Mesopo. Environ. j., 2016, 3(1) :1-9, 2016

ISSN 2410-2598

Mesopotemia Environmental journal

journal homepage:**www.bumej.com**

Activity of cynophyta algal extracts (*Anabaena azolla*) against some species of fungi in local habitats

Jasim M. Salman [1] **Mays M. Weber[1]**

[1]Department of Biology, College of Science, University of Babylon, Iraq.

Corresponding author: jasimsalman@uobabylon.edu.iq

To cite this article:

Salman J. M, Weber M.M. Activity of cynophyta algal extracts (*Anabaena azolla*) against some species of fungi in local habitats *Mesop. environ. j*., 2016, Vol.3, No.1.;1-9.

Abstract:

This study was done to indicate the activity of secondary metabolites of cyanophyta algal extracts for *Anabaena azolla* which isolate from one of running water system in Hilla city nearby Babylon university campus and development in BG11 medium to study the effective of secondary metabolites in *An.azolla* extracts (cold water, hot water, acetone and ethanol extracts) to inhibition two species of fungi (*Fusarium oxysporum* and *Penecillium expansum*) also used of HPLC and FT-IR analysis to detected some of active secondary metabolites was done only for acetone and ethanol extracts also to preliminary statements that used of four extracts of blue green alga *Anabaena azolla*. The role of *An.azolla* extracts to inhibition growth of these fungi was detected through measured diameter and percentage of inhibition zone which was increased with concentrations and the results showed these extracts had high correlation ratio and fungi shows high sensitivity to these extract. The mode of mechanism action to inhibition was on fungi cell wall by inhibition active enzymes, excelled treatment for cold water extract . The most important active compounds had been detected in HPLC and FT-IR were phenolic compounds which consists of (Tannic acid, Gallic acid and Coummarin) and flavonoids compounds consists of (Kaempherol and Quercetin) these all compounds detected specially in acetone extract of *An.azolla* , also to introductory statements which were detected six of active groups in extracts like (Terpens, glycosides, saponins, alkaloids, phenols and flavonoids) .

Keywords: Cyanophyceae, *Anabaena azolla*, inhibition growth, Fungi ,Secondary compounds, HPLC,FT-IR.

Introduction:

Anabaena azolla is one of cyanophyta type which is contain of heterocyst and gas vacuole [1] Anabaena can produce toxins in fresh aquatic water ecology and the bloom of *An.azolla* lead to produce alkaloids neurotoxins anatoxin[2] show that Anabaena can produce three types of toxins like anatoxins(s) which is organophosphate toxins, carbmate toxins and saxitoxins (LD_{50} is 10-30 mg/kg) [3]. Anabaena also produce microcystin-LR which is type of hepato toxins and there (LD_{50} is 40-70 mg/kg) and produce

cylindrospermopsin which mechanism is to inhibition of Protein synthesis of fungi [4]. Anabaena produce these toxins as secondary compounds that used to inhibition growth of pathogenic fungi and the unsaturated fatty acids consider important source to produce toxins because its contain of toxic chemical properties and these secondary compounds contracts with hormonal and toxically effects against microbes[5]. This algae can produce lipopolysaccharides endotoxins inhibition of fungi [6] because of this algae can produce many types of toxins and for this reason we can used Anabaena extracts as antibiotics against *Fusariumoxysporum* and *Penecilliumexpansum* [7]. *An.azolla* also used in biological control to inhibition of undesirable microbes because its contain many benefits with estats against fungi and this way is more safety for ecology by compare with synthesis fungal pesticides[8] .

Materials and Methods:

Sample collection and Identification:

Algal samples collected from running lotic system nearby Babylon university and isolate from aquatic plant *Ceratophyllum demersum* as attached algae (9) . Then sampled carried to the laboratory at once to be incubated in suitable conditions for algae growth (10). Experimental cultures were incubated at cool white fluorescent lamps (200 µE/m^2) with light/dark cycle of 16/8 h and 25±1Ċ [11].

Preparation and Sterilization of media:

BG11 medium prepared according to method of Andersen and Kawachi [12] and according to procedure of [13].Original solution preparation from all elements in Table (1) then solutes in 1L of distilled water and sterilized in autoclave (121Ċ for 15min) and measured of pH near at 7.4 by used HCl (1N) with NaOH (1N) to prevent precipitation .

