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Antifungal activity of volatiles emitted from living cultures of *Chlorella vulgaris, Desertifilum tharense,* and *Navicula arenaria*

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ABSTRACT

The potential of algae, plants, fungi, and bacteria to produce a wide spectrum of volatile organic compounds (VOCs) including terpenes, alkanes, alkenes, alcohols, ketones, aldehydes, benzenoids, esters, and sulfo compounds is remarkable. Thus, the antifungal activity of volatiles emitted from *D. tharense*, *C. vulgaris,* and *N. arenaria* was evaluated against *M. phaseolina* and *F. oxysporum* under living conditions through a system that allows the exchange of VOCs between algae and phytopathogenic fungi without physical contact between them. Volatiles released from *C. vulgaris* and *D. tharense* cultures cause a significant growth reduction for *F. oxysporum* and *M. phaseolina*, and the highest activity was recorded by *C. vulgaris*. Volatiles of *C. vulgaris* which exhibited the highest antifungal activity were collected under natural living conditions by the headspace method and analyzed by GC/MS. GC/MS analysis of *C. vulgaris* volatiles proved that a complex mixture of bioactive and inactive natural compounds was produced by this alga. The identified compounds produced by *C. vulgaris* cells were belonging to different chemical classes, such as alkanes, cyclic alkanes, phenols, halogenated compounds, amides, acids, alcohols, ketones, lactones, fatty esters, fatty acid methyl esters, phthalic acid derivatives, and alcohols. The antifungal activity of *C. vulgaris* is attributed to the synergetic effect of all potent antifungal compounds produced by the alga cells.

INTRODUCTION

Algae produce a wide variety of metabolic organic compounds including intracellular and extracellular types and volatiles **(Lee *et al*., 2017)**. Algal volatile organic compounds (AVOCs) are considered secondary metabolites; they are produced in small quantities compared to other secondary metabolites **(Abdullah *et al.*, 2015)**. Volatiles have a significant role in the ecosystem, especially in the biological ecology. In addition, VOCs are essential for understanding the interaction between living organisms **(Rowan, 2011)**. In terrestrial ecosystem, microbial volatiles have a beneficial ecological functions

in plants, such as defensing against herbivores and pathogens, and enhancement or inhibition of seed germination or seedlings growth (**Zuo, 2019**).

Volatile organic compounds are odorous and small compounds (< C15) with lipophilic moiety, low molecular weight (< 300 Da), high vapor pressure, and low boiling point, and these properties ease their evaporation and diffusion. Microbial volatile organic compounds may be alkenes, aldehydes, ketones, alcohols, terpenes, sulfides, pyrazines and benzenoids (**Schulz-Bohm *et al*., 2017**). Numerous biotic and a biotic factors influencing the production rate and the quantity of AVOCs e.g. algal growth phase, algae species or strain, absence or presence of predators, type of culture, aeration, type of nutrients, temperature, light intensity, pH, salinity and seasonal changes (**Delgadillo-Hinojosa *et al*., 1997; Zuo, 2019**) .

Headspace sampling method of VOCs is a non-invasive and non-destructive technology for *in vivo* collection of volatiles (**Delgadillo-Hinojosa *et al*., 1997; Snow & Slack, 2002; Gressler *et al*., 2009**). There are several techniques for headspace sampling of volatiles such as solvent micro-extraction (SME), in which VOCs are collected and concentrated on a drop of solvent, suspended from the tip of a syringe needle above the sample (**Theis *et al*., 2001**). In solid phase extraction method (SPE), an adsorbent is used and VOCs are eluted using solvents. An advanced method over SPE is solid phase micro-extraction method (SPME), which involves the partitioning of analytes in both gas and liquid phase (**Spietelun *et al*., 2010**). The analytes are desorbed from the fiber inside the GC/MS inlet portion for analysis (**Martin *et al*., 2010; Spietelun *et al*., 2010**). After headspace sampling, volatiles are analyzed by GC/MS.

Ikawa *et al*. (2001) revealed that, VOCs produced from cyanobacteria and algae in lakes can change water odor and inhibit the growth of other types of algae such as *Chlorella pyrenoidosa* due to their allelopathic action. **Bravo-Linares *et al*. (2010)** used solid-phase micro-extraction method to quantify VOCs produced from *Ascophyllum nodosum, Fucus vesiculosus, Fucus serratus, Laminaria digitata, Ulva lactuca, Enteromorpha intestinalis,, Palmaria palmate* and *Griffithsia flosculosa*. **Milovanović *et al*. (2015)** determined the organic volatile compounds emitted from cyanobacterium strains e.g. *Spirulina*, *Anabaena*, and *Nostoc* to assess the best species for incorporation in food and feed products.

To our knowledge, no reports were conducted on the antifungal activity of VOCs emitted from microalgae cultures. Therefore, the present work aimed to investigate *in vitro* the antifungal activity of VOCs produced by living cultures of *Desertifilum tharense*, *Chlorella vulgaris,* and *Navicula arenaria* against *Macrophomina phaseolina* and *Fusarium oxysporum* through a system, which allows the exchange of VOCs between algae and phytopathogenic fungi without physical contact between them.

MATERIALS AND METHODS

2.1 Antifungal activity of algal volatiles

The estimation of antifungal activity of algae was carried out the following modified method and design of **Pettersson *et al.* (1999)** and **Zuo *et al.*, (2012)**. *Desertifilum tharense* was grown in BG11 liquid medium, while *Chlorella vulagris* and *Navicula arenaria* were grown in F/2 liquid medium. Algae were cultured at a temperature of 22°C in a light/dark area (16/8 h), with an illumination of 36 µmol/ms and continuous air flow of 3.5 L/min. Phytopathogenic fungi were grown in liquid potato dextrose agar (PDA) medium. Twenty millimeter of fresh prepared algae culture was added to algae media and 1.0 mL of fungal spore suspension was added to fungi media. Three treatments were carried out in this experiment (Fig. 1); the test was the 1st treatment, the 2nd was the alga control, and the 3rd was the fungal control. For test, both of the alga and the fungus bottles were connected to each other through a pipe (pipe 3), and the alga bottle was connected to air pump through another pipe (pipe 2). The bottle of the alga control was connected only to a pump through a pipe. In case of the fungal control; the fungus bottle was not exposed to the alga volatiles and connected to a bottle contain media only through a pipe (pipe 6), and the media bottle was connected by a pipe with an air pump. Air was pumped through a pump from pipe 2 into algae bottles for algal aeration and acceleration of the passage of algae volatiles into the fungus bottle through pipe "3". Each treatment had three replicates. After 7 days, fungi and algae biomass were harvested through centrifugation at 10000 rpm and dried in an oven for 24 hours to calculate their dry weight.

