].

2.4 Antifungal activity of algal extracts (*in vitro* assay)

The antifungal activity of algal extracts against two phytopathogenic fungal candidates were assayed *in vitro* on PDA media; 200g sliced potato, 20g dextrose, 15g agar and 1000mL distilled water [18] and a well cut technique was adopted [19]. Two wells with 5 mm in diameter were punched in solid PDA medium and one was filled with 100 µl of seaweeds extract and the other was filled with 100 µl of DMSO and this is

considered a negative control. Between the two wells fungal pathogen disk was inoculated. Positive control was performed by using two synthetic antifungals miconazole and nystatin.

Plates were incubated at 30^{O}C and the percentage of fungal radial growth inhibition was recorded after three & four days in case of *M. phaseolina* and till 6 days in case of *F. oxysporum*. Percentage of mycelia growth inhibition (MGI %) was calculated using the following index [6]:

$$MGI\% = [(Control - Test)/Control] \times 100$$

(Control = fungus radial growth from the middle of fungal disc toward the negative control well, Test= fungus radial growth from the middle of fungal disc toward the extract well).

2.5 Minimal inhibitory concentration (MIC) assay

Seaweeds extracts were serially diluted by DMSO to obtain diluted concentrations in the range of 25, 50 and 75 %. Four wells were punched in PDA media. All wells were inoculated with 100 µl of diluted extracts except the fourth well which was considered as negative control was inoculated with 100 µl DMSO. The fungal disk was inoculated in the center. The MIC value was taken as the lowest concentration of extract which inhibit the growth of the tested phytopathogenic fungi [20].

2.6 Analysis of *U. fasciata* metabolic profiles by GC-MS

U. fasciata acetone extract which showed the highest antifungal activity against *M. phaseolina* and *F. oxysporum* was analyzed using GC-MS.

Mass spectra were recorded using Shimadzu GCMS-QP2010 (Koyoto, Japan) equipped with Rtx-5MS fused bonded column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Restek, USA) equipped with a split–splitless injector. The initial column temperature was kept at 50°C for 3 min (isothermal) and programmed to 200°C at a rate of 15 °C/min, and kept constant at 200°C for 5 min (isothermal). Then the Temperature was programmed to 240°C at a rate of 3°C/min, and kept constant at 240°C for 10 min (isothermal). Finally, the temperature was programmed to 300°C at a rate of 4°C/min, and kept constant at 300°C for 10 min (isothermal). Injector temperature was 280°C. Helium carrier gas flow rate was 1.41 ml/min. All the mass spectra were recorded applying the following condition: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 220°C. Diluted samples (1% v/v) were injected with split mode (split ratio 1: 15).

2.7 Statistical analysis

The results were expressed as mean of three replicates ± standard error. Data was statistically analyzed by using analysis of variance (ANOVA) at $P < 0.05$ and means were separated using the least significant difference (LSD). Statistical analysis was done with IBM SPSS Statistics version 25 [21].

3. RESULTS AND DISCUSSION

3.1 Identification of the collected seaweeds

Seaweeds collected from the Mediterranean seashores at Port said were identified as (A) *Ulva lactuca* L, (B) *Ulva fasciata* Delile and (C) *Cladophora sericea* (Hudson) Kützing as shown in Fig. 2.

3.2 Percentage of Crude extract of seaweeds according to different organic solvents

Organic solvent used in extraction process has a significant effect on extract concentration as shown in Fig. 3. The total crude extract was about 8.73, 12.87 and 15.605 in case of *U. fasciata*, *U. lactuca* and *C. sericea* respectively as shown in fig. 3. Methanol maintained the highest percentage of extract in all algal species and that similar to Abdel-Aal *et al.* [14] who use five organic solvents to extract metabolites of *Spirogyra longata*.

Fig. 2. Morphological features of the collected marine algal species; A. *U. lactuca*, B. *U. fasciata* and C. *C. sericea*.