Table1. BG-11 medium components

Components	Stock solution g.L-1dH_2O	Quantity Used	Concentration in Final Medium (M)
$NaNO_3$		1.5g	$1.76x10^{-2}$
K_2HPO_4. $3H_2O$	40	1 ml	$1.75x10^{-4}$
$MgSO_4 \cdot 7H_2O$	75	1 ml	$3.04x10^{-4}$
$CaCl_2 \cdot 2H_2O$	36	1 ml	$2.45x10^{-4}$
Na_2CO_3	20	1 ml	$1.89x10^{-4}$
Citric acid	6	1 ml	$3.12x10^{-5}$
Na-EDTA	1	1 ml	$2.79x10^{-6}$
Ferric ammonium citrate	6	1 ml	$\sim 3x10^{-5}$
Trace metals mix A5	As shown below	1ml	

Concentration in Final Medium(M)	Trace metals mix A5	Amount (g)
$4.63x10^{-5}$	H_3BO_3	2.86
$9.15x10^{-6}$	$Mncl_2.4H_2O$	1.81
$7.65x10^{-7}$	$ZnSO_4.7H_2O$	0.22
$1.61x10^{-6}$	$NaMnO_4.2H_2O$	0.39
$3.16x10^{-7}$	$CuSO_4.5H_2$	0.079
$1.70x10^{-7}$	$CO(NO_3)_2.6H_2O$	0.049
	Distilled water	1L

Serial dilution cultures (SDC) :

Dilution of sample started by adding (1ml) of inoculated sample and complement to (9ml) of BG11 medium ,this procedure pledge many times and the algae is microscopically examined to get single algal cultures and then addition (20ml) of BG11 medium to (2ml) of algae culture to enhancement solution then incubated under suitable conditions for algae growth then the culture till to greenish color [14] .

Algal cultivation for biomass:

Used conical flasks (250ml) which contain (100ml) BG11 and adding (10ml) from isolated algae for two weeks, then transferred to Glass pools (1000ml) of BG11and incubated for 14 days [15].

HPLC analysis:

Analysis of acetone and ethanol extracts of *An.azolla* was done by used HPLC system (Shimadzu, Japan) by used column (5µm x 4.6 mm x 25cm)for Quercetin and Kaempherol with Detector (UV-366nm) and Flow rate (1.0 ml/min)and Coummarin with Detector (UV-280nm) and Flow rate (1.00 ml/min)and Gallic acid with Detector (UV-270nm) and Flow rate (0.7 ml/min) and Tannic acid with Detector(UV-254nm) andFlow rate (1.00 ml min) .

The standard sample were injected in to the HPLC system after they filtered by mile pore filter paper (0.45µm. pore size) and the time required by a sample to pass through the system is recorded as its retention time and calculate according to the following equation:

$$C_{sample} = \frac{C_{standard} \times A_{sample}}{A_{standard}} \times \frac{D.F}{Wt.or\ V}$$

C: Concentration , A: Area under the peak, D.F: Dilution factor, Wt: Weight , V: Volum

FT-IR analysis:

Analysis of acetone and ethanol extracts of *An.azolla* was done by used FT-IR system (Shimadzu, Japan) by used liquid cells and after analysis the identification of active groups in extracts was done with used (400-700 cm^{-1}) .

Statistical analysis :

For statistical analysis of the current study Duncan design was used for laboratory experiments and data were analyzed to study the inhibition capacity of *An.azolla* and at least significant difference was used to compare the significant difference between means at $P<0.05$.

Result and Discussion:

The results show the highest inhibition percentage for *Fus.oxysporum* was (21.764%) in cold water extract and lowest inhibition percentage was(6.470%) in acetone extract show in Table (2) and for *Pen.expansum* the highest inhibition percentage was(17.647%) in hot water and ethanol extracts and lowest inhibition percentage was (8.235%) in acetone extract show in Table(3) and the highest inhibition zone diameter for *Fus.oxysporum*was (18.5nm)cold water extract and the lowest inhibition zone diameter was (5.5nm) in acetone extract and for *Pen.expansum*the highest inhibition zone diameter was (15nm) in hot water extract and the lowest inhibition zone diameter was (6nm) in ethanol extract .