Percentage of fungi mycelia growth inhibition (MGI %) was measured using the following index (**De Corato *et al*., 2017**):

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

(Control = dry weight of unexposed fungus to alga volatiles, Test = dry weight of exposed fungus to alga volatiles).

2.2 Headspace collection and GC/MS analysis of algal volatiles

2.2.1 Headspace collection of algal volatiles

Volatiles of *Chlorella vulgaris* culture which exhibited the highest antifungal activity and F/2 media were collected using headspace sampling according to the modified method of **Kottb *et al.* (2015)**. A sterilized column was inserted in the bottle of *C. vulgaris* culture and F/2 media. The column contained active charcoal in the middle; active charcoal is considered a volatiles- trapping material and cotton at the ends. The air suction pump was connected to the column through pipe 5 for the acceleration of volatiles trapping by active charcoal (Fig. 2), while the air pump was connected to the bottle of *C. vulgaris* culture and F/2 media through pipe "2". After 3 days of the

incubation period, volatiles were eluted from the trapping material using dichloromethane.

2.2.2 GC/MS analysis of algal volatiles

Volatiles collected from *C. vulgaris* culture and F/2 medium were analyzed by GC/MS. Mass spectra were recorded using Shimadzu GCMS-QP2010 (Koyoto, Japan), equipped with Rtx-5MS fused bonded column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) (Restek, USA) equipped with a split–splitless injector. The initial column temperature was kept at 50°C for 3min (isothermal), programmed to 200°C at a rate of 15°C/min, and kept constant at 200°C for 5min (isothermal). Then, the temperature was programmed to 240°C at a rate of 3°C/min, and kept constant at 240°C for 10min (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 ratio1:5).

2.3 Statistical analysis

Data were compared using one-way ANOVA at $P < 0.05$, and means were separated using the least significant difference (LSD). The standard error for results out of three replicates was considered. Statistical analysis was performed using IBM SPSS Statistics version 25 **(Gomez & Gomez, 1984)**.

RESULTS AND DISCUSSION

3.1. Effect of microalgae headspace volatile metabolites on the growth of *M. phaseolina* and *F. oxysporum*

Volatiles emitted from *D. tharense* culture recorded a reverse effect on the growth of *M. phaseolina* (45.73%) and *F. oxysporum* (19.54%) (Table 1 & Figs. 3, 4). Volatiles from *C. vulgaris* culture significantly inhibit mycelium growth of both tested phyto-pathogens; the percent of mycelium growth inhibition of *M. phaseolina* reached 57.57% (Fig. 5); whilst in the case of *F. oxysporum*, the reduction in MGI% was 33.41% (Fig. 6). The results revealed that both of *D. tharense* and *C. vulgaris* have the capability to inhibit the growth of eukaryotes. In compatible with our results, **Ikawa *et al.* (2001)** reported that volatile organic compounds produced by cyanobacteria and algae in freshwater lakes have the ability to inhibit the growth of eukaryotic green alga *Chlorella pyrenoidosa*.

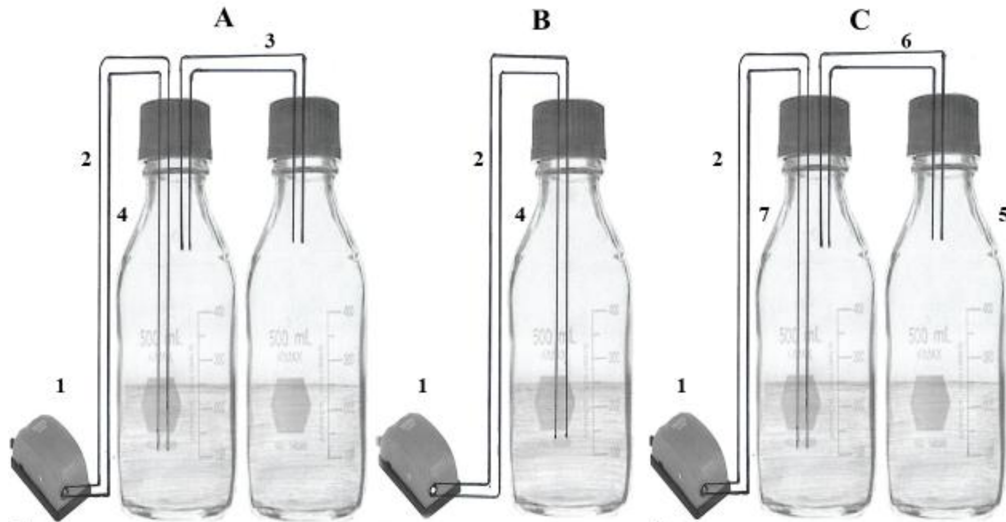

Fig.1. The design of the experiment treatments showing: **(A)** Test in which the fungus was exposed to the algal volatiles through a pipe “3”; **(B)** The algal control, and **(C)** The fungus control in which the fungus was not exposed to the algal volatiles.
(1) Air pump; (2) Pipe in which air was pumped from the pump through it to algal culture; (3) Pipe that connects between the alga and the fungus; (4) Algal culture bottle; (5) Fungus culture bottle; (6) Pipe that connect between control bottle and the fungus culture, and (7) Control bottle containing media only.