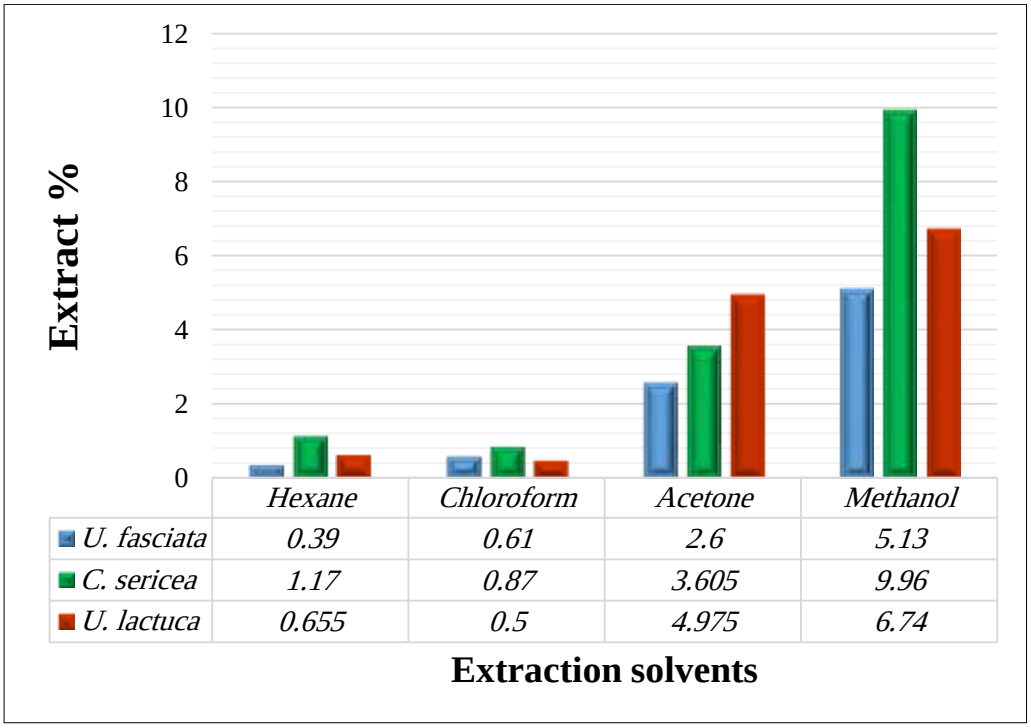

Fig. 3. Percentage of crude extracts from *U. fasciata*, *C. sericea* and *U. lactuca* by four organic solvents.

3.3 Antifungal activity of seaweeds extracts against *M. phaseolina* and *F. oxysporum* (in *vitro* assay)

Data of tables 1 and 2 showed that both of phytopathogenic fungal candidates were inhibited by *U. fasciata* acetone extract and the highest inhibitory effect recorded against *M. phaseolina* reached up to 28.98%. In table 2, there is no significant difference between miconazole and acetone extract of *U. fasciata* in their inhibitory action against *M. phaseolina*. The inhibitory effect of *U. fasciata* acetone extract (24.78 mg/ml) against *F. oxysporum* and both of *U. lactuca* hexane extract (6.5 mg/ml) and *C. sericea* methanol extract (49.8 mg/ml) against *M. phaseolina* was higher than miconazole and nystatin. Similar finding was matching with El-Sheekh & Soliman [22] who found that *U. fasciata* was effective bioagent in inhibiting the mycelial radial growth of *F. solani* and *Rhizoctonia solani* on PDA medium in comparing with control.

U. lactuca methanol extract was not effective in mycelium growth inhibition of *F. oxysporum* and *M. phaseolina* as shown in table 3 and 4 and these results disagree with Zouaoui & Ghalem [23] who reported that methanol extract obtained from *U. lactuca* showed antifungal activity for *Candida albicans*. There is no significant difference in the inhibitory effect between positive control miconazole and *U. lactuca* hexane extract at 4.91 mg/ml concentration.