The results show that *An.azolla* extracts had important role to inhibition of fungi also show this algae can produce active compounds like (Phenols, Flavonoids, Alkaloids, Terpens, Glycosides, Saponins) in natural aquatic ecology because the cold and hot water extracts show highly inhibition zone for *Fus.oxysporum* and *Pen.expansum* [16] .

The lag phase for Anabaena algae continuous for long time after adding active extracts and this lead to remain algal cell active and secretion active compounds to inhibition *Fus.oxysporum* and *Pen.expansum* because these extract maybe influenced with enzyme activity of these fungi and caused inhibition [17] .

These fungi show high sensitive to these extracts specially for cold and hot water extracts this maybe because the acetone and ethanol lead to reduce activity of some active compounds which present by measured the inhibition zone and inhibition percentage, phenolic compounds in these extracts can be used as antibiotics [18] .

The mechanism of inhibition for these extracts was facultative for active function in fungi without any side effects [19] and inhibition of *Fus.oxysporum* and *Pen.expansum* may be caused by effect on Mycotoxins or on spores functions or synthesis of B-(1,3)D-glucan or by effect on cell wall that lead to inhibition fungal cell or by influence with cell permeability [20] or effect on *Fus.oxysporum* and *Pen.expansum* fatty acids by decreased of saturated and unsaturated fatty acids because these extract contain mixture of active compounds leads to inhibition of fungi [21] .

An.azolla extracts may be acts in three mechanisms on these two type of fungi, the first one these extracts effect on ergosterol(azoles) [22] . The second mechanism was effect to inhibition of polyenes that contract with enzymes inside cell and the third mechanism was mainly effect on enzymes that made fungal cell wall and inhibition of cell membrane fatty acids synthesis then all of these lead to inhibition these fungi [23] .

Table (2) Secondary compounds groups in blue green alga *An.azolla* detected by HPLC system analysis

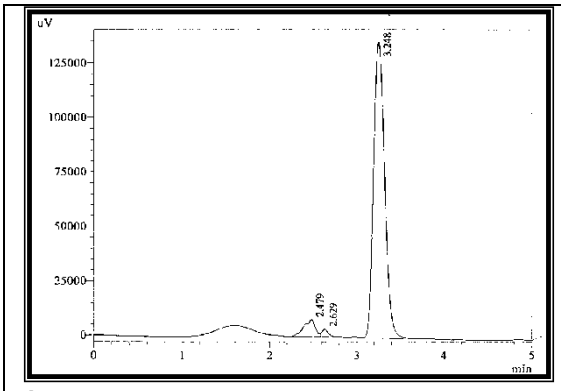
Compounds	Extract	
	Acetone	Ethanol
Tannic acid	33.1 ppm	11.3 ppm
Galic acid	43.1 ppm	N.D
Coummarin	17.2 ppm	N.D
Quercetin	43.7 ppm	22.6 ppm
Kaempherol	27.3 ppm	35.8 ppm

Table (3) Percentage of inhibition zone of algae extracts against *Fus.oxysporum*

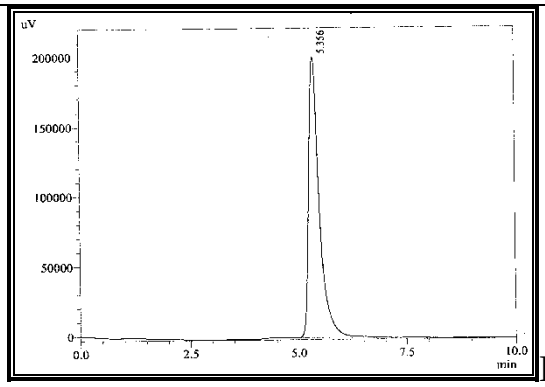
Con. Mg/ml	Inhibition zone %			
	Cold water	Hot water	Acetone	Ethanol
10	11.176	10.588	6.470	11.176
25	14.705	15.882	8.823	11.764
50	21.764	17.058	12.352	14.705

