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Allelopathic Effect of *Asparagus officinalis* Extracts on the Growth of *Chlorella vulgaris*

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Abstract | **Original Research Article**

In laboratory study was evaluated the allelopathic effect of *Asparagus officinalis* organs (shoot, tubers and roots) aqueous extracts on growth of Chlorella vulgaris. In this study were assessed *A. officinalis* organs cold aqueous extract at different concentrations (25, 50, 75 and 100%). The results suggested that *A. officinalis* shoot, tubers and roots cold aqueous extract appeared to have allelopathic effect on the *C. vulgaris* compared to untreated control. The growth of *C. vulgaris* was significantly reduced gradually with the increase of aqueous extract concentration levels. However the reduction was varied and could be parts of the *A. officinalis* and extract concentration dependent. All *A. officinalis* organs extracts had different degrees of allelopathic effect against the tested algae, the highest allelopathic activity was recorded for tubers extract at concentration 75 and 100% followed by the roots extract at 75 % whereas, the lowest allelopathic activity was achieved for shoot extract. Extensive studies should be undertaken for the ethanolic extract of *A. officinalis* organs as a strong allelopathic activity against green algae.

Keywords: Allelopathy, *Asparagus officinalis*, *Chlorella Vulgaris*, Aqueous extract.

INTRODUCTION

The term "Allelopathy" means an any process involving secondary metabolites produced by plants, micro-organisms, viruses and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects" [1]. Chemicals released from plants and imposing allelopathic influences are known as allelochemicals. Most allelochemicals are classified as secondary metabolites such as phenolic compounds, tannins, flavonoids, terpenoids, alkaloids, steroids, quinons and glycosides ,There are hundreds of secondary metabolites in the plant kingdom and many are known to be phytotoxic [2]. These allelochemicals are reported to be present in almost all plant parts, including stems, leaves, flowers, buds, pollen grains, seeds, fruits, roots, and rhizomes [3]. *A. officinalis* is an important garden vegetable plant, which is cultivated in temperate and subtropical areas. Asparagus is an herbaceous, perennial, and belonging to asparagus order, asparagaceae family and asparagus genus. This species is an economically important genus of *asparagus* with high nutritional, pharmaceutical and industrial values [4]. *Asparagus* cultivation is growing due to rich anti-cancer compounds and antioxidants, such as saponin, aspartic acid, rotin, protodioscin, glutathione, phenolic compounds, flavonoid, and A, B, C, and E vitamins as well as zinc and fiber content it has been used as an anti-inflammatory [5], and antifungal activities [6]**.** *C. vulgaris* is a photosynthetic microorganisms and eukaryotic from family of chlorellaceae. This organism is a unicellular green microalga and has spherical cells with diameter of 2 to 10 micrometers, which has asexual reproduction in which; a mother cell reproduces 4 daughter cells, so that its growth rate is higher and found in both fresh and marine water **[**7]. Therefore, the aim of this study was to evaluate the allelopathic effect of *A. officinalis* organs (shoot, tubers and roots) aqueous extracts on growth of *C. vulgaris.*

Microalgae Isolation and Culturing

C. vulgaris were taken from soil sample of Alexandria city. In this work, the method described by [8]. Soil samples were added into Petri dishes containing solution (enrichment culture) and then cultured in a constant temperature (25°C) with light (4000 LUX intensity of light). The media used for isolating the green algae was (Chu 10) [9]: (Ca (NO3)2 0.04g/l, K2HPO4, 0.01g/l, MgSO4.7H2O 0.025g/l, Na2CO30.02g/l, Na2SiO3.5H2O 0.025g/l, FeCl30.0008g/l.). This medium was used to isolate Chlorophyta .The isolation of the algae were carried out using the moist plate method recommended by [10]. *C. vulgaris* was cultivated with media Chu 10 in 500 ml flasks containing 200 ml of culture under controlled conditions at

laboratory temperature. Light was provided by cool-white fluorescent lamps at 4000 Lux with a dark/light cycle of 16:8 h for 16 days.

Plant sampling and preparation of extracts

The fresh samples of shoot, tubers and roots were collected from the wild *Asparagus* (*Asparagus officinalis* L.) shrubs growing in the Al-Jabal Al-Akhdhar region–Libya. The samples were further identified by Taxonomist at the Department of Botany Herbarium, Faculty of Science, and Omar Al-Mukhtar University. The collected samples were washed and crushed and extracted with cold distilled water [11]. Different concentrations of cold aqueous extract (25, 50, 75 and 100% w/v) were prepared, in addition to the control (distilled water). The cold aqueous extract used directly to treatment the algal cultures and the algae growth rate each 2 days for 16 days were recorded each two days for 16 days at the end of the experiment, the calculation of the algae growth rate were recorded according to the following equation [12].

$$\text{Growth rate} = \ln (B_1 - B_0) / (t_1 - t_0)$$

Where:

B_1 = the number of cell at time t1.