C. sericea methanol extract at 49.8 mg/ml and *U. fasciata* acetone extract at 24.78 mg/ml have a role in inhibiting the growth of *M. phaseolina* as shown in table 2 and 6. The results are not matching with El-Sheekh & Soliman [22] and Soliman *et al*. [8] who reported that there is no linear growth reduction of *M. phaseolina* by extracts of *Gracilaria confervoides, U. fasciata* and *Enteromorpha flexuosa*.

C. sericea hexane, chloroform and acetone extracts show non-significant results in both *F. oxysporum* and *M. phaseolina* as shown in table 5. *F. oxysporum* mycelium growth was inhibited by *C. sericea* methanol extract (17.01%). Methanol extract of *C. sericea* was found high effective against *F. oxysporum* and *M. phaseolina* and methanol extract of other seaweeds was not effective against any pathogens. Khan *et al.* [24] reported that most of the seaweeds inhibited growth of *F. oxypsorum, M. phaseolina* and *R. solani*. The highest antifungal activities were observed in both aqueous and methanol extracts and this matching with our data.

Obtained data revealed that acetone was the most effective extraction solvent with significant antifungal activity followed by methanol and this finding is contrasted to Al-Saif *et al.* [25] who found that chloroform was the most effective solvent.

M. phaseolina was more sensitive and showed higher radial growth reduction by seaweed extracts than *F. oxysporum*. The results are in agreement with the results of Galal *et al.*[9] who proved that *F. oxysporum* was less affected to algal extract.

The difference in this study results and other studies could attributed to difference in tested pathogenic fungi, type of algal species, extraction solvent and extraction method. The difference in chemical composition of seaweeds differs according to seaweeds species, habitats and environmental conditions.

3.4 Minimal inhibitory concentration (MIC) assay

Most of seaweeds extracts inhibit the growth of *M. phaseolina* and *F. oxysporum* at concentration of the crude extract. *U. lactuca* hexane extract inhibit the growth of *M. phaseolina* at concentrations of 6.55 mg/ml and 4.91 mg/ml and both concentrations were not effective against *F. oxysporum* as shown in table 3. In table 5, mycelium reduction of *M. phaseolina* and *F. oxysporum* was by methanol extract of *C. sericea* at 49.8 mg/ml only. Recorded results was not matched with Ibraheem *et al*., [26] who found that methnol extract of *Sargassum latifolium* and *Padina gymnospora* was effective in mycelium reduction of *Fusarium solani* and *Rhizoctonium solani* at all concentrations.

Inhibitory effect of different concentration of *F. oxysporum* by methanol extract of *C. sericea* was clear in Fig.4 (I), While Fig.5 showed *M. phaseolina* mycelium inhibition at 24.7 mg/ml concentration by *C. sericea*

methanol extract and *U. fasciata* acetone extract. Fig 6. clarifies that M. phaseolina growth inhibited at 4.91 mg/ml (75%) and other lower concentrations were not effective in growth inhibition.

Table 1. Determination of MIC for different extraction solvents of *U. fasciata* against *F. oxysporum* and *M. phaseolina*

Hexane	Concentration (mg/ml)	**1.3**	**2.6**	**3.9**	**5.2**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	-	-
Chloroform	Concentration (mg/ml)	**1.52**	**3.05**	**4.57**	**6.1**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	-	-
Acetone	Concentration (mg/ml)	**6.19**	**12.39**	**18.58**	**24.78**
	F. oxysporum	-	-	-	+
	M. phaseolina	-	-	-	+
Methanol	Concentration (mg/ml)	**8.41**	**16.83**	**25.25**	**33.67**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	-	-

(+) means there is an antifungal activity to extract and (–) means there is no antifungal activity was detected to extract

Table 2. MGI % of phytopathogenic fungal candidates by different extraction solvents of *U. fasciata* resulting from MIC

