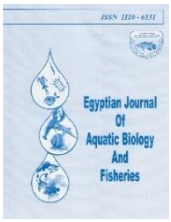
Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(1): 1829 – 1848 (2024)
www.ejabf.journals.ekb.eg



Genotoxicity of *Withania somnifera* Leaf Extracts on the Marine Diatom *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin

Hanan M. Khairy*[1], Ashraf Haider[2], Mohammed Halawa[2], Rasha Ramadan[2]
[1]National Institute of Oceanography and Fishery, NIOF, Egypt
[2]Botany Department, Faculty of Science, University of Tanta, Egypt
***Corresponding Author: hanan_khairy@yahoo.com**

ARTICLE INFO

Article History:
Received: Dec. 5, 2023
Accepted: Feb.8, 2024
Online: Feb. 28, 2024

Keywords:
Withania somnifera,
Cylindrotheca closterium,
Allelochemicals,
Growth,
Gene expression

ABSTRACT

Allelochemicals disintegrate more readily and tend to pose less of a risk to non-target organisms, making them a safer, less intrusive, and ecologically friendly alternative to conventional pest management methods. The production of allelochemicals with algicidal properties was demonstrated in this research. Methanol, ethyl acetate, hexane and pet-ether extracts of *Withania somnifera* were used to inhibit the growth of *Cylindrotheca closterium*. The results demonstrated that *C. closterium* growth in the hexane extract of *W. somnifera* showed a decreasing trend, peaking at 0.021 at 0 minutes and decreasing to 0.009 after 120 minutes. After being exposed to *W. somnifera* hexane extract, algal cells shrank in size and took on a pale, nearly colorless appearance. In addition, the cell wall shrank and became irregular. The results demonstrated the primary ingredient in several extracts was different fatty acids. The primer (5'd[GGTGCGGGAA]-3') that was applied had the highest polymorphism and reflected 71% of the genetic variation for *C. closterium* after it was exposed to four solvents containing *W. somnifera* extract as the active component. In contemporary research, the emphasis is largely on with molecular and genetic pathways receiving significant attention. In contrast, micro-perspectives including gene expression studies are comparatively rare.

INTRODUCTION

In response to pressures like eutrophication and favorable physicochemical circumstances, some phytoplankton species release a macromolecule called mucilage **(Ergul *et al.*, 2021)**. **Turk *et al.* (2010)** stated that marine mucilage is an intricate organic mass with a unique microbial ecology. Most of these secretions are composed of hetero-polysaccharides **(Svetličić *et al.*, 2011)**. The overabundance of marine mucilage is associated with phytoplankton algae blooms, which can be hazardous (and can result in the discharge of toxins) and negatively impact the local ecology, fishing, and aquaculture activities **(Pierre & Vallet, 2023)**.

Mucilage can endanger a variety of species and, in certain cases, crucial habitats when it develops in tiny bodies of water with poor water circulation. Mucilage damages

sensitive environments, such as seagrass beds and coral reefs according to **Lorenti *et al.* (2005)**. Building accumulation of mucilaginous debris impedes fisheries and fishing, interfering with fishing networks, suffocating marine life, endangering the economy, degrading beach water quality, and affecting tourism **(Degobbis *et al.*, 1995; Savun-Hekimolu & Savun-Hekimolu, 2021)**.

Diatoms were believed to be the main source of the mucilage polysaccharide matrix **(Degobbis *et al.*, 1995; Thornton, 2002)**. The mucilage-associated phytoplankton population benefits greatly from the presence of *Cylindrotheca closterium* in particular **(Degobbis *et al.*, 1995; Staats *et al.*, 1999, 2000)**. According to **Özalp (2021)**, the creation of hypoxic conditions and the shrouding of the benthic zone have an effect on all trophic levels.

Various methods such as herbicides, metals, photosensitizers, and some compounds have been effectively employed to combat dangerous algal blooms. Chemical procedures are thought to be efficient and quick to work, but compared to other strategies, they have a higher risk of hazardous quality and non-target reaction. Harmful algal blooms have been crushed or broken down using algaecides and other chemical methods such as copper sulphate **(Jancula & Maršálek, 2011)**.

According to **Uddin *et al.* (2017)**, plants have the ability to emit chemicals into the biological community that can either directly or indirectly aid in the growth and development of other nearby species as well as themselves. **Rice (1984)** defined allelopathy as the extracellular production of allelochemicals that have an inhibitory or stimulatory effect on target species. Numerous species are impacted by allelochemical poisoning, which also causes financial losses **(Mathieu, 2022)**. Furthermore, allelochemicals are readily broken down secondary metabolites of biosynthesis. They can be categorized as phenolic acids, terpenoids, alkaloids, and among others, based on their various characteristics and synthesis routes **(Zhao *et al.*, 2019)**. The development of *Microcystis aeruginosa* can be significantly inhibited by phenolic acids **(Zhang *et al.*, 2010)**, linoleic acids **(Ni *et al.*, 2015)**, flavonoids, and tannins **(Tazart *et al.*, 2019)**. These compounds may be effective substitutes for algae management.

Allelochemicals have been suggested as a biocontrol agent to manage pollution and promote the proliferation of microalgae **(Mendes & Vermelho, 2013)**. Plant allelopathy for controlling algal blooms has drawn a lot of interest **(Liu *et al.*, 2021).** The use of plant allelopathy to control algal blooms has attracted notable attention as a result of the increased frequency of hazardous algal blooms in the world in recent years **(Ma *et al.*, 2018; Zhao *et al.*, 2019)**. More than 20 distinct variations of plant allelochemicals, including terpenes, fatty acids, phenolic acids, and others, have been found **(Tan *et al.*, 2019)**.

Zhou *et al.* (2019) investigated the effects of an aqueous extract of *Artemisia ordosica* leaves on *Nostoc* sp. and *Chlorella vulgaris*. Aqueous extracts include organic acids, phenols, saccharides, alcohols, and humic and fulvic acids. Strong growth

inhibitory activity was demonstrated by the allelochemical ethyl 2-methylacetoacetate (EMA) isolated from *Phragmites communis* Tris on *Microcystis aeruginosa* and *Chlorella pyrenoidosa* **(Li *et al*., 2005)**.

According to earlier studies, certain chemicals released by plants are primarily responsible for controlling physiological responses including photosynthesis and destroying cellular structures, which in turn control the growth of algae **(Yu *et al.*, 2019)**. However, different species and tissues emit distinct allelochemicals from plants **(Moosavi *et al*., 2011)**.

Shaltout *et al.* (2009) noted that *Withania somnifera* (Solanaceae) is a common plant in Egypt. It has dwindling sugars, a few flavonoids, and a few alkaloids **(Padmavathi *et al*., 2005)**. The most prevalent biologically active substances in the Solanaceae family are called alkaloids, and they have a significant physiological effect on all mammals, including humans. *W. somnifera* is a crucial medicinal plant that is sometimes referred to as Indian ginseng. The plant extract and a few distinct compounds have a variety of poly pharmaceutical applications, including anti-stress, anti-inflammatory, relaxing, and cancer prevention **(Winters *et al*., 2006)**. **Wagner *et al.* (1984)** reported that starches, alkaloids, glycosides, fixed oils, and lipids were detected using phytochemical screening of ethanol extract. Alkaloid extracts from *W. somnifera*'s roots, stems, leaves, and fruits showed antibacterial activity against Agrobacterium tumefaciens, *Bacillus subtilis*, *Enterobacter aerogens*, and *Klebsiella pneumoniae*. The capacity of these substances to damage the phospholipid cell membrane of microorganisms may also account for their antibacterial properties **(Cowan, 1999)**.