B_0 = the number of cell at time t2.

t_1 = time at the beginning of the experiment.

t_2 = time at the end of the experiment.

RESULTS

The allelopathic effect of different concentration of *A. officinalis* organs (shoot, tubers and roots) aqueous extract (25, 50, 75 and 100%) on *C. vulgaris* growth rate under laboratory conditions are represented in Tables 1, 2, 3 and 4 respectively. The data demonstrated that the number of cells and growth rate of *C. vulgaris* were affected by applying the different concentrations of the shoot, tubers and roots aqueous extract of *A. officinalis*. Generally, number of cells and growth rate decreased with the increase in treatment concentrations. Compared to control, the reduction increased as the concentration of the donor species increased. The reduction was varied and was parts of the donor species dependent.100% concentration level had the greatest inhibitory effect.

Table-1: Effect of different concentration levels of *Asparagus officinalis* Shoot aqueous extract on growth rate of *Chlorella vulgaris.*

Concentration Levels% / Time (days)	0		25		50		75		100	
	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R
0	1.25	-	1.25	-	1.25	-	1.25	-	1.25	-
2	6.00	0.78	6.00	0.78	7.00	0.86	6.50	0.82	8.25	0.90
4	7.50	0.11	4.75	- 0.12	8.75	0.11	6.25	- 0.01	6.50	- 0.12
6	9.00	0.09	5.25	0.05	9.00	0.01	5.25	- 0.08	6.25	- 0.02
8	10.3	0.07	4.75	0.05	10.0	0.05	4.00	- 0.13	5.50	- 0.06
10	12.0	0.08	9.00	0.32	9.50	- 0.03	3.25	- 0.01	5.25	- 0.02
12	12. 5	0.01	8.50	- 0.03	7.25	- 0.16	2.75	- 0.08	4.00	-0.14
14	11.0	- 0.06	7.50	- 0.06	6.00	- 0.09	2.50	- 0.04	3.50	-0.06
16	10.3	- 0.03	6.00	- 0.11	5.50	- 0.04	2.00	- 0.11	3.00	-0.07

Cell No: Number of cell G.R: Growth rate

Table-2: Effect of different concentration levels of *Asparagus officinalis* Root aqueous extract on growth rate of *Chlorella vulgaris*

Concentration Levels% / Time (days)	0		25		50		75		100	
	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R
0	1.25	-	1.25	-	1.25	-	1.25	-	1.25	-
2	6.00	0.78	3.25	0.74	5.50	0.74	6.00	0.78	5.25	0.72
4	7.50	0.11	4.25	0.56	5.00	- 0.05	5.00	-0.1	7.50	0.18
6	9.00	0.09	5.00	0.11	4.75	- 0.03	4.50	-0.05	4.50	0.45
8	10.3	0.07	8.00	0.23	4.25	- 0.06	4.00	- 0.06	5.25	0.10
10	12.0	0.08	6.75	- 0.12	3.75	- 0.06	3.50	- 0.07	6.00	0.07
12	12. 5	0.01	5.75	- 0.08	3.00	-0.11	2.25	- 0.22	5.50	-0.04
14	11.0	- 0.06	5.00	- 0.07	3.00	0.00	2.00	- 0.06	5.00	-0.05
16	10.3	- 0.03	4.75	- 0.03	2.50	- 0.09	2.00	0.00	4.25	- 0.08

Cell No. : Number of cell G.R: Growth rate

Table-3: Effect of different concentration levels of *Asparagus officinalis* Tuber aqueous extract on growth rate of *Chlorella vulgaris*