Treatments	**Conc**	***F. oxysporum*** **MGI %**	***M. phaseolina*** **MGI %**
Miconazole	0.03 mg/ml	**24 ± 1.76** [a]	**10.76 ± 2.98** [e]
Nystatin	0.03 mg/ml	**12.2 ± 2.32** [c]	**14.86 ± 0.63** [f]
Hexane	5.2 mg/ml	0 [b]	0 [d]
Chloroform	6.1 mg/ml	0 [b]	0 [d]
Acetone	24.78 mg/ml	**23.58 ± 0.25** [a]	**28.98 ± 0.74** [g]
Methanol	33.67 mg/ml	0 [b]	0 [d]
LSD at $p \leq 0.05$		**3.69**	**3.95**

All values are mean (n=3) ± standard error.

Values with the same letters in the same column are not significantly different at $p \leq 0.05$

Table 3. Determination of MIC for different extraction solvents of *U. lactuca* against *F. oxysporum* and *M. phaseolina*

Hexane	Concentration (mg/ml)	**1.63**	**3.27**	**4.91**	**6.55**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	+	+
Chloroform	Concentration (mg/ml)	**1**	**2**	**3**	**4**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	-	-
Acetone	Concentration (mg/ml)	**6.21**	**12.43**	**18.65**	**24.87**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	-	-
Methanol	Concentration (mg/ml)	**6.12**	**12.24**	**18.36**	**24.48**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	-	-

(+) means there is an antifungal activity to extract and (–) means there is no antifungal activity was detected to extract

Table 4. MGI % of phytopathogenic fungal candidates by different extraction solvents of *U. lactuca* resulting from MIC

Treatments	**Conc**	***F. oxysporum*** **MGI %**	***M. phaseolina*** **MGI %**
Miconazole	0.03 mg/ml	**24 ± 1.76** [a]	**10.76 ± 2.98** [e]
Nystatin	0.03 mg/ml	**12.2 ± 2.32** [b]	**14.86 ± 0.63** [f]
Hexane	6.5 mg/ml	0 [c]	**22.97 ± 0.98** [g]
Hexane	4.91 mg/ml	0 [c]	**10.46 ± 0.12** [e]
Chloroform	4 mg/ml	0 [c]	0 [d]
Acetone	24.8 mg/ml	0 [c]	0 [d]
Methanol	24.4 mg/ml	0 [c]	0 [d]
LSD at $p \leq 0.05$		**3.35**	**3.68**

All values are mean (n=3) ± standard error.

Values with the same letters in the same column are not significantly different at $p \leq 0.05$

Table 5. Determination of MIC for different extraction solvents of *C. sericea* against *F. oxysporum* and *M. phaseolina*

Hexane	Concentration (mg/ml)	**4.87**	**9.75**	**14.62**	**19.5**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	-	-
Chloroform	Concentration (mg/ml)	**1.45**	**2.9**	**4.35**	**5.8**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	-	-
Acetone	Concentration (mg/ml)	**5.63**	**11.26**	**16.89**	**22.53**
	F. oxysporum	-	-	-	-
	M. phaseolina	-	-	-	-
Methanol	Concentration (mg/ml)	**12.45**	**24.9**	**37.35**	**49.8**
	F. oxysporum	-	-	-	+
	M. phaseolina	-	-	-	+

(+) means there is an antifungal activity to extract and (–) means there is no antifungal activity was detected to extract

Table 6. MGI % of phytopathogenic fungal candidates by different extraction solvents of *C. sericea* resulting from MIC

Treatments	**Conc**	***F. oxysporum*** **MGI %**	***M. phaseolina*** **MGI %**
Miconazole	0.03 mg/ml	**24 ± 1.76** [a]	**10.76 ± 2.98** [f]
Nystatin	0.03 mg/ml	**12.2 ± 2.32** [b]	**14.86 ± 0.63** [g]
Hexane	19.5 mg/ml	0 [c]	0 [e]
Chloroform	5.8 mg/ml	0 [c]	0 [e]
Acetone	22.5 mg/ml	0 [c]	0 [e]
Methanol	49.8 mg/ml	**17.01 ± 0.17** [d]	**24.77 ± 0.79** [h]
LSD at $p \leq 0.05$		**3.68**	**3.97**

All values are mean (n=3) ± standard error.