Nevertheless, definitive evidence about *Cylindrotheca closterium*'s function in the production of mucilage remains to be acquired. The characterization of *C. closterium* extracellular polymeric substances and/or the identification of factors that regulate their production, such as unbalanced nutrients, temperature, irradiance, and bacterial activity, have been the subject of numerous studies **(Grossart, 1999; Staats *et al*., 2000; Wolfstein *et al*., 2002)**.

Mucilage development, which negatively impacts the entire ecology, especially benthic species, is a rising problem in semi-enclosed areas like Alexandria's Eastern Harbor. This work's primary goal was to investigate the mutagenic effects of *W. somnifera* extracts on the dangerous alga *C. closterium* that was isolated from the coastal region of Alexandria's EH in order to bio-control the growth of these algae as an alternative to algaecides and other chemical methods.

MATERIALS AND METHODS

1. Algal species and culture medium

The study's algal species, which is depicted in Fig. (1), was isolated from the coastal area of Alexandria's Eastern Harbor and subsequently identified genetically as *Cylindrotheca closterium* **(Ramadan *et al.*, 2020)**. The isolated species were cultivated

on F/2 medium **(Guillard, 1975)**. The growth curves for each were created by graphing the optical density versus the duration of incubation.

Fig. 1. *Cylindrotheca closterium* as shown under light microscope (Magnification power 40x)

2. Plant material

After the plant was identified in the Tanta University Herbarium, the leaves of *Withania somnifera* were harvested from the university's garden.

3. Preparation of leaves extracts

The *W. somnifera* leaves collected were cleaned in a vessel with distilled water and then allowed to dry. Using a grinder, dried leaves were ground into a powder. The powder was dissolved by weight of a 10gm per 100ml in methanol, ethyl acetate, hexane, and petroleum ether. The mixture was then shaken for 72 hours at 24- 28°C. After again filtering through cheese cloth, all extracts were placed in an incubator to allow the solvent evaporate. For further study, the dried extracts were collected in falcon tubes and stored in the freezer.

4. Treatment the alga culture with plant extracts

The crude extract dissolved in 100% Dimethyl sulfoxide (DMSO), and then different concentrations were prepared, as represented in Table (1).

Table 1. Different concentrations used in treatment of *Cylindrotheca closterium*

Plant extract	Positive control	Negative control
40 ml of F/2 media	40ml of F/2 media	40ml of F/2 media
1ml of algal culture	1ml of algal extract	1ml of algal culture
1ml of plant extracts (0.05%)	1ml of distilled water	1ml of DMSO

5. GC–MS analysis

At Tanta University's Central Laboratory, GC-MS analysis of *Withania somnifera* leaf extracts in methanol, ethyl acetate, hexane, and pet-ether. The analysis was performed using a GC-MS system model Inst. (Perkin Elmer model: Clarus 580/560 S) with a column (Rxi-5Sil) measuring 30 meters in length, 0.25mm in internal diameter, and a film thickness of 0.25µm. The oven temperature was programmed to start at 60°C for 2 minutes, followed by a ramp at 10°C/ min to 280°C, and held for 6 minutes. The injector volume was 1µl, split was 20:1, and the carrier gas was helium. 1ml/ min was the flow rate. Scan was 50 to 500Da, transfer temperature was 250°C, source temperature was 200°C, and solvent delay was 5.00min. By contrasting the retention time and mass spectrum of the *W. somnifera* leaf extracts with those obtained from the GC-MS system and published data, the constituent parts of the extracts were determined. Peak area measurements were used to calculate each component's concentration, and then converted to a percentage, according to the method of **Farag *et al.* (1986)**.

6. Effects of *Withania somnifera* leave extracts on alga growth and morphology

The growth measured by optical density of the control and the treated cultured species were obtained, and the morphological changes were examined by light microscope. The growth of the isolated species was assessed by measuring the optical density of the cultures at 680nm (OD680) using a UNICO UV/Visible spectrophotometer, model 2000UV, power supply, and AC220V/ 50HZ.

7. Extraction of total genomic DNA from *Cylindrotheca closterium*

Total genomic DNA was isolated from affected algal cells in accordance with Omega Co.'s (USA) manufacturing protocol, utilizing the method described by **Besteiro *et al.* (2011)**. The DNA was precipitated using 250µl of 100% ethanol after homogenising algal cells in 250µl of 10mM Tris-HCl. At 70°C, DNA was eluted in distilled H_2O after being bonded to a HiBind® DNA column.

8. PCR amplification of *rbcL* gene

The rbcL gene fragment was amplified specifically by PCR utilizing the Gene Amp PCR Cycler (Creacon, Thermo Cycler, Holand). Table (2) lists the specific rbcL forward and reverse PCR primers. The cycling parameters were started at 94°C for 4 minutes, then there were 30 cycles of denaturing for 30 seconds at 94°C, annealing for 60 seconds at 55°C, and extending for 90 seconds at 72°C, with a further extension lasting for 10 minutes at 72°C **(Haitao *et al.*, 2007)**.

Table 2. Features of *rbc*L gene primers

Primer	Sequence	Tm (°C)	GC%
*rbc*L-F	5’-CGKTACGAATCTGGWG-3’	43.06	57
*rbc*L-R	5’-CCAATWGTACCACCACCRAAT-3’	54.86	47

9. RAPD-PCR analysis

The algal DNA was subjected to a random amplification of polymorphic DNA (RAPD-PCR) analysis in accordance with the ready-to-go RAPD analysis beads (GE Healthcare Life Sciences) manufacturer's procedure. The amplification process consists of 45 cycles, each comprising the following steps: initial denaturation at 95°C for 1 minute, annealing at 36°C for 1 minute, extension at 72°C for 1 minute, followed by a final extension cycle at 72°C for 5 minutes. Table (3) displays the random primer sequences employed in the amplification process.

Table 3. Sequences of random primers used for RAPD analysis

Primer	Sequence
Primer 1	5’-GGTGCGGGAA-3’
Primer 2	5’-GTTTCGCTCC-3’
Primer 3	5’-GTAGACCCGT-3’
Primer 4	5’-AAGAGCCCGT-3’
Primer 5	5’-AACGCGCAAC-3’

10. Agarose gel electrophoresis

The PCR products were resolved in 1.5% agarose gels at 80 volts for 20min. The DNA bands were visualized with ethidium bromide **(Ausubel *et al*., 1994)**.

DNA sequencing

According to **Linderman (1998)**, the PCR products were extracted from agarose gels using Micro spin filters (E.Z.N.A.®Gel Extraction Kit, D2500-01, OMEGA Bio-Tech, USA) and quantified spectrophotometrically. The ABI PRISM® 3100 genetic analyzer (Micron-Corp. Korea) was used for sequence analysis.

11. Data analysis

The data analysis was conducted using the gel documentation system (Geldoc-It, UVP, England) and Totallab analysis software (Ver.1.0.1), available at www.totallab.com. In order to verify the identity of aligned sequences, BLAST analysis was performed on the NCBI website (http://www.ncbi.nlm.nih.gov/webcite). By employing the pairwise distance method and the ClusteralW software analysis (www.ClusteralW.com), the genetic distances and multi-alignments were calculated. The *Cylindrotheca* sample sequences that are stored in the GenBank were also compared with the nucleotide sequences.