Table (4) Percentage of inhibition zone of algae extracts against *Pen.expansum*

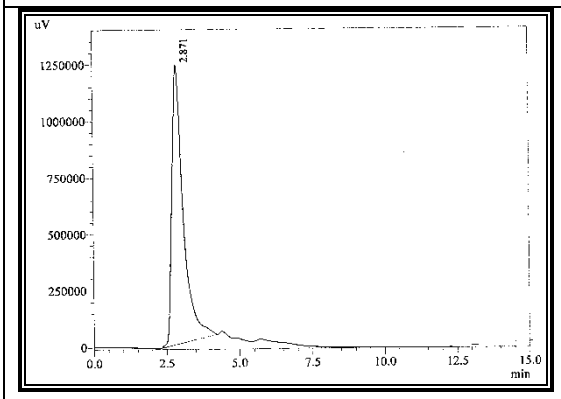
Con. Mg/ml	Inhibition zone %			
	Cold water	Hot water	Acetone	Ethanol
10	10	12.352	8.235	7.058
25	11.764	14.705	12.352	15.294
50	17.058	17.647	14.705	17.647



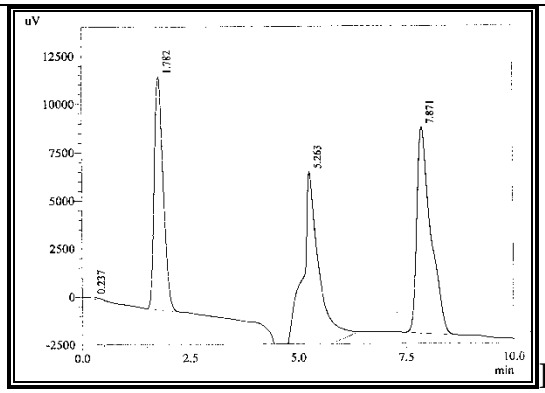
A



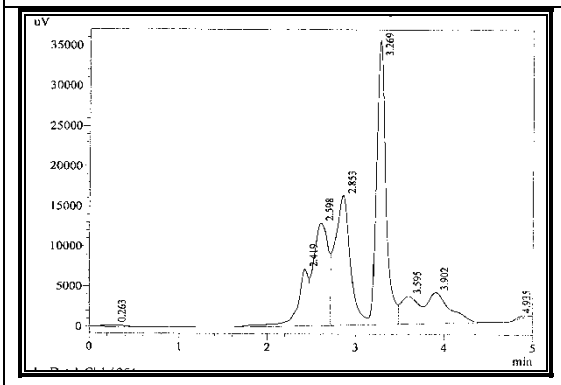
B



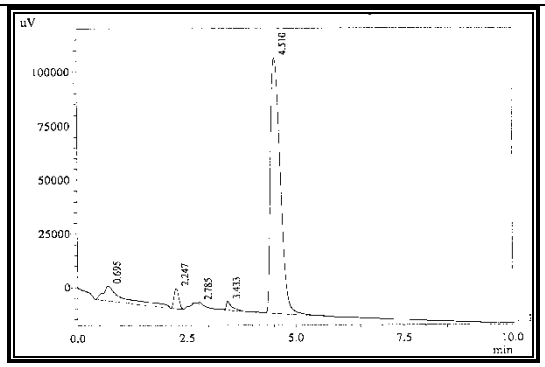
C



D

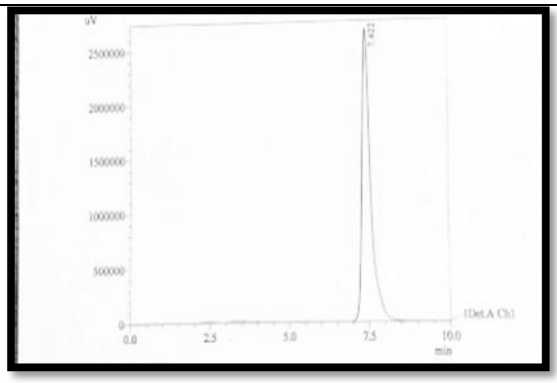


E

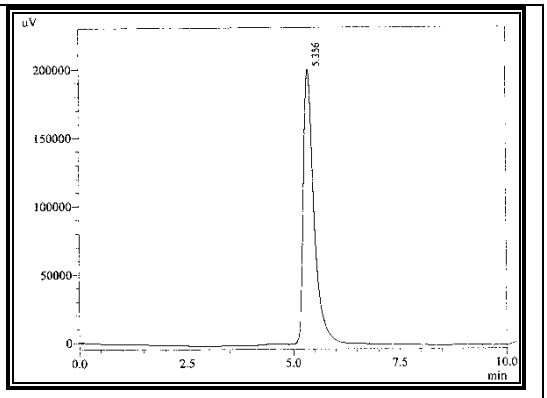


F

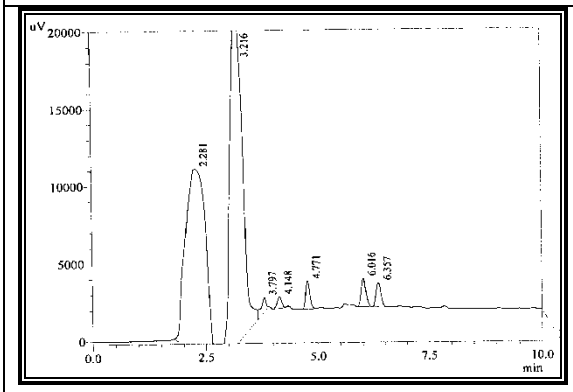
Fig.1 Chromotogram of HPLC to blue green alga *An. azolla*:
(A) Tannic acid standard.
(B) Gallic acid standard.
(C) Tannic acid in acetone extract.
(D) Gallic acid in acetone extract.
(E) Tannic acid in ethanol extract.
(F) Gallic acid in ethanol extract.