Values with the same letters in the same column are not significantly different at $p \leq 0.05$

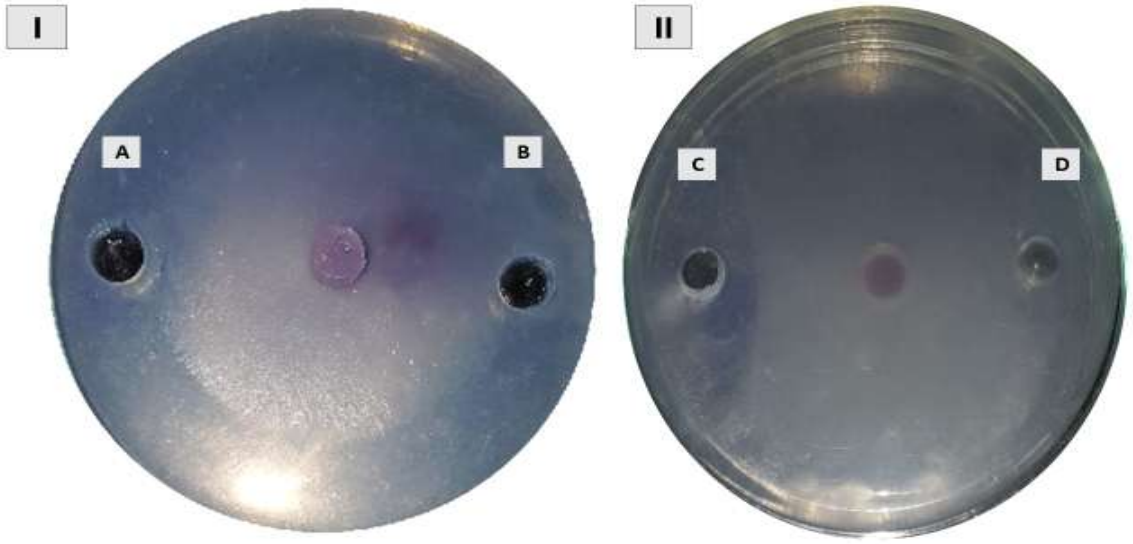

Fig.4. **(I)** Mycelial growth inhibition of *F. oxysporum* by methanol extract of *C. sericea*; A. represents methanol extract *C. sericea* dissolved in DMSO and B. represents DMSO only. **(II)** Effect of miconazole (positive control) on *F. oxysporum* growth; C. refers to miconazole that is dissolved in water and D. refers to water.