Concentration Levels% / Time (days)	0		25		50		75		100	
	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R
0	1.25	-	1.25	-	1.25	-	1.25	-	1.25	-
2	6.00	0.78	4.00	0.85	5.00	0.78	4.5	0.64	7.00	0.80
4	7.50	0.11	5.25	0.14	5.50	0.05	4.75	0.03	6.75	- 0.02
6	9.00	0.09	8.00	0.21	5.00	- 0.04	3.75	-0.12	4.50	- 0.20
8	10.3	0.07	7.00	- 0.06	4.00	- 0.11	3.50	- 0.03	4.00	- 0.05
10	12.0	0.08	6.75	- 0.02	3.75	- 0.0 3	2.25	- 0.22	3.50	- 0.06
12	12. 5	0.01	5.75	- 0.08	3.25	- 0.07	2.00	- 0.05	3.00	- 0.07
14	11.0	- 0.06	5.00	- 0.07	3.00	- 0.04	2.00	0.00	2.75	- 0.04
16	10.3	- 0.03	4.00	- 0.11	2.75	- 0.04	1.75	0.06	2.25	- 0.10

Cell No: Number of cell G.R: Growth rate

Table-4: Effect of different concentration levels of *Asparagus officinalis* Mixture aqueous extract on growth rate of *Chlorella vulgaris*

Concentration Levels% / Time (days)	0		25		50		75		100	
	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R	Cell No. $\times 10^6$ Cell l/ml	G.R
0	1.25	-	1.25	-	1.25	-	1.25	-	1.25	-
2	6.00	0.78	4.25	0.61	4.75	0.66	3.00	0.43	8.50	0.90
4	7.50	0.11	3.75	- 0.06	5.25	0.05	6.00	0.34	6.00	- 0.17
6	9.00	0.09	3.75	0.00	4.00	- 0.14	6.50	0.04	5.50	- 0.04
8	10.3	0.07	4.50	0.09	3.25	- 0.10	8.50	0.13	5.25	- 0.02
10	12.0	0.08	4.25	- 0.03	3.00	- 0.04	7.25	- 0.08	4.75	- 0.05
12	12. 5	0.01	4.00	- 0.03	2.50	- 0.09	6.25	- 0.07	4.00	- 0.08
14	11.0	- 0.06	3.50	- 0.07	2.25	- 0.05	5.00	- 0.11	3.50	- 0.06
16	10.3	- 0.03	3.25	- 0.04	2.00	- 0.06	4.75	- 0.03	2.75	-0 .12

Cell No. : Number of cell G.R: Growth rate

DISCUSSION

Allelopathy is widely understood as the harmful effect that one plant has on another plant due to the chemicals it releases into the environment [13]. This work dealt with the allelopathic effects of different concentrations (25, 50, 75, 100%) of Aqueous extract of *A. officinalis* plant (root, shoot, tuber, mixture). On growth rate of green alga *C. vulgaris* as compared the

effect of extracts of root, shoot, tuber, mixture, we founded the minimum values of growth rate when we added the extract of the tuber and root the results cleared that growth rate decrease gradually by increasing the period of culturing this may be due to the *A. officinalis* plant have some allelopathic character. Another study agreement with this explanation, *A. officinalis* plant contains some compounds such as, phenol, steroidal saponins [14], phenolic compounds [15], which considered allelochemical compounds [16,17]. Told that when *Asparagus* is planted in a field where it has been grown before, there are often lower yields and greater loss of young plants. One of the causes of these phenomena is allelopathy. The same case recorded with crude extracts from the roots and crown residues were bioassay on many different fungal isolates on Petri plates and were found to inhibit the growth of oomycetous fungi. Extracts from the roots were found to be more active than extracts from other portions of the *Asparagus* plants [18]. The mode of action of the compound depends on the nature of the interaction between donor and target organisms, the activity of allelopathic compounds being directed against either competitors or predators. In the context of competition, this is mainly with other photoautotrophic organisms, allelopathic compounds. May inhibit photosynthesis, kill the competition or exclude it from the donor vicinity [19]. And allelochemicals from Phragmites communis could destroy the membrane structure of *Chlorella pyrenoidosa* and *Microcystis aeruginosa*. By analyzing the types and relative content of phospholipid fatty acids in the algal cell membrane [20]. And this supports our findings. Its effectiveness on growth suggests that Aqueous extract of *A. officinalis* plant (root, shoot, tuber, mixture) may act as a source of allelochemicals after being released into soil or after decomposition.

CONCLUSIONS

The present investigation revealed that aqueous extract of *A. officinalis* different concentration levels inhibited the growth rate of *C*. vulgaris. Its effectiveness on germination and growth suggests that aqueous extract (root, shoot, tuber, mixture) of *A. officinalis* act as a source of allelochemicals after being released into soil or after decomposition. The presence of allelochemicals negatively affects the algae and plants. Further studies are suggested to clarify the possible physiological mechanism to allelopathic effect on algae.