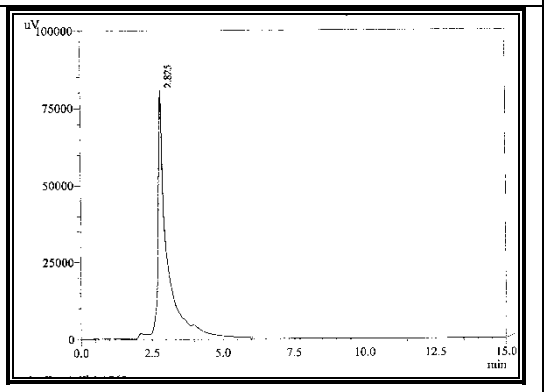
A



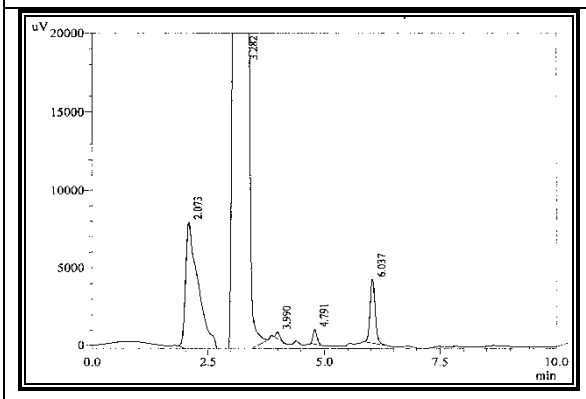
B



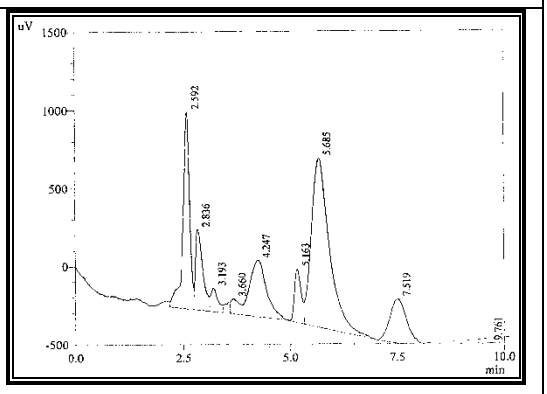
C



D

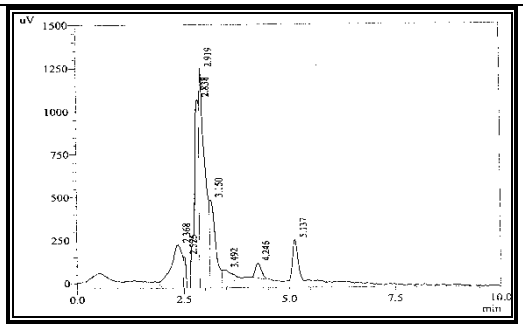


E

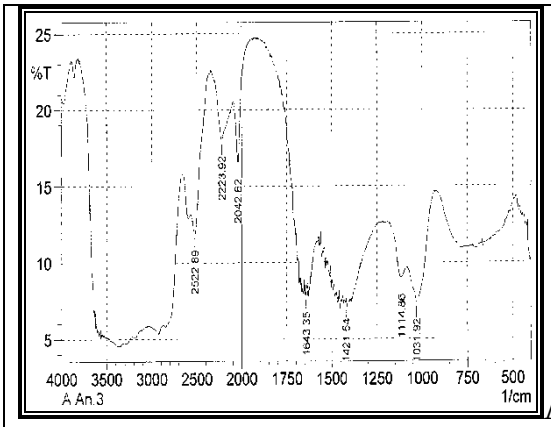


F

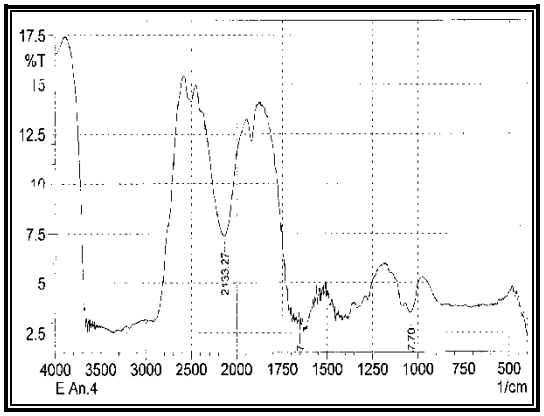
Fig.2: Chromotogram of HPLC to blue green alga *An. azolla*:
(A)Chromatogram of HPLC for Coummarin standard.
(B)Chromatogram of HPLC for Quercetin standard.
(D) Chromatogram of HPLC for Kaempherol standard.
(C) Chromatogram of HPLC for Coummarin in acetone extract.
(E) Chromatogram of HPLC for Coummarin in ethanol extract .
F) Chromatogram of HPLC for Quercetin and Kaempherol in acetone extract .
(G) Chromatogram of HPLC for



G



A-



B

Fig.3: Secondary compounds analysis in FT-IR system:
(A) Infrared FT-IR analysis for active compounds in acetone extract.
(B) Infrared FT-IR analysis for active compounds in acetone extract

.

Conclusions:

1-Anabaena extracts show high inhibition zone percentage on fungi species under study.
2- This study show that the cold water extract had highest activity to inhibition fungi under study that improve of this alga can secretion active compounds under natural growth conditions.

Acknowledgment

All material of this study was supported by department of Biology, College of Science and Environmental Research Center, University of Babylon.

References:

[1] Rippka, R.; Deruelles, J.; Waterbury, J.B.; Herdman, M. and Stanier, R.Y. Generic assignments, strain histories and properties of pure cultures ofcyanobacteria. Journal of General Microbiology,.111(1): 1-61. 1979.

[2] Sivonen ,K; and Jones, G. Cyanobacterial toxins. In: Chorus, I. and J. Bartram(Eds.),toxic cyanobacteria in water :Aguide to their public health , consequences , monitoring and management, London: WHO:41-111. 1999.

[3] WHO, Guidelines for safe recreational water environments – Volume 1: coastal and fresh waters. World Health Organization: Geneva. 2003.

[4] Griffiths, D.J; and Saker, M.L. The Palm Island mystery disease 20 years on: a review of research on the cyanotoxin cylindrospermopsin. Environ Toxicol, 18(2): p. 78-93. 2003.

[5] Moe, C.L. Waterborne transmission of infectious agents, in Manual of environmental microbiology, C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach and M.V. Walter, Editors. American Society for Microbiology: Washington, 136-52. 1997.