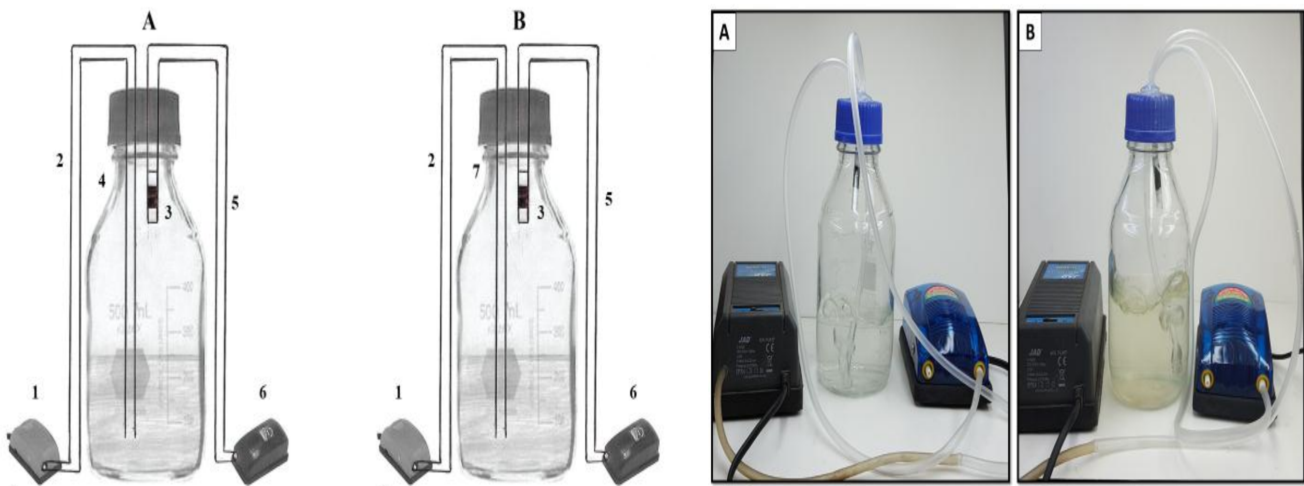

Fig. 2. The design of volatiles collection: **(A)** Control in which the bottle contains media only, and **(B)** Test in which the bottle contains *Chlorella vulgaris* culture.

(1) Air pump, (2) Pipe that is connected to air pump; air was pushed to algal culture and media through it, (3) Sterilized column that contains active charcoal in the middle and cotton at the ends, (4) Bottle contain media only, (5) Pipe that is connected to air suction pump and column, (6) Air suction pump, and (7) Bottle contain *C. vulgaris* culture.

Table 1. Effect of volatiles emitted from microalgae on the growth of *Macrophomina phaseolina* and *Fusarium oxysporum*

	Phytopathogenic fungi Percentage of MGI	
Algal species	***M. phaseolina***	***F. oxysporum***
D. tharense	45.7357 ± 0.99 [a]	19.5478 ± 1.75 [d]
C. vulgaris	57.5743 ± 1.35 [b]	33.4134 ± 2.68 [e]
N. arenaria	No inhibition	7.8837 ± 1.12 [f]
LSD at p ≤ 0.05	**6.8005**	**5.155**

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

Values with different letters are significantly different at $P \leq 0.05$

Table 2. Effect of *M. phaseolina* and *F. oxysporum* volatiles on the dry weight of *D. tharense*, *C. vulgaris,* and *N. arenaria*

		Phytopathogenic fungi		
		M. phaseolina	***F. oxysporum***	**LSD at p ≤ 0.05**
D. tharense	Control	0.064 ± 0.007 [a]	0.063 ± 0.0067 [a]	**0.075**
	Test	0.1134 ± 0.044 [a]	0.0562 ± 0.006 [a]	
C. vulgaris	Control	0.3401 ± 0.074 [b]	0.221 ± 0.051 [c]	**0.1755**
	Test	0.3794 ± 0.054 [b]	0.4569 ± 0.019 [d]	
N. arenaria	Control	0.3664 ± 0.0765 [e]	0.350 ± 0.11 [e]	**0.377**
	Test	0.350 ± 0.11 [e]	0.8122 ± 0.182 [f]	

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

Values with the same letters are not significantly different at $P \leq 0.05$

The results of the present study investigate that volatiles emitted from algae cultivation medium did not have any effect on fungi growth, but their growth was affected only by volatiles emitted from algae. Volatiles emitted from *N. arenaria* enhanced the growth of *M. phaseolina* (Fig. 7), but *F. oxysporum* growth was inhibited (Fig. 8).

3. 2 Effect of fungal culture volatiles on microalgae growth

There was no significant difference between the dry weight of algae exposed to *M. phaseolina* volatiles and unexposed algae (control). In addition, *C. vulgaris* and *N. arenaria* growth was promoted by *F. oxysporum* volatiles (Table 2).

3.3 Volatile profile of *Chlorella vulgaris* by HS-GC/MS analysis

Volatiles trapped from headspace of *C. vulgaris* culture that exhibit the highest antifungal activity were adsorbed by charcoal and injected in GC/MS (Shimadzu GCMS-QP2010, Koyoto, Japan). Fifty- nine compounds were identified from *C. vulgaris* culture after 3 days of cultivation, and 23 compounds were identified from f/2 media (Table 3). Volatile compounds emitted from *C. vulgaris* cells belong to different chemical classes; namely, alkanes, cyclic alkanes, phenols, halogenated compounds, amides, acids, alcohols, ketones, lactones, fatty esters, fatty acid methyl esters, phthalic acid derivatives and alcohols (Fig. 9). The most abundant classes were alkanes (72.88%), followed by alcohols (6.77%). The highest peak area was identified as Phenol, 2,4-bis(1,1-dimethylethyl)-; Peak No. 20 (Fig. 10). Pentadecane, hexadecane and heptadecane compounds were emitted by *C. vulgaris* and have antifungal properties. Similar finding was reported by **Milovanović *et al.* (2015)** who found that strains of *Spirulina platensis*, *Nostoc* sp. and *Anabaena* sp. produce a wide range of volatiles including pentadecane, hexadecane and heptadecane. Furthermore, *Chlorella vulgaris* is able to produce tetradecane with antimicrobial properties. This agrees with the finding of Milovanović et al., (2015) who reported that tetradecane was produced by *Spirulina platensis* samples. It was proved that the lipophilic or hydrophilic properties of bioactive compounds are related with antimicrobial properties. The order of antifungal activity of various phyto-compounds is phenols, aldehydes, ketones, alcohols, esters and hydrocarbons. The antifungal activity of bioactive compounds increases when the carbon chain length of compounds increases **(Bendiabdellah *et al.*, 2012)**. The antagonistic mechanisms of volatiles emitted from *C. vulgaris* cells may be attributed to one of potent identified volatile compounds or due to synergetic relation between all volatiles.