12. Statistical analysis

The means of three replicates, standard deviation, and the one-way analysis of variance [ANOVA] test were utilized in the statistical presentation and analysis of the current study. SPSS V2 was utilized to compare quantitative data collected at different periods within the same group.

RESULTS

1. Effect of *Withania somnifera* leaves extracts on *Cylindrotheca closterium*

The effect of several *Withania somnifera* leaf extracts on *Cylindrotheca closterium*'s optical density (OD) was investigated in the current study (Table 4). The findings showed that the optical density (OD) of the algal culture increased steadily over time, starting at 0.012 at 0 minutes and reaching 0.048 at 120 minutes despite the absence of the plant extract. When treated with a methanol extract of *W. somnifera* leaves, the growth of the algal culture, as assessed by OD, decreases, starting at 0.027 at 0min and reaching 0.010 at 120min. On the other hand, when *C. closterium* was treated with ethyl acetate extract, its OD increased from 0.014 at 0min to 0.006 at 120min. As seen in Table (4), the growth of the algal culture in the case of petroleum ether leaf extract starts at 0.012 at 0min and reaches 0.001 at 120min. In conclusion, the hexane extract exhibits a declining trend in algal growth, starting at 0.021 at 0min and reaching 0.009 after 120min.

2. Effect of *Withania somnifera* leaves extracts on the morphology of *Cylindrotheca closterium* cells

Under a light microscope at a magnification of 40x, *C. closterium* cells that had not been treated with plant extracts (control) had a normal form with two long ends and a regular cell wall (Fig. 2a). On the other hand, algal cells treated with *W. somnifera* hexane extract displayed a reduction in cell size and a pale or almost colorless appearance. Furthermore, the internal cell divisions were numerous, and the cell wall contracted and became uneven (Fig. 2b).

Table 4. Effect of *Withania somnifera* different extracts on *Cylindrotheca closterium* growth measured as optical density

Extract \ Time	30min	60min	90min	120min
+ve control	0.012±0.02	0.035±0.002	0.040±0.005	0.048±0.002
Methanol	0.024±0.01	0.018±0.003 [a]	0.014±0.006 [a]	0.009±0.003 [a]
Ethyle acetate	0.011±0.01	0.008±0.001 [a]	0.008±0.004 [a]	0.006±0.001 [a]
Hexane	0.020±0.02	0.018±0.002 [a]	0.015±0.006 [a]	0.010±0.001 [a]
Pet-ether	**0.009±0.001 [a]**	**0.006±0.001 [abc]**	**0.004±0.001 [a]**	**0.003±0.001 [b]**

The results represented by means of three replicate ± Standard deviation

*The same small letters indicates there was no significant difference between the two groups, while the difference letters indicates there was a significant difference between these two groups at $P < 0.05$ level of probability.

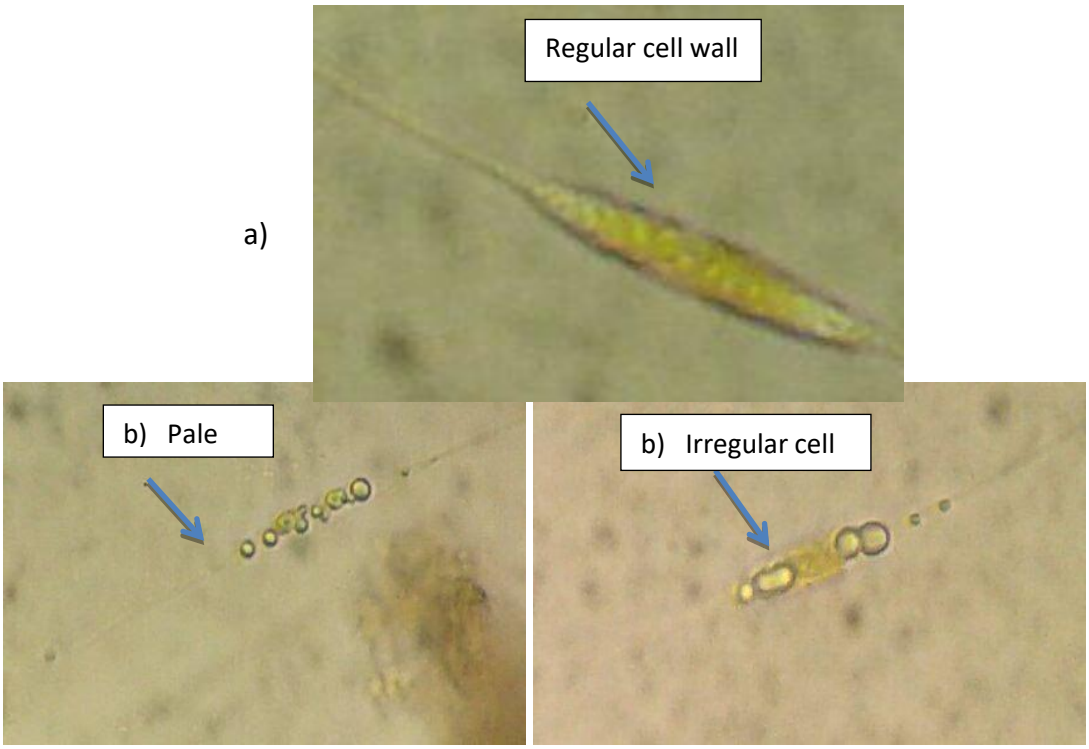


Fig. 2. Morphological changes caused by *Withania somnifera* leaf extracts on *Cylindrotheca closterium* cells. a) Control algal cells with regular cell wall, **b)** Algal cells treated with hexane extract show pale color and abnormal cell divisions

3. GC-MS analysis of different extracts from *Withania somnifera* leaves

The findings in Table (5) and Fig. (2a) clearly illustrate the presence of distinct components of various fatty acids in the methanol extract of *W. somnifera* leaves. The primary content, accounting for 25.08%, was cyclohexanetetrol. Meanwhile, the fatty acid and sterol component of hexane extract (Table 5 & Fig. 2b) was represented by the derivatives of decanoic acid, which was the main fatty acid with total area percentage (56.97%). Table (6) and Fig. (2c) illustrate the fatty acid and sterol content of the ethyl acetate extract of *W. somnifera* leaves. The major fatty acids, with a total area percentage of 54.44%, were derivatives of decanoic acid. Conversely, the results obtained, as presented in Table (6) and Fig. (2d), demonstrate the presence of decanoic acid derivatives with a total area percentage of 27.44% in the pet-ether extract of *W. somnifera* leaves.

4. Random amplified polymorphic DNA (RAPD) and polymorphism

The random amplified polymorphic DNA (RAPD) technique was used to assess the impact of four solvents used to prepare *W. somnifera* active ingredient on treated *C. closterium*. Table (7) summarizes the findings as follows:

Primer 1 (5'd[GGTGCGGGAA]-3') with the genomic DNA from *C. closterium*, fifty two genomic fragments with various sizes range were amplified. **Primer 2** (5'-d[GTTTCGCTCC]3') showed 51 fragments with various sizes range were recorded. **Primer 3** (5'-d[GTAGACCCGT]-3'), fifty nine fragments were obtained, in addition, 32 polymorphic fragments were recorded with 54% of polymorphism. **Primer 4** (5'-d[AAGAGCCCGT]-3'), 51 fragments with various sizes range were recorded for *C. closterium* genomic. **Primer 5** (5'-d[AACGCGCAAC]-3'), fifty fragments with various sizes range were recorded for *C. closterium* genomic which amplified with fifth primer.