[6] Blahova, L;Adamovsky, O; Kubala, L; Svihalkovd, L; Zounkova, R; and Blaha, L. The isolation and characterization of lipopolysaccharides from Microcystaeruginosa, aprominent toxic water bloom forming cyanobacteria Toxicon 76:187-196. 2013.

[7] Yousif, D.Y.M. Activity of some Macro-algae Extracts Against phytopathognic Fungi, Ph.D. thesis, Coll. Of science, University of Baghdad, Iraq. 2014.

[8] Salman, J.M; and Abdul-Adel, E. Potential use of cyanophyta species *Oscillatorialimnetica*in bioremediation of organophosphorus herbicide glyphosate. Mesop. Environ. J, 1(4): 15-26. 2015.

[9] Priya-Gopinath, T. and Ajit Kumar, K. G. Micro algal Diversity of the OF THE Fresh Water lake In Thiruvananthapuram district, Kerala. International Journal of Plant, Animal and Environmental Sciences, Vol.5, No.1, pp. .2015.

[10] Shoker R. M. H. and Jawad, A. M. Evaluation of Isolated Compounds Activity from Convolvulus Arvensis Against Algae. Iraqi Journal of Science, Vol.54,No.1,pp.62-71.2013.

[11] Rippka, R.; Deruelles, J.; Waterbury, J.B.; Herdman, M. and Stanier, R.Y. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology,.111(1): 1-61. 1979.

[12] Andersen, R. A. and Kawachi, M. Traditional Microalgae Isolation Techniques In: Andersen, R.A. [Ed.] Algal Culturing Techniques. Academic Press, San Diego.578. 2005.

[13] Jawad, A.L.M. Interactions Between Cyanobacteria and Other Microorganisms. Ph.D Thesis , Liverpool University, 123-125. 1982.

[14] Desikaghary, F.R. Cyanophyta, London, Acad. Press. 1959.

[15] (15)Tredici, M.R. Mass production of microalgae: photobioreactors. In: Richmond A.[Eds.] Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Oxford: Blackwell Science. 2004.

[16] Rossana, A.C.; Valdirene, M.G.; Ana, F.U.; Candvania, M. Effect of proteins from the red seaweed Hypneamusciformis (Wulfen) Lamouroux on the growth of human pathogen yeasts. Brazilian of Biology and Technol., 49:915-921. 2006.

[17] Hellio C, De La Broise, D., Dufossé, L., Le Gal, Y. &Bourgougnon , N . Inhibition of marine bacteria byextracts of macroalgae: Potential use for environmentallyfriendly antifouling paints. Marine Environmental Research, 52: 231-247. 2001.

[18] Abd El-Aty, M; Mohamed, A; Samhan , A. In vitro antioxidant and antibacterial activities of two fresh water Cyanobacterial species, *Oscillatoriaagardhii* and *Anabaena sphaerica*. Journal of Applied Pharmaceutical Science, 4 (7): 069-075. 2014.

[19] Salvador, N., Gómez, G. A., Lavelli, L. and Ribera, M. A. Antimicrobial activity of Iberian macroalgae. Scientia Marina 71: 101-113. 2007.

[20] G**upta, C. P.; Dubey, R. C.; Kang, S. C. and Maheshwari, D. K.** Antibiosismediatednecrotrophic effect of *Pseudomonas* GRC2 against two plant pathogens. Curr Sci. 81(1): 91-94. 2001.

[21] Persson F, R Svensson, GM Nylund, NJ Fredriksson, H Pavia & M Hermansson. Ecological role of asecondary metabolite for a colonizing bacterial community.Biofouling 27: 579-588. 2011.

[22] Sheehan D J, Hitchcock C A, Sibley C M. Current and emerging azole antifungal agents. ClinMicrobiol Rev. 12:40–79. 1999.

[23] Nozawa Y, Morita T. Molecular mechanisms of antifungal agents associated with membrane ergosterol. Dysfunction of membrane ergosterol and inhibition of ergosterol biosynthesis. In: Iwata K, VandenBossche H, editors. In vitro and in vivo evaluation of antifungal agents. Amsterdam, The Netherlands: Elsevier Science Publishers, B. V.; p. 111. 1989.