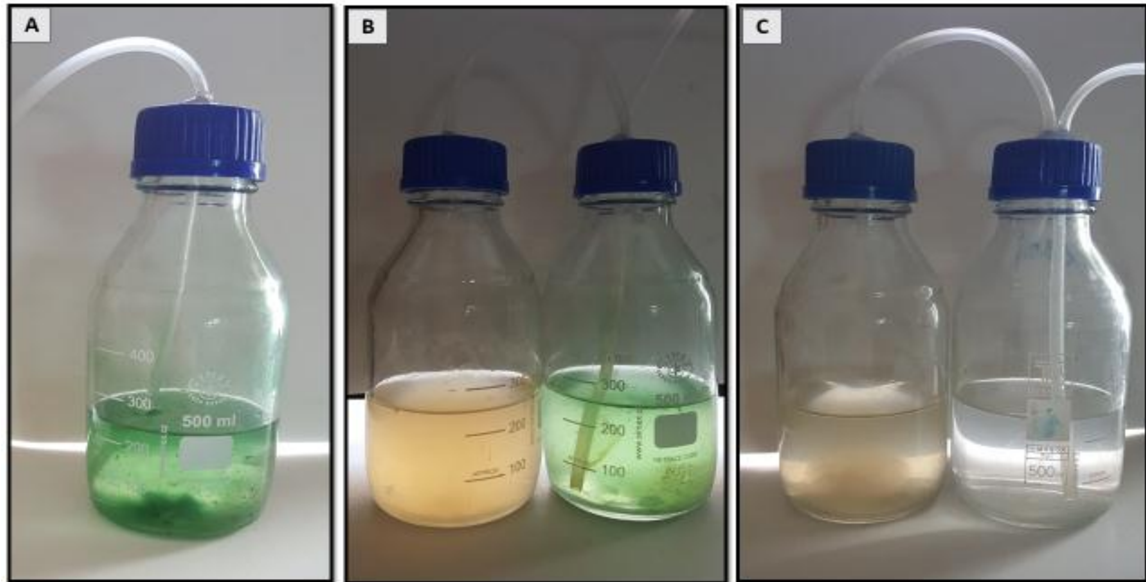

Fig. 3. Effect of *D. tharense* volatiles on the growth of *M. phaseolina*: **(A)** Algal control, **(B)** Test in which the fungus was exposed to *D. tharense* volatiles, and **(C)** Fungus control.

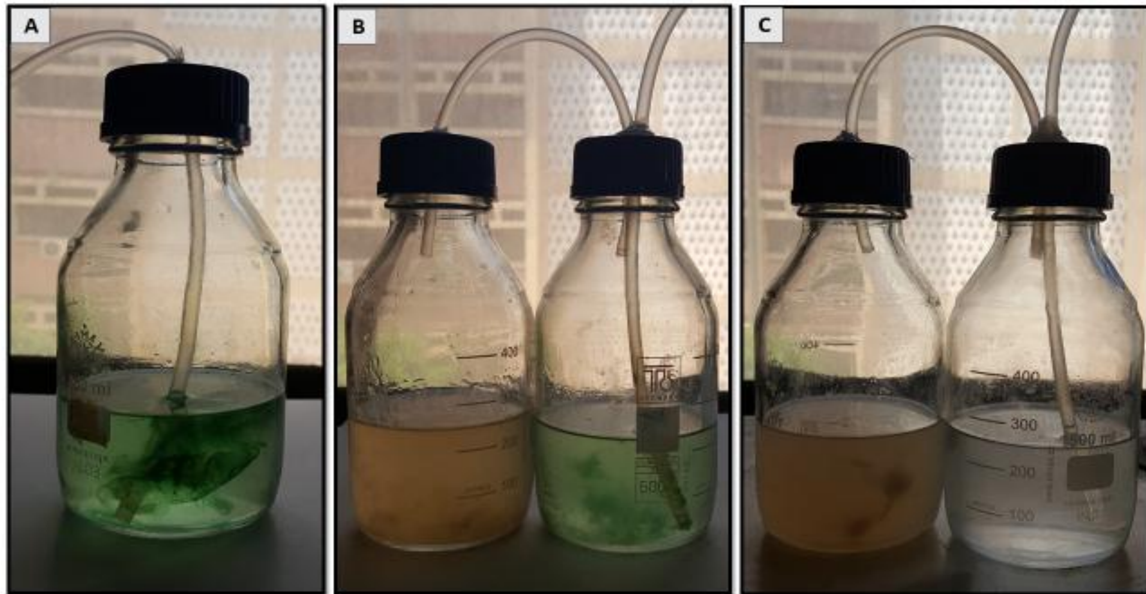

Fig. 4. Effect of *D. tharense* volatiles on the growth of *F. oxysporum*: **(A)** Algal control, **(B)** Test in which the fungus was exposed to *D. tharense* volatiles, and **(C)** Fungus control.