The data obtained from RAPD fingerprinting (Table 8) of *C. closterium* exposed to four solvents of *Withania somnifera* leaf extract indicates that the highest genetic polymorphism and variation (23.8% polymorphism) occurred with the application of hexane solvent, compared to the other solvents. The genetic variation of *C. closterium* after exposure to four solvents for *W. somnifera* extract was arranged in descending order as follows: hexane (23.8%), ethyl acetate (19.6%), positive control (19.4%), methanol (19.2%), and pet ether solvents (18.2%).

Table 5. Bioactive compounds identified in the methanol and hexane leaf extract of *Withania somnifera* by GC-MS analysis showing area percentage and name of each compound

Methanol	Area %	**Hexane**	Area %
Acetic acid,nonyl ester	3.01	Oxirane,tetradecyl(hexadecan	1.98
Benzen acetaldehide	2.24	Palmitic acid ME (hexadecane	2.49
2-Pyrrolidinone,1-methyle	3.10	n-Hexadecanoic acid	5.08
2-Pyrrolidinone	6.12	(E)-13-Docosenoic acid heptadecanedicarboxylic acid)	2.79
2,4-Hexadienal,(E,E)	1.26	13-Heptadecyn-1-ol	2.25
Phenylacetaldehyde	1.18	17-Pentatriacontene	2.03
1H-pyrrole-2-carboxaldehyde	1.22	9,12-Octadecadienoic acid, methyl ester.	6.97
2-Methoxy-4-vinylphenol	2.09	9-Octadecenoic acid, methyl ester	15.59
2-Oxo-1-methyl-3 isopropylpyrazine	3.95	Phytol (2-Hexadecen-1-ol)	4.37
Furane-2-carboxylicacid,5-{4-1methyl propyl)phenoxymethyl}	1.24	Oleic Acid (9-Octadecenoic acid)	0.42
Formyl isoglutamine	1.95	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	2.74
1H-Pyrrole-2,5-dione,3-ethyl-4-methyl (Maleimide,)	1.53	Cyclopropaneoctanoic acid, 2-[(2-pentylcyclopropyl)methyl]-, methyl ester	6.65
Formamide,N-{(1-{(cyano-2-methyl propyl)hydroxyamino}-2-methylpropyl}-	3.45	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	1.76
1,7-Dioxa-10-thia-4,13-diazacyclopentadeca	1.53	i-Propyl 9-tetradecenoate	1.90
12-Tridecynoic acid,methyl ester	2.23	Diisooctyl phthalate	4.82
Pyridine,2-phenyl	2.71	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-	2.34
9-Octadecanoic acid,1,2,3-propanetriyl ester,(E,E,E)-	8.85	2',4'-Dihydroxyacetophenone, bis (trimethylsilyl)ether	4.16
D-Allosa	1.21	Arsenous acid, tris(trimethylsilyl) ester	1.86
2-Naphthalenamine	1.24	1,2-Benzisothiazol-3-amine tbdms	4.00
3-Methyl-4-phenyl-1H-pyrrole	1.63	Octamethylcyclotetrasiloxane	2.57
1,2,3,5-Cyclohexanetetrol.	25.08	Cyclotetrasiloxane, octamethyl-	3.01

à-D-Glucopyranoside, O-à-D-glucopyranosyl-(1.fwdarw.3)-á-D-fructofuranosyl	3.02	1-Monolinoleoylglycerol trimethylsilyl ether	1.89
Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, oxime,	1.37	Monolinoleoylglycerol trimethylsilyl ether	2.62
n-Hexadecanoic acid	3.03	1-(2-Acetoxyethyl)-3,6- diazahomoadamantan-9-one oxime(Chloroethyl acetate)	3.72
Desulphosinigrin	2.41	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	0.79
cis-Vaccenic acid	5.31	(nonadeca-2,6,10(cis)-triene)	2.78
Oleic Acid	3.83	5,7,9(11)-Androstatriene, 3-hydroxy-17-oxo-(Methyl 12-oxo-9-dodecenoate)	1.83
Octadecanoic acid	1.50	1-Monolinoleoylglycerol trimethylsilyl ether	2.10
Codeine	1.40	1-Monolinoleoylglycerol trimethylsilyl ether	1.75
1,4,7-Androstatrien-3,17-dione	1.31	Spirost-8-en-11-one, 3-hydroxy(3á,5à,14á,20á,22á,25R)-(Spirost-5-en-3-ol,)	2.74

Table 6. Bioactive compounds identified in the ethyl acetate and petroleum ether leaf extract of *Withania somnifera* by GC-MS analysis showing area percentage and name of each compound

Ethyl acitate	Area %	**Pet-ether**	Area %
Nonadecane	2.80	Diisooctyl phthalate	2.84
7,9-Di-tert-butyl-oxaspiro(4,5)deca-6,9-diene-2,8-dione	3.17	9-Octadecenoic acid (Z)-, methyl ester	4.00
Pentadecanoic acid, 14-methyl-, methyl ester	2.83	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	4.70
11,14-Eicosadienoic acid, methyl ester	3.44	Diisooctyl phthalate	5.46
9-Octadecenoic acid (Z)-, methyl ester	11.31	Octadecane,3-ethyl-5-(2ethylbutyl)-	3.40
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	4.57	Hexamethylcyclotrisiloxane	1.67
Octadecane, 3-ethyl-5-(2-ethylbutyl)-	1.51	Trimethyl[4-(1,1,3,3,-tetramethylbutyl)	6.59
Hexanedioic acid, mono(2-ethylhexyl)ester	1.71	phenoxy]silane	5.09
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	2.14	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl)ester	2.55
Di-n-octyl phthalate	3.60	2-Bromo-4,5-dimethoxycinnamic acid	1.50
Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-	1.79	16-itrobicyclo[10.4.0]hexadecan-1-ol-13-one	1.81
Medazepam	2.16	Cyclotrisiloxane, hexamethyl	2.42
Piperazine,1-[2-(4-chlorophenoxy)acetyl]-4-(2furanylcarbonyl)--	2.62	cis-10-Heptadecenoic acid, methyl ester	1.49
2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	2.35	1-Monolinoleoylglycerol trimethylsilyl	2.93
1,2-Benzisothiazol-3-amine tbdms	2.25	Ether	2.76
Trimethylsiloxybenzaldehyde oxime, trimethylsilyl-	1.72	16-Nitrobicyclo[10.4.0]hexadecan-1-ol-13-one	5.86
1,2-Benzisothiazol-3-amine tbdms	2.65	2,4,6-Cycloheptatrien-1-one,3,5-bis-trimethylsilyl-	2.35
5-(4,5-Dihydro-3H-pyrrol-2-ylmethylene)-4,4-dimethylpyrrolidine-2-thione	2.17	2,4,6-Cycloheptatrien-1-one,3,5-bis-trimethylsilyl-	1.83
á-Asarone	1.86	1,4-Bis(trimethylsilyl)benzene	1.91
9-Octadecene, 1-[2-(octadecyloxy)ethoxy	2.10	Phenol, 2,6-dichloro-4-nitro-	1.42
Oleic acid, eicosyl ester	1.95	Etamiphyllin	2.21
Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-	**18.53**	Hexasiloxane,1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	2.13
Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-	2.59	4a,7,7,10a-Tetramethyldodecahydrobenzo	3.12
9-Hexadecenoic acid, methyl ester, (Z)-	3.24	[f]chromen-3-ylamine	4.86