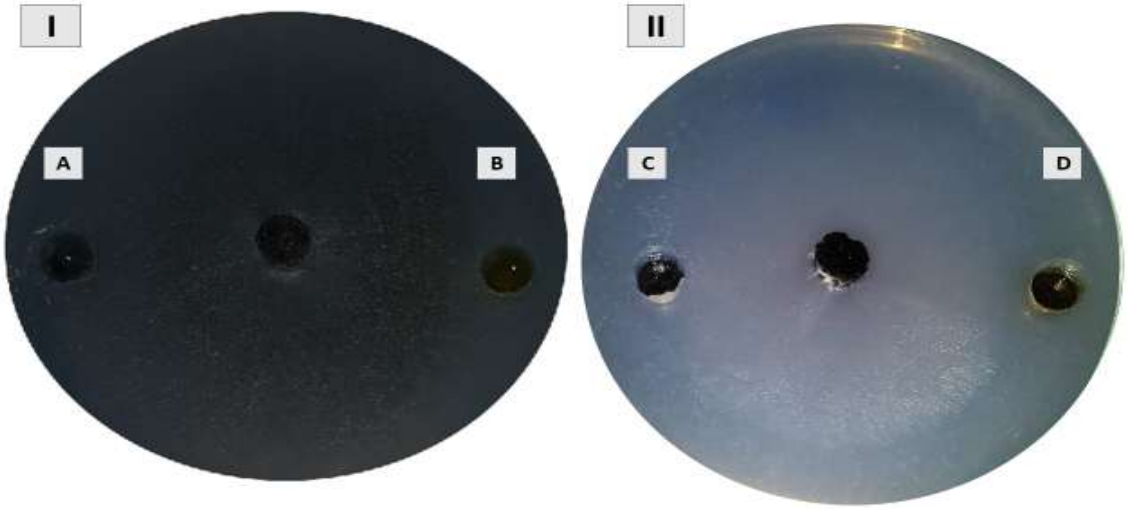

Fig. 5. **(I)** Mycelial growth inhibition of *M. phaseolina* by *C. sericea* methanol extract; A. refers to DMSO only and B. refers to *C. sericea* methanol extract dissolved in DMSO. **(II)** Growth inhibition of *M. phaseolina* by *U. fasciata* acetone extract; C. refers to DMSO only and D. refers to by *U. fasciata* acetone extract.

Fig. 6. MIC of *U. lactuca* hexane extract; A. represents 75% concentration of extract (4.91 mg/ml) and this concentration inhibit *M. phaseolina* growth, B. represents 50% concentration of extract (3.27 mg/ml), C. represents 25% concentration of extract (1.63 mg/ml) and D. represents DMSO only. B, C and D show that there is no effect of DMSO and extract at these concentrations (50 and 25%) on *M. phaseolina* growth.

3.5 Analysis of *U. fasciata* metabolic profiles by GC-MS

Results of table 7 revealed that eleven bioactive compounds were detected in *U. fasciata* acetone fraction using GC/MS. The most abundant compounds are gamma Sitosterol**,** Didecyl phthalate, 1,2-Benzenedicarboxylic acid, diisodecyl ester**,** Cyclononasiloxane, octadecamethyl- and Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl-. Compounds result from GC/MS analysis may be the reason of *M. phaseolina* and *F. oxysporum* growth inhibition by *U. fasciata* acetone extract.

Seaweeds can be considered as a diverse source of bioactive metabolites due to harsh environmental conditions in which they exist. Macroalgae metabolites act as effective control measures and possess antifungal, antibacterial, antiviral and other biological activities [27]. Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- that is detected by GC-MS is one of the powerful compound with antifungal, antibacterial, antiseptic, antioxidant and germicidal properties due to its toxic potential [28]. It was reported that gamma-sitosterol has antimicrobial and antiviral activity [29]. It was proven that plants or algae such as *Lythrum* sp. and *Sargassum* sp. that usually grow in water flow can synthesize phthalates that are known of their antimicrobial activity [30].

Results suggest that the antifungal activity of *U. fasciata* acetone extract may be due to the activity of only one compound or through a synergetic effect between all identified powerful bioactive compounds with antifungal action.