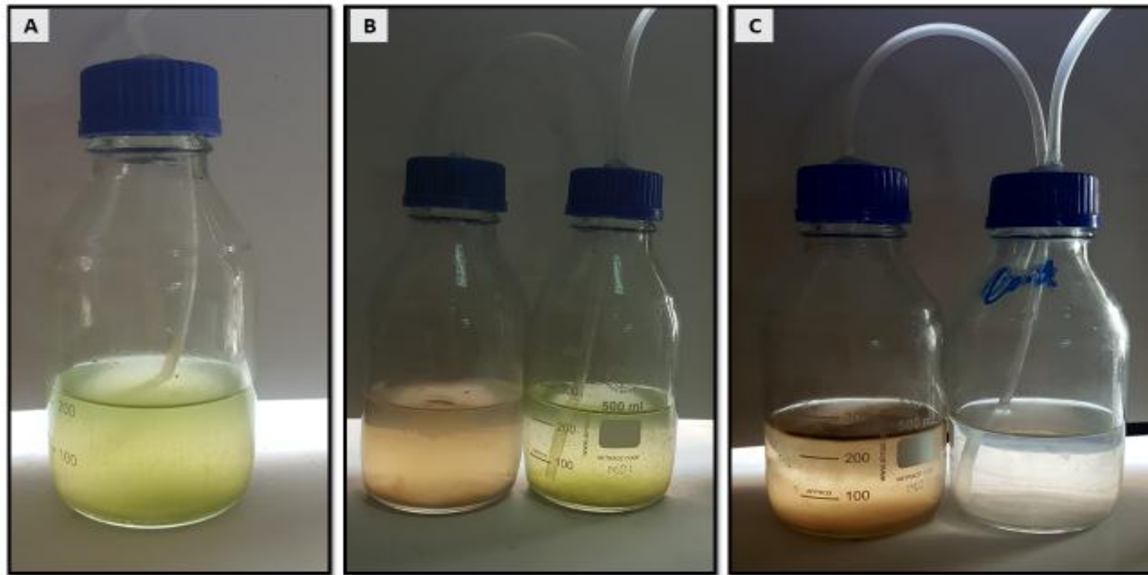

Fig. 5. Effect of *C. vulgaris* volatiles on the growth of *M. phaseolina*: **(A)** Algal control, **(B)** Test in which the fungus was exposed to *C. vulgaris* volatiles, and **(C)** Fungus control.

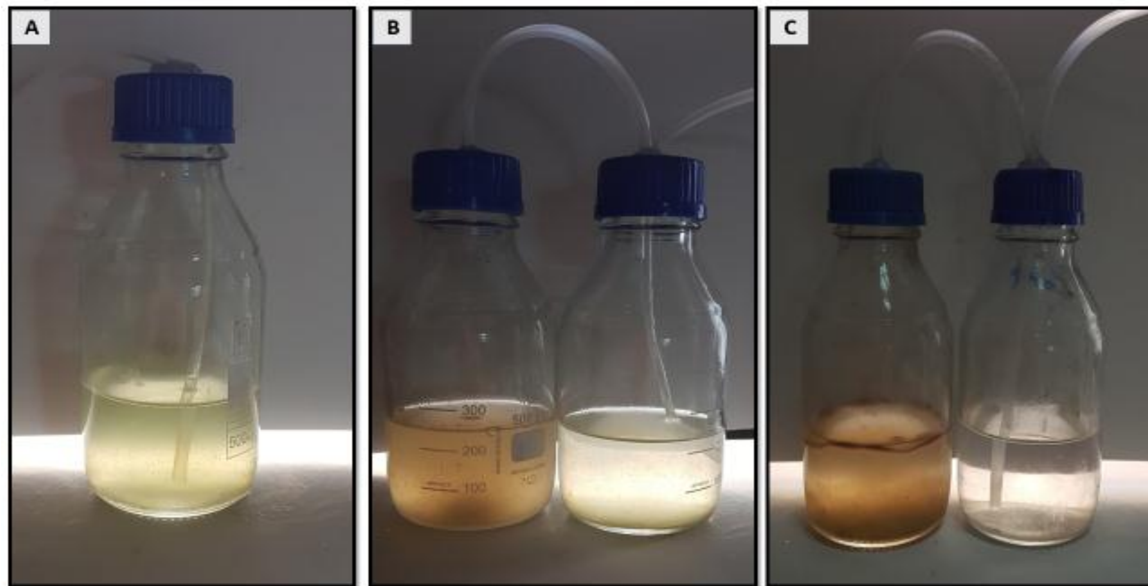

Fig. 6. Effect of *C. vulgaris* volatiles on the growth of *F. oxysporum*: **(A)** Algal control, **(B)** Test in which the fungus was exposed to *C. vulgaris* volatiles, and **(C)** Fungus control.

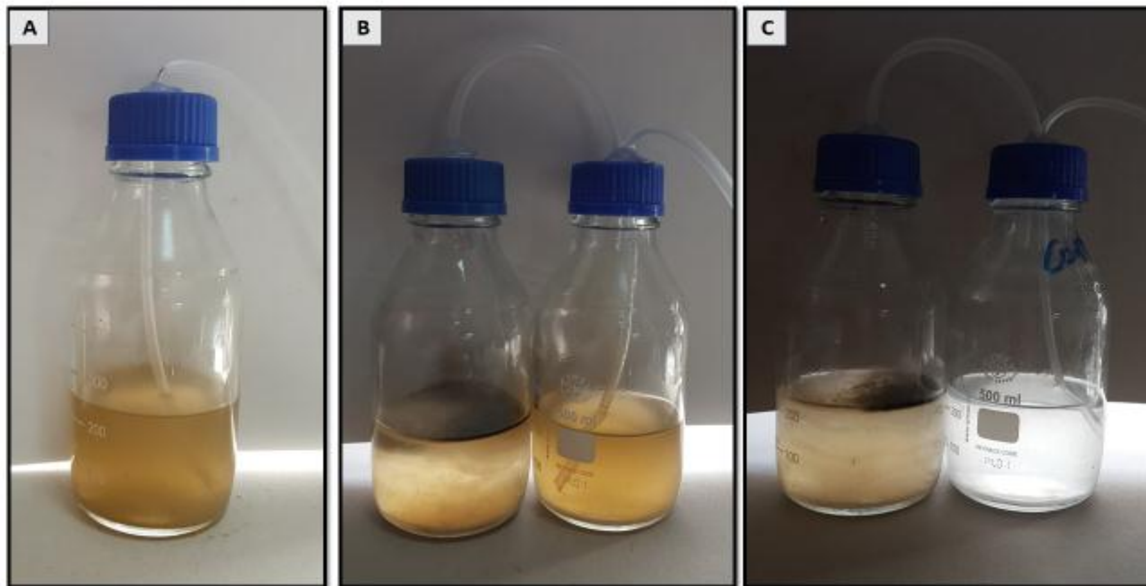

Fig. 7. Effect of *N. arenaria* volatiles on the growth of *M. phaseolina*: **(A)** Algal control, **(B)** Test in which the fungus was exposed to *N. arenaria* volatiles, and **(C)** Fungus control.