Silicic acid, diethyl bis(trimethylsilyl) ester	2.28	1-Monolinoleoylglycerol trimethylsilyl ether	1.66
Cyclotetrasiloxane, octamethyl-	5.47	2,4,6-Cycloheptatrien-1-one,3,5-bis trimethylsilyl	2.22
Tris(tert-butyldimethylsilyloxy)arsane	1.92	O,ctadecane, 3-ethyl-5-(2-ethylbutyl)-	1.63
Cyclohexane, 1,1'-dodecylidenebis [4-methyl-	1.44	Cyclotetrasiloxane, octamethyl	1.52
Octamethylcyclotetrasiloxane	1.79	Hexamethylcyclotrisiloxane	14.09
8,14-Seco-3,19-epoxyandrostane-8,14-dione, 17- acetoxy-3á-methoxy-4,4-dimethyl- and may be 2 Oleyl oleate	2.04	1-Methylcyclooctene	3.98

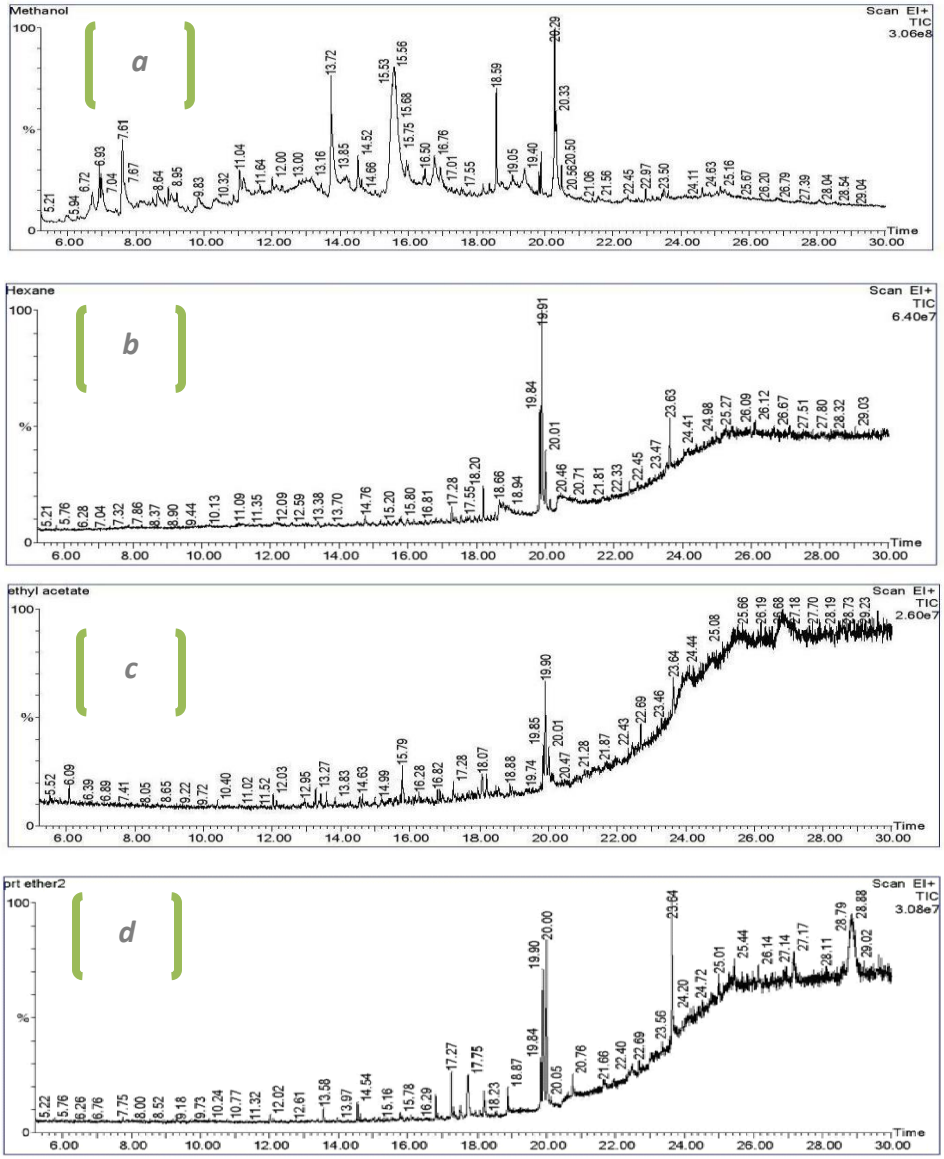
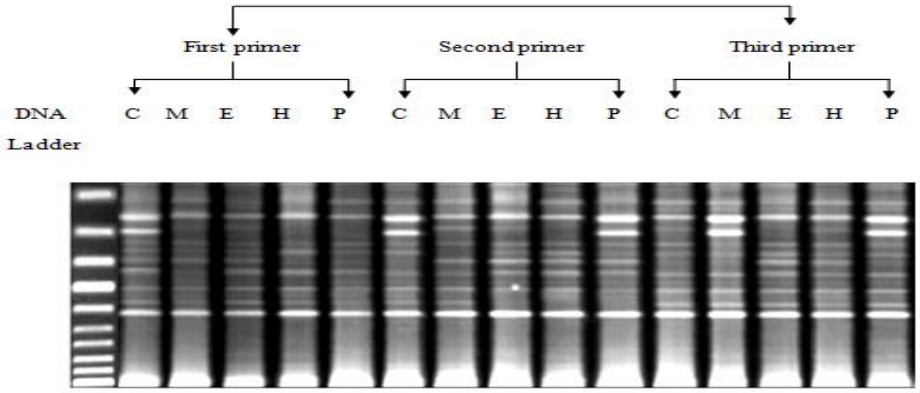


Fig. 3. Chromatograph of GC-MS fractions of **a)** Methanol extract, **b)** Hexane extract**, c)** Ethyl acetate extract, and **d)** Petroleum ether extract of *Withania somnifera* leaves

Table 7. Polymorphism % of RAPD primers

Primer	Total amplified fragment	Polymorphic fragment	Monomorphic fragment	Polymorphism %
Primer1	52	37	15	71
Primer2	51	33	18	64
Primer3	59	32	27	54
Primer4	51	27	24	53
Primer5	50	27	23	54



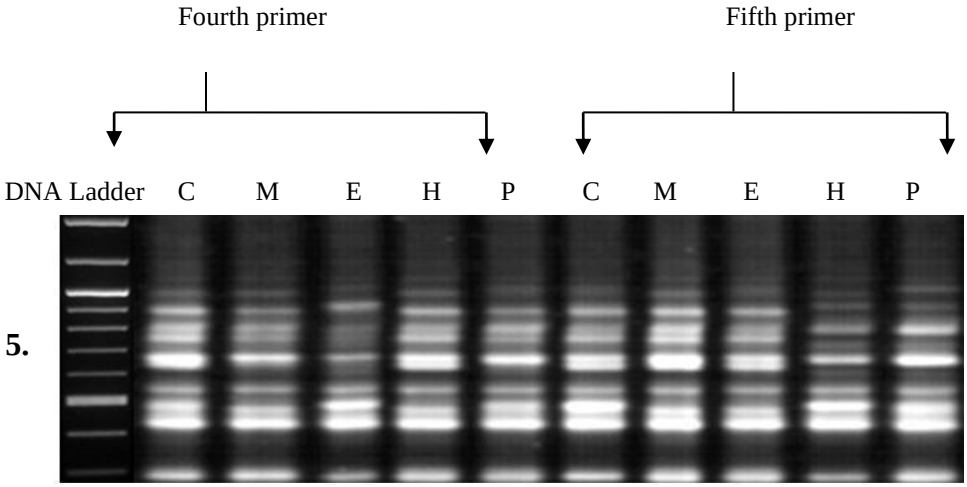


Fig. 5. DNA (RAPD) fingerprinting technique of *Cylindrotheca closterium* treated with *Withania somnifera* leaf extract dissolved in different solvents. (C: Positive control, M: Methanol, E: Ethanol, H: Hexane, and P: petroleum ether)