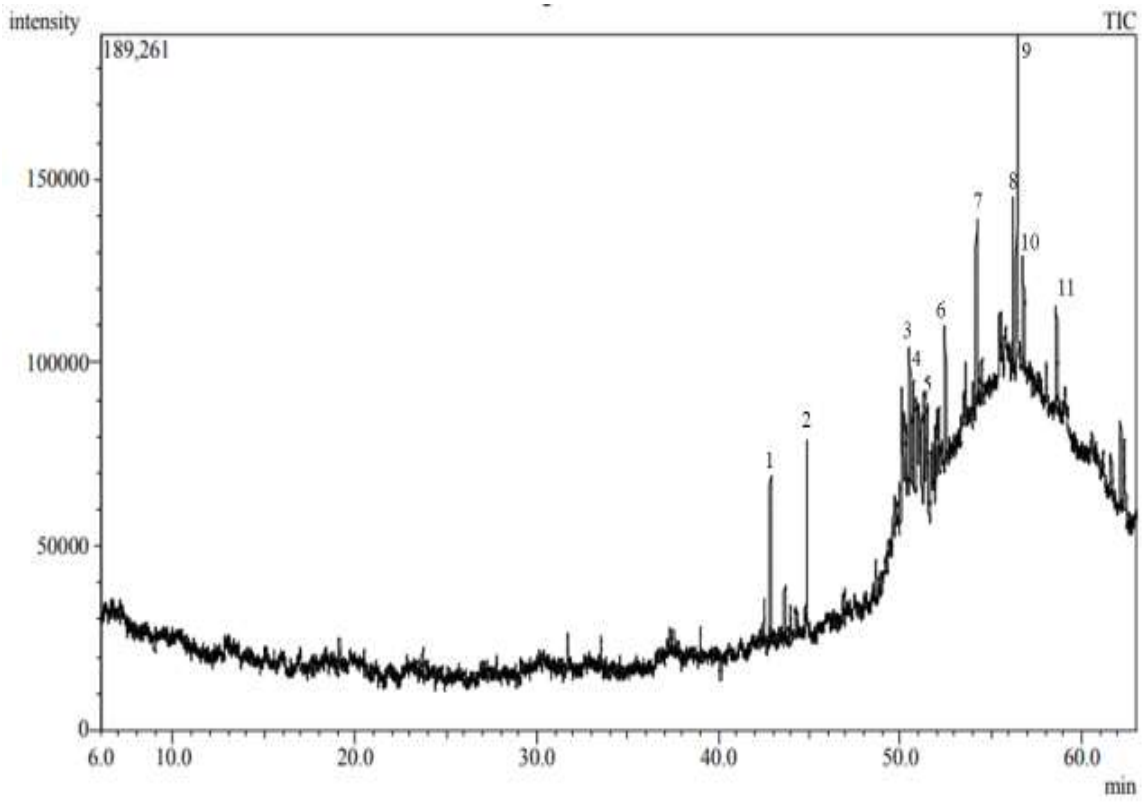

Fig. 7. Total ion chromatogram of GC-MS of *U. fasciata* acetone extract.

Table 7. GC/MS analysis of bioactive volatile compounds of *U. fasciata* acetone extract

No.	R_t	Compound name	Molecular formula	Molecular weight	Base peak m/z	Area %
1	42.852	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- (Isomer 1)	$C_{23}H_{32}O_2$	340	177.10	7.08
2	44.841	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	149.05	7.03
3	50.506	1,2-Benzenedicarboxylic acid, diisodecyl ester	$C_{28}H_{46}O_4$	446	149	8.93
4	50.720	Didecyl phthalate	$C_{28}H_{46}O_4$	446	148.95	10.08
5	51.328	Phthalic acid, bis(7-methyloctyl) ester	$C_{26}H_{42}O_4$	418	149	6.14
6	52.475	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- (Isomer 2)	$C_{23}H_{32}O_2$	340	177.10	4.45
7	54.196	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- (Isomer 3)	$C_{23}H_{32}O_2$	340	177.10	7.45
8	56.192	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- (Isomer 4)	$C_{23}H_{32}O_2$	340	177.10	8.10
9	56.429	.gamma.-Sitosterol	$C_{29}H_{50}O$	414	43.05	21.77
10	56.764	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	$C_{30}H_{50}O$	426	55.05	6.32
11	58.596	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	73.05	8.17
Total identified						95.52

4. CONCULSION

The results of the present study revealed that *U. fasciata* acetone extract was the most effective extract in mycelial growth reduction of *M. phaseolina* and *F. oxysporum*. It confirmed by GC-MS analysis that several bioactive compounds were recorded in *U. fasciata* acetone extract like Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- and gamma-sitosterol. Thus, this potent extract has a promising future scope to be used as biocontrol measure against diseases caused by soil borne phytopathogenic fungi; as eco-friendly, natural and cheap fungicide.

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