REFERENCES

1. Torres A, Oliva RM, Castellano D, Cross P. First world congress on allelopathy-a science of the future. Cadiz, Spain. 1996.
2. Batish DR, Kaur S, Singh HP, Kohli RK. Role of root-mediated interactions in phytotoxic interference of Ageratum conyzoides with rice (Oryza sativa). Flora-Morphology, Distribution, Functional Ecology of Plants. 2009 Jan 1;204(5):388-95.
3. Singh A, Singh D, Singh NB. Allelochemical stress produced by aqueous leachate of Nicotiana plumbaginifolia Viv. Plant Growth Regulation. 2009 Jun 1;58(2):163-71.
4. Sarabi B, Hassandokht MR, Hassani ME, Ramak MT. Evaluation of morphological characteristics of Iranian edible wild asparagus (Asparagus officinalis L.). *Iran J Horticultural Sci* 2010 ; 41: 197-207.
5. Jang DS, Cuendet M, Fong HH, Pezzuto JM, Kinghorn AD. Constituents of Asparagus officinalis evaluated for inhibitory activity against cyclooxygenase-2. Journal of agricultural and food chemistry. 2004 Apr 21;52(8):2218-22.
6. Shimoyamada M, Suzuki M, Sonta H, Maruyama M, Okubo K. Antifungal activity of the saponin fraction obtained from Asparagus officinalis L. and its active principle. Agricultural and Biological Chemistry. 1990;54(10):2553-7.
7. Ortiz Montoya EY, Casazza AA, Aliakbarian B, Perego P, Converti A, de Carvalho JC. Production of Chlorella vulgaris as a source of essential fatty acids in a tubular photobioreactor continuously fed with air enriched with CO2 at different concentrations. Biotechnology progress. 2014 Jul 8;30(4):916-22.
8. Cameron FA, Morelli and GB Blank. Soil studies-Microflora of desert regions. IV. Soil extract as a culture medium JPL. Space Programs Summary.1965: 37-32.
9. Chu SP. The influence of the mineral composition of the medium on the growth of planktonic algae: part I. Methods and culture media. The Journal of Ecology. 1942 Aug 1:284-325.
10. Al-Wathnani H, Ara I, Tahmaz RR, Al-Dayel TH, Bakir MA. Bioactivity of natural compounds isolated from cyanobacteria and green algae against human pathogenic bacteria and yeast. Journal of Medicinal Plants Research. 2012 May 16;6(18):3425-33.
11. Al-Abed AS, Qasem JR, Abu-Blan HA. Antifungal effects of some common wild plant species on certain plant pathogenic fungi. Dirasat. Series B, Pure and Applied Sciences. 1993;20(3):149-58.
12. Robert RLG. Growth measurements. Division rate. In: *Physiological Methods. Culture and Growth Measurements*. R.J. Stein (Ed.), Cambridge University Press, Cambridge. 1979. p 275.
13. Willis RJ. Terminology and trends in allelopathy. Allelopathy Journal. 1994;1(1):6-28.
14. Fukushi E, Onodera S, Yamamori A, Shiomi N, Kawabata J. NMR analysis of tri-and tetrasaccharides from asparagus. Magnetic Resonance in Chemistry. 2000 Dec;38(12):1005-11.
15. Zhang SH, Wang ZY, Zhang CY and Sun CH, Extraction and adsorption of *Asparagus* flavonoids with macroporous resin. *J Henan UnivTechnol (Nat SciEdn)* 26:78–83. 2005.
16. Bais HP, Loyola-Vargas VM, Flores HE, Vivanco JM. Root-specific metabolism: the biology and

biochemistry of underground organs. In Vitro Cellular & Developmental Biology-Plant. 2001 Nov 1;37(6):730-41.

17. Motoki S, Ozawa T, Hattori T, Komura T, Oka J, Komatsu K, Tsukada M. Allelopathy in asparagus 1: reduction of the allelopathic effect on asparagus by the flowable agent in activated carbon. Acta horticulturae. 2002.
18. Hartung AC, Stephens CT. Effects of allelopathic substances produced by asparagus on incidence and severity of asparagus decline due toFusarium crown rot. Journal of chemical ecology. 1983 Aug 1;9(8):1163-74.
19. Leflaive JP, Ten-Hage LO. Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. Freshwater Biology. 2007 Feb;52(2):199-214.
20. Li FM. *Inhibition effect of allelochemicals from macrophytes on harmful algal growth* (Doctoral dissertation, Dissertation for the Doctoral Degree. Beijing: Tsinghua University. 2005.