Table 8. Results of *Cylindrotheca closterium* treated with *Withania somnifera* leaf extract dissolved in four solvents were compared to a control group using random amplified polymorphic DNA (RAPD) fingerprinting, which showed total amplified bands, polymorphic bands, and polymorphism percentage

Primer	Total amplified band						Polymorphic band						Polymorphism %				
	C	M	E	H	P	Total amplified	C	M	E	H	P	Total Polymorphic bands	C	M	E	H	P
1	9	11	10	12	10		6	7	6	8	5	32	19	22	19	**25**	16
2	11	10	9	11	10	51	5	4	3	5	6	23	22	17	13	**22**	26
3	11	11	13	13	11	59	6	5	6	8	4	29	21	17	21	**27**	14
4	10	10	10	11	10	51	4	5	5	5	4	23	17	22	22	**22**	17
5	10	10	11	10	9	50	4	4	5	5	4	22	18	18	23	**23**	18
Average of Polymorphism %													**19.4**	**19.2**	**19.6**	**23.8**	**18.2**

C: Positive control, M: Methanol extract, E: Ethyl acetate solvent, H: Hexane, and P: Petroleum ether.

DISCUSSION

The process by which a microbe or plant produces secondary metabolites, through both direct and indirect interactions, either promote or impede the growth and development of another, which is one of the widest definitions of allelopathy that has been proposed **(Malik & Williams, 2005)**.

The effects of several *Withania somnifera* leaf extracts on *Cylindrotheca closterium* alga growth were investigated in the current study. The findings showed that adding no leaf extract to the *C. closterium* culture increased growth as shown by the optical density. The investigation of **Li *et al.* (2016)** regarding the effect of *Sagittaria trifolia* tubers extract on *Microcystis aeruginosa* likewise discovered the same inhibitory pattern. Following a 96-hour treatment with 0.9g/L root extract, *M. aerogenosa* cells showed an 80.48% growth inhibition rate. Algae growth, however, declines in response to various leaf extract treatments. In accordance with our results, **Mahesh (2008)** observed that hexane extract exhibited the strongest inhibitory effect, starting at 0.021 at 0 minutes and reaching 0.009 at 120 minutes. This suggests that *W. somnifera* has an anti-algal action.

The identification of novel naturally occurring bioactive chemicals may result from phytochemical characterization and active chemical screening investigations on plant-based substances. Several extracts of *W. somnifera* leaves were made for this study in order to look at how this plant affected the growth of a particular alga *C. closterium*. The solvents that were employed were hexane, petroleum ether, methanol, and ethyl acetate. Subsequently, these extracts underwent GC-MS analysis to determine the constituent elements of various *W. somnifera* leaf extracts. The findings demonstrated that *W. somnifera* leaf hexane extract significantly inhibited *C. closterium* growth, as indicated by optical density. These findings might be explained by the fact that the main fatty acids with the highest percentage of total area (56.97) were derivatives of decanoic acid.

Pseudokirchneriella subcapitata's susceptibility to fatty acids was investigated by **Kamaya *et al.* (2003)**. Fatty acids have been proposed by many studies as potential allelochemicals that influence aquatic creatures **(Chiang *et al.*, 2004)**. **Wu *et al.* (2006)** speculate that the lytic action of fatty acids on stressed cells, which results in the rupture of cell plasma membranes, may be the origin of the deadly effects of fatty acids. **Zhou *et al*. (2019)** demonstrated that high concentrations of *Artemisia ordosica* leaf extract inhibit the growth and photosynthetic activity of both *Chlorella vulgaris* and *Nostoc* sp., while low concentrations enhance the chlorophyll fluorescence yield and growth rate of *C. vulgaris*. Fatty acids also have the additional impact of causing phycobilins to separate from the membranes of thylakoids. According to **Hamed *et al.* (1999)**, phenolic chemicals can impede various essential biological activities, such as respiration, cell division, mineral absorption, photosynthesis, protein chlorophyll production, phytohormone action, and nucleic acid metabolism, to varying degrees. In the current study, derivatives of decanoic acid constituted the majority of the ethyl acetate extract. **Kumar *et al.* (2015)** reported similar findings, i.e., alcohol extract of *W. somnifera* included propane, oleic acid, 1-diethoxy 2methyl, azabicyclooctan-3-ol, and 8-methyl-endo. **Siddique and colleagues (2015)** reported the formation of benzyl ether and either arachidonic acid or phytanic acid from the acetone leaf extract of *W. somnifera*. On the other hand, **Kaur *et al.* (2013)** identified more than 35 chemical components found in *W. somnifera* roots. According to **Pandit *et al.* (2013)**, a methanol extract of *W. somnifera* exhibited a wide range of antibacterial activity against *Streptococcus mutans* and *Streptococcus sobrinus*. **Singh and Kumar (2014)** reported that extracts from *W. somnifera* may have antimicrobial properties.

A certain amount of allelochemicals will alter the shape of the cell, causing the protoplast to shrink and split from the cell wall, the nucleus to contract, and the chloroplasts' structure to be disturbed **(Qian *et al.*, 2009)**. Our findings, which demonstrated that algal cells treated with *W. somnifera* hexane extract shrunk in size and almost completely lost color, were in line with this one. Under a light microscope, the cell wall contracted and became uneven, and several internal cell divisions were visible. Controlling the movement of matter and energy between cells and their surroundings depends on the cell membrane. The cell membrane may be harmed if it is not properly maintained **(Tan *et al.*, 2019)**. According to research by **Fm *et al.* (2007)** and **Qian *et al.* (2009)**, exposure to electromagnetic fields (EMA) increased the levels of unsaturated fatty acids in the cell membranes of *Chlorella vulgaris* and *Microcystis aeruginosa*, which caused metal ions like K + and Mg2+ to extravasate into the cells.

Algal growth was also inhibited by allelochemicals, which altered gene expression, protein synthesis, and respiration **(Vardi *et al.*, 2006; Zhang *et al.*, 2010)**. The majority of alkaloids show allelopathy and can bind strongly to target DNA, according to **Wink and Latz-Brüning (1995)**. This inhibits DNA translation and transcription and raises the temperature at which DNA may be cleaved by 5 ~C, all of which have an impact on the completion of protein synthesis.

The highest genetic polymorphism and genetic variation (23.8% polymorphism) was found in the applied hexane extract of *W. somnifera* when compared to other solvents, according to data on genetic polymorphism obtained from RAPD fingerprinting technique applied on *C. closterium* after exposer to four solvents for *W. somnifera* extract. The genetic variation of *C. closterium* was ordered discerningly for hexane, ethyl acetate, methanol, and petroleum ether with 23.8, 19.6, 19.2, and 18.2% of genetic polymorphism, respectively, following exposure to four solvents for *W. somnifera* extract. **Rynearson *et al.* (2000)** measured polymorphism patterns using DNA-based markers, such as ribosomal DNA sequences or restriction fragment-length. M13 DNA fingerprinting was utilized by **Oppermann *et al.* (1997)** to distinguish between two isolates of *Chlorella ellipsoidea* that were taken from distinct habitats and individuals and isolates of the same genus or species.