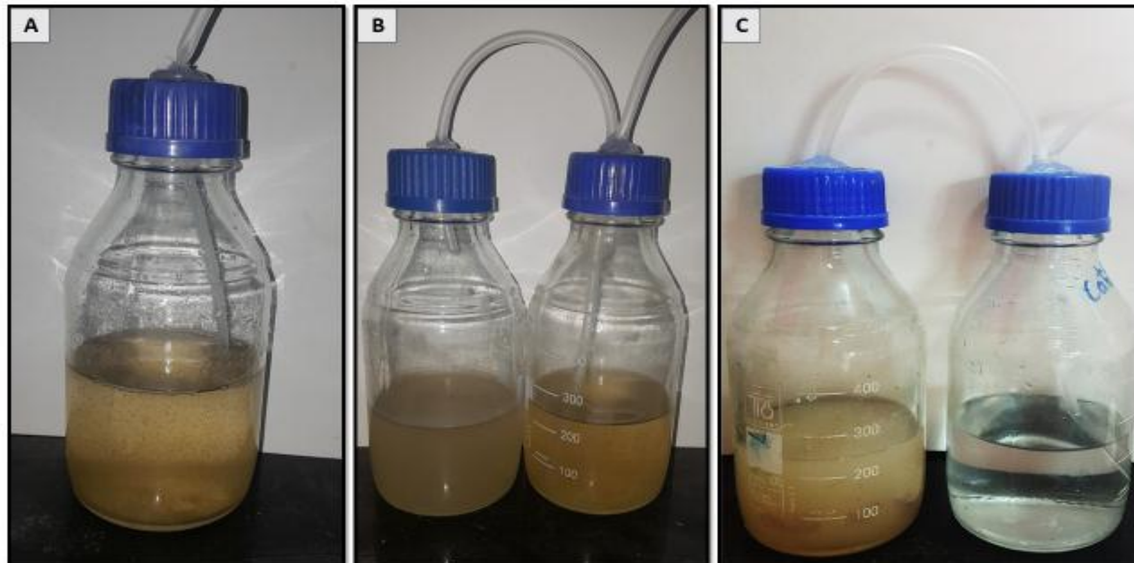

Fig. 8. Effect of *N. arenaria* volatiles on the growth of *F. oxysporum*: **(A)** Algal control, **(B)** Test in which the fungus was exposed to *N. arenaria* volatiles, and **(C)** Fungus control.

Table 3. Identified bioactive volatile compounds from headspace of *Chlorella vulgaris* culture and F/2 medium by GC/MS analysis

Peak No.	R_t	VOCs	Base peak m/z	Area %		Chemical group	Biological activities	References
				C. vulgaris	Media only			
1	7.650	Octane, 5-ethyl-2-methyl-	43.05	0.04	-	Alkanes	-	-
2	9.440	Tridecane	57.10	0.09	-	Alkanes	Antifungal, Antibacterial, Antiviral	(Sihag & Pathak, 2017)
3	9.555	Undecane, 2,6-dimethyl-	57.10	0.05	-	Alkanes	-	-
4	9.880	Undecane, 2,9-dimethyl-	57.10	0.06	-	Alkanes	-	-
5	9.988	Decane, 3,7-dimethyl-	43.05	0.09	-	Alkanes	-	-
6	10.204	Hexadecane, 2,6,11,15-tetramethyl	71.10	0.07	-	Alkanes	-	-
7	10.256	Hexadecane	57.10	2.88	5.23	Alkanes	Antibacterial activity	(Murugan K, Kalaivani P, Bothiraj K V, 2020)
8	10.630	Isopropyl-5-methyl-1-heptanol	43.05	0.65	0.40	Alcohol	Antifungal or Antimicrobial activity	(Mannaa & Kim, 2018)
9	10.923	Nonane, 5-(2-methylpropyl)-	57.10	0.13	-	Alkanes	-	-
10	11.003	Nonane, 5-butyl-	71.10	0.22	0.14	Alkanes	-	-
11	11.104	Tridecane, 2-methyl	57.10	0.05	-	Alkanes	Antimicrobial activity	(Chandrasekar *et al*., 2015)
12	11.454	Tetradecane	57.10	0.49	0.36	Alkanes	Antimicrobial activity	(Kavitha & Uduman, 2017)
13	11.559	Tridecane, 2,5-dimethyl	57.10	0.37	-	Alkanes	-	-

14	11.861	Dodecane, 4-methyl-	43.05	0.10	-	Alkanes	-	-
15	12.146	Heptadecane, 2,6,10,15-tetramethyl-	57.10	0.37	-	Alkanes	Antimicrobial activity	(Paradoxa & Alternata, 2015)
16	12.148	Heptadecane	57.10	2.68	3.92	Alkanes	Antimicrobial activity	(Paradoxa & Alternata, 2015)
17	12.182	Dodecane, 4,6-dimethyl-	71.10	0.8	0.56	Alkanes	-	-
18	12.464	Tetradecane, 5-methyl-	57.10	0.79	0.67	Alkanes	Antifungal, Antibacterial, Antiviral	(Sihag & Pathak, 2017)
19	12.501	Heptadecane, 8-methyl-	57.10	0.38	-	Alkanes	Antifungal and antibacterial activity	(Murugan K, Kalaivani P, Bothiraj K V, 2020)
20	12.58	Phenol, 2,4-bis(1,1-dimethylethyl)-	191.20	3.27	5.41	Phenol	Antifungal, Antioxidant activity	(Ren *et al.*, 2019)
21	12.693	1-Decanol, 2-hexyl-	57.10	0.45	1.58	Alcohol	Antimicrobial activity	(Krishnamoorthy & Subramaniam, 2014)
22	12.836	Eicosane	57.10	2.26	4.08	Alkanes	Antifungal activity	(Ahsan *et al.*, 2017)
23	13.067	5,5-Diethyltridecane	57.10	0.11	-	Alkanes	-	-
24	13.194	1-Hexadecene	57.10	0.28	-	Alkanes	Antibacterial activity	(Gideon, 2015)
25	13.251	Pentadecane	57.10	1.18	0.76	Alkanes	Antifungal, Antibacterial, Antiviral	(Sihag & Pathak, 2017)