Shao *et al.* (2010) investigated the physiological reactions of *Microcystis aeruginosa* NIES-843 under wheat bran leachate stress. They discovered that the gene for microcystin synthesis (mcyB) was somewhat up-regulated, whereas the expression of the genes for fatty acid synthesis (fabZ), the antioxidant protein peroxiredoxin (prx), and the D1 protein of photosynthetic processes (psbA) were down-regulated. Here, we showed a physiological model of antialgal sustainability under the stress of *W. Somnifera* extracts. The presented results concluded the production of natural products by tested plant extracts as a response to algal growth.

RECOMMENDATION

It is recommended to manage fisheries in a controlled manner to stop ship ballast and bilge water discharges, and to take the required actions to regulate industrial and household wastes, as well as terrestrial inputs that lead to the numerical abundance of these species and the formation of mucilage. Allelopathic inhibition is widely considered an eco-friendly method that presents a novel and exciting approach to controlling algal blooms. Future studies should also concentrate on creating algicides with many functionalities, including flocculation, sedimentation, and gradual release, that are made of biodegradable modifiers.

REFERENCES

Ausubel, F.M.; Brent, R.; Kingston, R.E.; Moore, D.D.; Seidman, J.G.; Smith, J.A. and Struhl, K. (1994). Current Protocols in Molecular Biology. Wiley, New York.

Besteiro, S.; Brooks, C.F.; Striepen, B.; and Dubremetz, J. F. (2011). Autophagy protein Atg3 is essential for maintaining mitochondrial integrity and for normal intracellular development of *Toxoplasma gondii* tachyzoites. PLoS Pathog. 7:e1002416. doi: 10.1371/journal.ppat.1002416

Chiang, I.Z.; Huang, W.Y. and Wu, J.T. (2004). Allelochemicals of *Botryococcus braunii* (Chlorophyceae). Journal of Phycology. **40**: 474-480.

Cowan, M.M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., **12**: 564-582.

Degobbis, D.; Fonda-Umani, S.; Franco, P.; Malij, A.; Precali, R. and Smodlaka, N. (1995). Changes in the northern Adriatic ecosystem and the hypertrophic appearance of gelatinous aggregates. Sci. Total Environ. **165:** 43–58**.**

Ergul, H.A.; Balkis-Ozdelice, N.; Koral, M.; Aksan, S.; Durmus, T.; Kaya, M.; Kayal, M.; Ekmekci, F. and Canli, O. (2021). The early stage of mucilage formation in the Marmara Sea during spring 2021. J. Black Sea/Mediterranean Environment. **27 (2)**: 232-257.

Farag, N.S.; Fawzi, F.G.; El-Said, S.I.A. and Mikhail, M.S. (1986). Streptomycin in relation to potato brown rot control. Acta Phytopatholgica et Entomologica Hungarica. **21**: 115–122.

Fm, L.; Hy, H.; Chong, Y.X.; Men, Y.J. and Guo, M.T. (2007). Influence of EMA isolated from *Phragmites communis* on physiological characters of *Microcystis aeruginosa*. China Environmental Science. **27**: 377–381.

Grossart, H.P. (1999). Interaction between marine bacteria and axenic diatoms *Cylindrotheca fusiformis*, *Nitzschia laevis*, and *Thalassiosira weissflogii* incubated under various conditions in the lab. Aquat. Microb. Ecol. **19**: 1–11.

Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. In Culture of Marine Invertebrate Animals. Plenum Press, New York. 29–60.

Haitao, L.; Guanpin, Y.; Ying, S.; Suihan, W. and Xiufang, Z. (2007). *Cylindrotheca closterium* is a species complex as was evidenced by the variations of rbcL gene and SSU Rdna. J. Ocean Univ. China. 6 **(2)**: 167-174.

Hamed, A.; AL-Wakeel, S. and Abu-ElSoud, M. (1999). Allelopathic potential of *Acacia nilotica* leaf extract of some metabolic activities of Pea seeds during germination. Journal of Union Arab Biology, Cairo. **7**: 1-16.

Jancula, D. and Maršálek, B. (2011). Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. Chemosphere. 85(9): 1415-1422.

Kamaya, Y.; Kurogi, Y. and Suzuki, K. (2003). Acute toxicity of fatty acids to the freshwater green alga *Selenastrum capricornutum*. Environmental Toxicology **18**: 289-294.

Kaur, N.; Niazi, J. and Bains, R. (2013). A Review on Pharmacological Profile of *Withania somnifera* (Ashwagandha). Research & Reviews of Journal Botanical. 2**(4)**: 6-14.

Kumar, M. and Gupta, R.P. (2015). In vitro antibacterial effect of *Withania somnifera* root extract on *Escherichia coli*. Vet World. 8 **(1)**: 57–60.

Li, F.M. and Hu, H.Y. (2005). Isolation and characterization of a novel antialgal allelochemical from Phragmites communis. Appl. Environ. Microbiol. **71**: 6545–6553.

Li, J.; Liu, Y.G.; Zhang, P.Y.; Zeng, G.M.; Cai, X.X.; Liu, S.B.; Yin, Y.C.; Hu, X. J.; Hu, X.; Tan, X.F. (2016). Growth inhibition and oxidative damage of

Microcystis aeruginosa induced by crude extract of *Sagittaria trifolia* tubers. – Journal of Environmental Sciences. **43**: 40-47.

Linderman, R.G. (1998). *Mycorrhiza* interactions with the rhizospheremicroflora: the mycorrhizosphere effect. Phytopatology. **78**: 366–371.

Liu, J.; Chang, Y.; Sun, L.; Du, F.; Cui, J.; Liu, X.; Li, N.; Wang, W.; Li, J. and Yao, D. (2021). Abundant allelochemicals and the inhibitory mechanism of the phenolic acids in water dropwort for the control of *Microcystis aeruginosa* Blooms. Plants. **10**: 2653.

Lorenti, M.; Buia, M.C.; Martino, V. and Modigh, M. (2005). Occurrence of mucous aggregates and their impact on *Posidonia oceanica* beds. Sci. Total. Environ. **353**: 369-379.

Ma, H.; Huang, L.; Zhang, J.; Shi, D. and Yang, J. (2018). Optical properties of straw-derived dissolved organic matter and growth inhibition of *Microcystis aeruginosa* by straw-derived dissolved organic matter via photo-generated hydrogen peroxide. Environ. Poll. **242**: 768–760.

Malik, M.A.B. and Williams, R.D. (2005). Allelopathic growth stimulation of plants and microorganisms. Allelopathy J. **16**: 175-198.

Mahesh, B. and Satish, S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. World journal of agricultural sciences. **4**: 839-843.

Mathieu, A. (2022). Advances in Bioengineering Study of Microalgae. J. Pet. Environ. Biotechnol. **13**: 1000453.

Mendes, L.B.B. and Vermelho, A.B. (2013). Allelopathy as a potential strategy to improve microalgae cultivation. Biotechnol. Biofuels. **6**: 152.

Moosavi, A.; Afshari, R.T.; Asadi, A. and Gharineh, M.H. (2011). Allelopathic effects of aqueous extract of leaf stem and root of sorghum bicolor on seed germination and seedling growth of *Vigna radiata* L. Not. Sci. Biol. **3**: 114–118.

Ni, L.X.; Jie, X.T.; Wang, P.F.; Li, S.Y.; Wang, G.X.; Li, Y.P.; Li,Y.; Acharya, K. (2015). Effect of linoleic acid sustained-release microspheres on *Microcystis aeruginosa* antioxidant enzymes activity and microcystins production and release. Chemosphere. **121**: 110-116.

Oppermann, B.; Karlovsky,P. and Reisser, W. (1997). M13 DNA fingerprinting in unicellular and filamentous green algae, Europ. J. Phycol. 32 **(2)**: 103-110.

Özalp, H.B. (2021). First massive mucilage event observed in deep waters of Çanakkale Strait (Dardanelles), Turkey. J Black Sea/Medit Environ 27 **(1)**: 49-66.

Padmavathi, B.; Rath, P.C.; Rao, A.R. and Singh, R.P. (2005). Roots of *Withania somnifera* Inhibit Forestomach and Skin Carcinogenesis in Mice. Evid Based Complement Alternat Med. 2 **(1)**: 99-105.

Pandit, S.; Chang, K.W. and Jeon, J.G. (2013). Effects of *Withania somnifera* on the growth and virulence properties of *Streptococcus mutans* and *Streptococcus sobrinus* at sub-MIC levels. Anaerobe. **19**: 1–8.

Qian, H.; Xu, X.; Chen, W.; Jiang, H.; Jin, Y.; Liu, W. and Fu, Z. (2009). Allelochemical stress causes oxidative damage and inhibition of photosynthesis in *Chlorella vulgaris*. Chemosphere. **75**: 368–375.

Ramadan, R.; Khairy, H.M.; Halawa, M. and Haider, A. (2020). Isolation and genetic identification of *Cylindrotheca closterium* algae from Alexandria coastal region. Sci. J. Basic Appl. Sci. 1-14.

Rice, E.L. (1984). Allelopathy, second ed. Academic Press, London, pp. 422.

Pierre, C. and Vallet, G. (2023). A mathematical model of marine mucilage, the case of the liga on the Basque coast. https://hal.science/hal-03930194

Rynearson, T.A. and Armbrust, V.E. (2000). DNA fingerprinting reveals extensive genetic diversity in a field population of the centric diatom *Ditylum brightwellii*. American Society of Limnology and Oceanography. 45 **(6)**: 1329–1340.

Savun-Hekimoğlu, B. and Savun-Hekimoğlu, C. (2021). Mucilage Problem in the Semi-Enclosed Seas: Recent Outbreak in the Sea of Marmara. International Journal of Environment and Geoinformatics. 8 **(4)**: 402-413.

Shaltout, H.K.; Sharaf El-Din, A. and Ahmed, A.D. (2009). Plant life in the Nile Delta. Dar El Kotub No.23263\195.

Shao, J.; Yu, G.; Wang, Z.; Wu, Z.; Peng, X. and Li, R. (2010). Towards clarification of the inhibitory mechanism of wheat bran leachate on *Microcystis aeruginosa* NIES-843 (Cyanobacteria): physiological responses. Ecotoxicology. **19**: 1634–1641.

Siddique, H.Y.; Mujtaba, F.S.; Faisal, M.; Jyoti, S. and Naz, F. (2015). Effect of *Withania somnifera* leaf extract on the dietary supplementation in transgenic Drosophila model of Parkinson's disease, The All Results Journals. 6 **(2)**: 16-23.

Singh, G. and Kumar, P. (2014). Antibacterial activity of flavonoids of *Withania Somnifera* L. Green pharmacy. **(16)**: 114-118.

Staats, N.; De Winder, B. and Stal, L. J. (1999). Isolation and characterization of extracellular polysaccharides from the epipelic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. Eur. J. Phycol. **34**: 161–169.

Staats, N.; Stal, L.J. and Mur, L. R. (2000). Exopolysaccharide production by the epipelic diatom *Cylindrotheca closterium*: effects of nutrient conditions. Journal of Experimental Marine Biology and Ecology. 249 **(1)**: 13-27.

Svetličić, V.; Žutić, V.; Radić, T.M.; Pletikapić, G.; Zimmermann, A.H. and Urbani, R. (2011). Polymer networks produced by marine diatoms in the Northern Adriatic Sea. Marine Drugs. **9**: 666-679.

Tan, K.; Huang, Z.; Ji, R.; Qiu, Y.; Wang, Z. and Liu, J. (2019). A review of allelopathy on microalgae. Microbiology. **165**: 587–592.

Tazart, Z.; Douma, M.; Tebaa, L.; Loudiki, M. (2019). Use of macrophytes allelopathy in the biocontrol of harmful *Microcystis aeruginosa* blooms. – Water Science & Technology Water Supply. 19 **(1)**: 245-253.

Thornton, D.C.O. (2002). Diatom aggregation in the sea: mechanisms and ecological implications. Eur. J. Phycol. **37**: 149–161.

Turk, V.; Hagström, A.; Kova, N. and Faganeli, J. (2010). Composition and function of mucilage macroaggregates in the northern Adriatic. Aquat Microb Ecol. 61 **(3)**: 279-289.

Uddin, M.N.; Robinson, R.W.; Buultjens, A.; AI Harun, M.A.Y.; Shampa, S.H. (2017). Role of allelopathy of Phragmites australis in its invasion processes. J. Exp. Mar. Biol. Ecol. **486**: 237-244.

Vardi, A.; Formiggini, F.; Casotti, R.; de Martino, A.; Ribalet F.; Miralto, A. and Bowler, C. (2006). A stress surveillance system based on calcium and nitric oxide in marine diatoms. PLoS Biol. **4**: 411–419.

Wagner, H.; Bladt, S. and Zagaiwski, E.M. (1984). Plant Drug Analysis, Springer-Verlag, Berlin/New York. 126-169.

Wink, M.; Latz-Brüning, B. (1995). Allelopathic Properties of Alkaloids and Other Natural Products.

Winters, M. (2006). Ancient Medicine, Modern Use: *Withania somnifera* and its Potential Role in Integrative Oncology. Alternative Medicine Review. **11**: 269-27.

Wolfstein, K.; de Brouwer, J. F.C. and Stal, L.J. (2002). Biochemical partitioning of photosynthetically fixed carbon by benthic diatoms during short-term incubations at different irradiances. Mar. Ecol. Prog. Ser. **245**: 21–31.

Wu, J.T.; Yin-Ru, C.; Wen-Ya, H. and Wann-Neng, J. (2006). Cytotoxic effects of free fatty acids on phytoplankton algae and cyanobacteria. Aquatic Toxicology **80**: 338-345.

Yu, S.; Li, C.; Xu, C.; Effiong, K. and Xiao, X. (2019). Understanding the inhibitory mechanism of antialgal allelochemical flavonoids from genetic variations: Photosynthesis, toxin synthesis and nutrient utility. Ecotoxicol. Environ. Saf., **177**: 18–24.

Zhang, T.T.; Zheng, C.Y.; Hu, W.; Xu, W.W.; Wang, H.F. (2010). The allelopathy and allelopathic mechanism of phenolic acids on toxic Microcystis aeruginosa. J. Appl. Phycol. **22**: 71–77.

Zhao, W.; Zheng, Z.; Zhang, J.; Roger, S.F. and Luo, X. (2019). Evaluation of the use of eucalyptus to control algae bloom and improve water quality. Sci. Total Environ., 667: 412–418.

Zhou, X.; Zhang, Y.; An, X.; De Philippis, R.; Ma, X.; Ye, C. and Chen, L. (2019). Identification of aqueous extracts from **Artemisia ordosica** and their allelopathic effects on desert soil algae. Chemoecology. **29**: 61–71.