26	13.444	2-methyltetracosane	43.05	0.62	-	Alkanes	-	-
27	13.697	Pentadecane, 2,6,10-trimethyl-	57.10	0.88	0.93	Alkanes	Antimicrobial activity	(Begum *et al*., 2016)
28	13.799	9-methylheptadecane	57.10	0.58	-	Alkanes	-	-
29	13.834	Pentadecane, 3-methyl-	57.10	0.60	-	Alkanes	-	-
30	13.919	Decane, 3,8-dimethyl-	57.10	0.31	-	Alkanes	-	-
31	14.137	5-Ethyl-5-methylheptadecane	71.10	0.32	-	Alkanes	-	-
32	14.676	1-Decanol, 2-octyl-	57.10	0.95	-	Alcohol	-	-
33	14.775	Dodecane, 2,6,11-trimethyl-	57.10	0.17	0.71	Alkanes	Antibacterial activity	(Begum *et al*., 2016)
34	14.914	Heptadecane, 2-methyl-	57.10	0.79	0.84	Alkanes	Antimicrobial activity	(Paradoxa & Aternata, 2015)
35	15.155	Nonane, 5-methyl-5-propyl-	71.10	0.18	-	Alkanes	-	-
36	15.358	Octadecane	57.10	1.1	1.83	Alkanes	-	-
37	15.415	1-Dodecanol, 2-hexyl-	57.10	0.40	1.19	Alcohol	-	-
38	15.482	Hexadecane, 2,6,10,14-tetramethyl-	57.10	1.57	1.63	Alkanes	-	-
39	15.611	2-Bromotetradecane	57.10	0.15	0.36	Halogenated compounds	Antimicrobial activity	(Sawant *et al*., 2018)
40	15.638	Decane, 2,3,4-trimethyl-	43.05	0.27	-	Alkanes	-	-
41	15.684	Tetradecane, 4-methyl-	43.05	021	0.27	Alkanes	Antimicrobial activity	(Begum *et al*., 2016)

42	15.723	Isopropyl myristate	43.05	0.88	0.73	Fatty ester	Antimicrobial, antioxidant activity	(Begum *et al.*, 2016)
43	16.381	Octadecane, 3-methyl-	57.10	0.27	-	Alkanes	-	-
44	16.831	Heneicosane	57.10	1.48	1.63	Alkanes	Antimicrobial activity	(Kawuri & Darmayasa, 2019)
45	17.374	Hexadecanoic acid, methyl ester	74.05	0.69	-	Fatty acid methyl esters	Antimicrobial activity	(Kawuri & Darmayasa, 2019)
46	17.576	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	57.10	2.25	3.10	Lactones	-	-
47	18.185	Heptadecane, 8,8-dipentyl-	43.05	0.40	-	Alkanes	Antimicrobial activity	(Paradoxa & Alternata, 2015)
48	18.349	Dibutyl phthalate	149.10	0.47	-	Phthalic acid derivative	Antifungal activity	(Ahsan *et al.*, 2017)
49	19.800	Heptane, 2,2,3,3,5,6,6-heptamethyl-	57.10	0.08	-	Alkanes	-	-
50	20.086	Nonadecane, 9-methyl-	57.10	0.27	-	Alkanes	-	-
51	21.873	5,8-Tridecadione	85.10	0.15	-	Ketones	-	-
52	22.170	5,5-Diethylpentadecane	57.05	0.04	-	Alkanes	-	-
53	22.675	Decane, 1-iodo-	85.10	0.007	-	Halogenated compounds	-	-
54	23.370	Dodecane, 5-cyclohexyl-	57.10	0.12	-	Cyclo alkane	-	-
55	24.061	Dodecane, 2-methyl-	57.10	0.12	-	Alkanes	-	-

56	25.694	Dodecane, 2-cyclohexyl-	82.10	0.05	-	Cyclo alkane	-	-
57	27.005	2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	178.10	1.01	0.79	Acids	-	-
58	30.548	Octanamide, N,N-dimethyl-	87.10	0.12	-	Amides	-	-
59	32.497	5,5-Diethylheptadecane	57.10	0.06	-	Alkanes	-	-

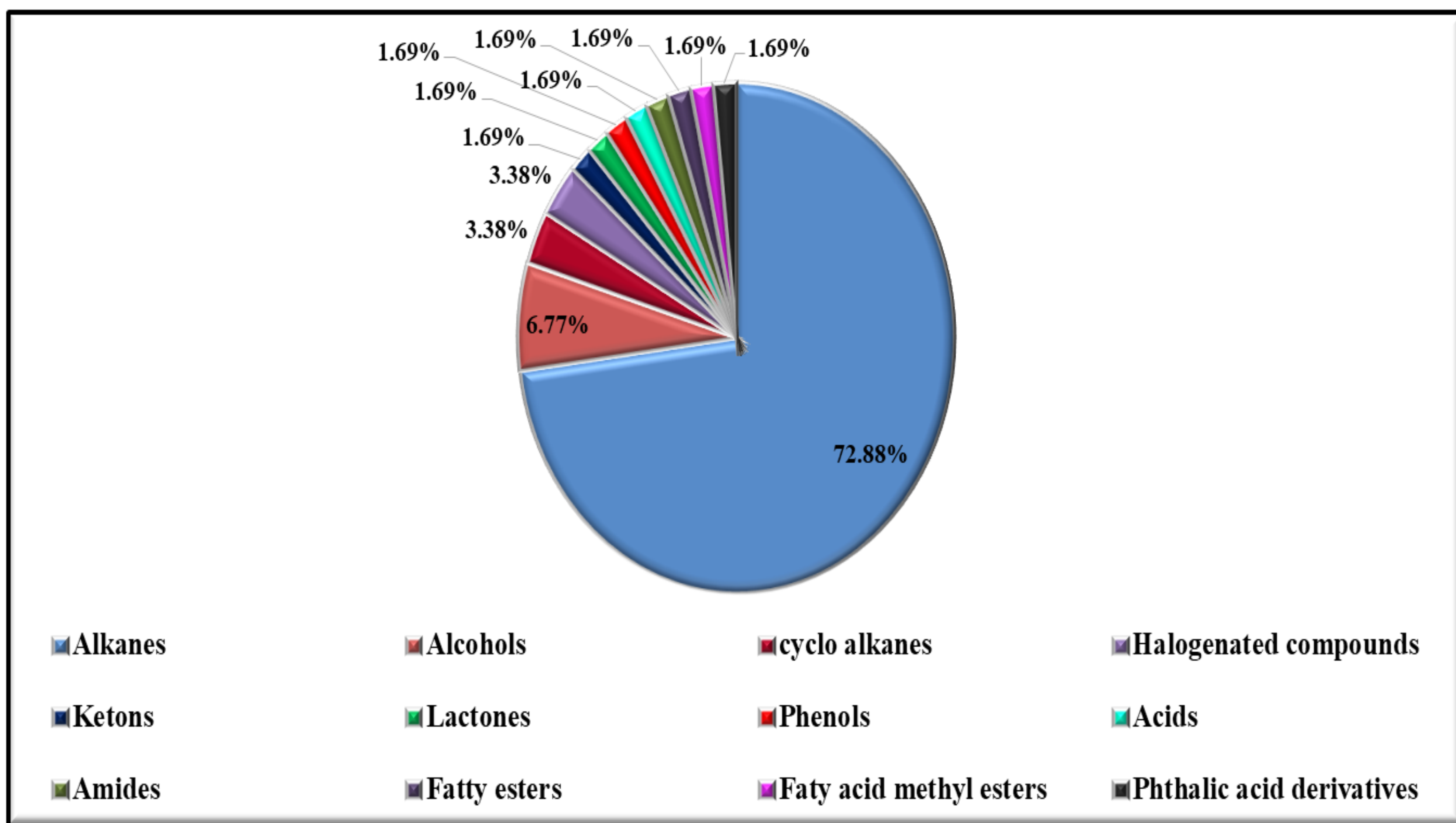

Fig. 9. Percentage of VOCs chemical groups identified in *C. vulgaris* culture headspace.

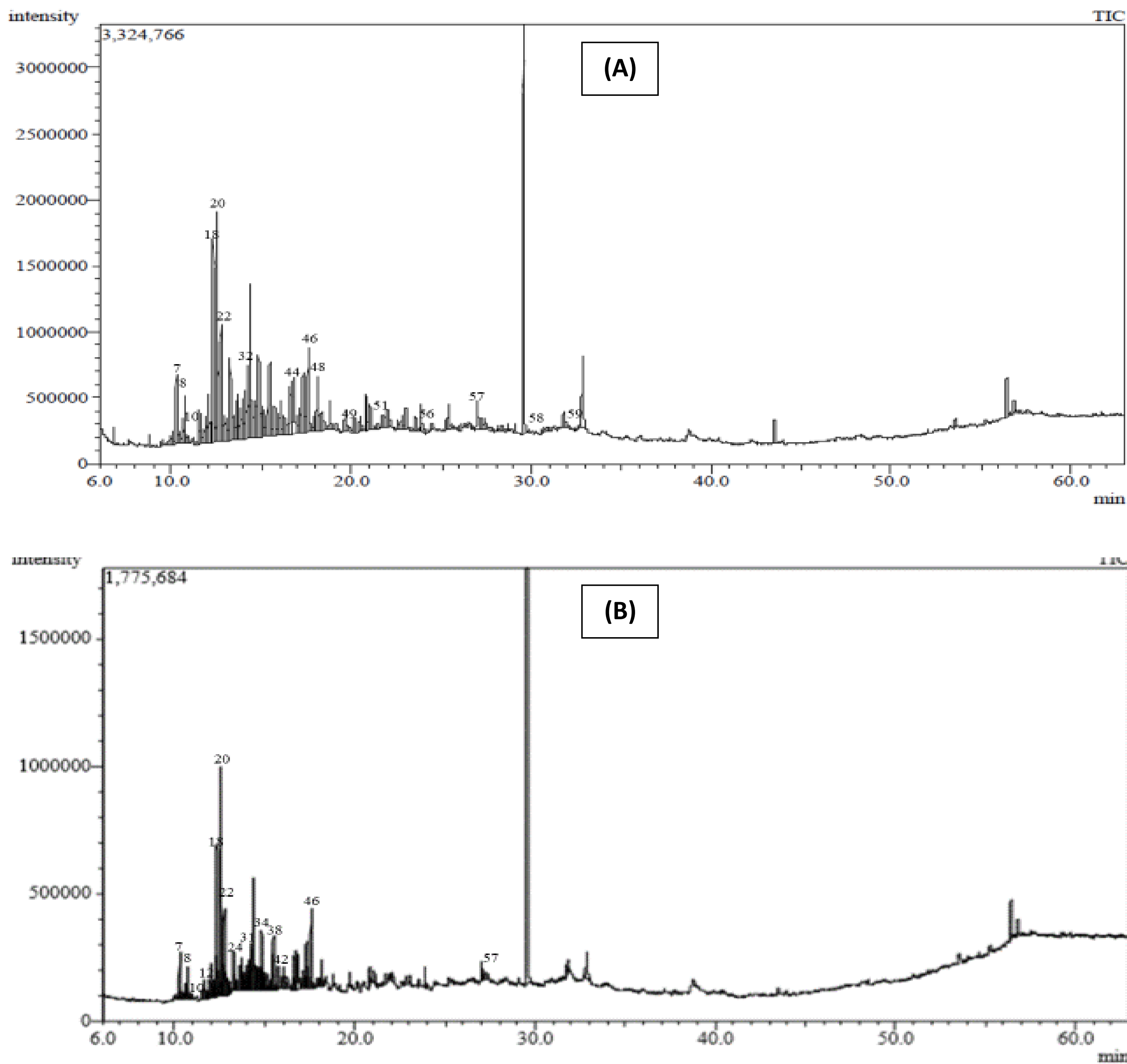

Fig. 10. **(A)** Total ion chromatogram of GC-MS of VOCs of *C. vulgaris* culture and **(B)** total ion chromatogram of GC-MS of F/2 media VOCs

CONCLUSION

Algal volatile organic compounds emitted from living cultures of *C. vulgaris* are effective in the growth inhibition of soil-borne phytopathogenic *F. oxysporum* and *M. phaseolina*. Thus, *C. vulgaris* is recommended to be applied in the agriculture field under greenhouse condition in the biological control of plant diseases caused by *F. oxysporum* and *M. phaseolina*.

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