

## Study alcoholic extract effecting of *Actinidia* in the growth of two types of algae *Microcystis* sp. & *Chroococcus* sp.

Zainab Mohsin Ibrahim<sup>1</sup> & Athraa Oodaa Hussain<sup>2</sup>

<sup>1,2</sup> Department of Biology, College of Science, Thi-Qar University, Iraq

### ABSTRACT:

The main objective of this study is examine the effect of (*Actinidia*) Kiwi Peel extract against growth two species of blue-green algae (Cyanobacteria) : *Chroococcus* sp and *Microcystis* sp. the growth rate of algae counted directly by Chamber. The algae farms were divided in to Three groups as following : the first group (C1): Treatment control concentration 0% , the second group (C2): Treatment of alcoholic extract concentration of the Kiwi plant 1% , the third group (C3): alcoholic extract concentration of the Kiwi plant 5% . All groups were noted during the time periods.

The results showed the (*Actinidia*) Peel extract caused a significant inhibition ( $p < 0.05$ ) in the rate of growth of algae at concentration 5% for two species, and the colonies were reached in *Microcystis* sp (72) cell in ml compared with control , in *Chroococcus* sp. was least cells (60) . *Microcystis* sp was more resistance for *Actinidia* Peel extract.

**KEYWORDS:** Cyanobacteria, *Chroococcus* algae, *Microcystis* algae, (*Actinidia*) Kiwi Peel alcoholic extract,

### INTRODUCTION:

The pollution of the problems facing the world, especially water pollution, which is defined as the change in the river's water conditions directly or indirectly as a result of the activities of the human problem and thus it causes changes in environmental ecosystems and constitutes a danger to human health, can some types of algae live in water contaminated with feces sewage, as used large amounts of nitrogen compounds and phosphates in waste during its growth (sprue, 1992). Nutrient direct impact on the density of phytoplankton, is the large increase in nutrients.

The large increase in plant nutrients (nitrogen and phosphorus) in the water of the problems that threaten the water ecosystem is, and this increase grow large numbers of algae and prosperity on the surface of the water layer, causing Algale Blooming phenomenon and which are effects adverse to the aquatic environment and organisms that live in them and so lead to a reduction in the diversity of algae (2003, Klug), and that the viability of algae make the water taste and smell is not good, and it has the ability to change the pH and water color and turbidity. Considering algae from organisms that change the chemical and physical properties important to the water, such as turbidity, temperature, color and materials radioactive and organic materials, bio-oxygen demand (BOD) and pH and dissolved oxygen (DO) (Perscott, 1975). The use of some immune extracts found in some fruits and vegetables that feed the human in the daily food *Actinidia* protect the body from damage resulting from toxins microbiology because they contain effective groups at high rates have the ability to protect body cells from damage these toxins by increasing the production of enzymes antioxidants when strep (Freeman, and Kodera, 1995; Lawson and Hughes, 1992) These are rich source of vitamin-C (Ascorbic acid). The peel of the *Actinidia* contains various active constituents and essential oils.

**Actinidia** peel is one of the important dietary sources of antioxidant phenolics ( Jayaprakasha et al.,2008)

Kiwi(**Actinidia**) is an important medicinal plant of the family Actinidaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities and the antibacterial potential in crude extracts of different parts (awaii *et al.*, 2000). Many polymethoxylated flavones have several important bioactivities, which are very rare in other plants (Ahmad *et al.*,2006).

The aim of the study to know the vital effects of alcoholic extract of **Actinidia** peel, in terms of the fact that this extract Stimulator or an inhibitor of the growth of two types of algae Cyanophayta namely: *Chroococcus* sp. & *Microcystis* sp.

#### **MATERIAL AND METHODS :**

##### **Washing and sterilizing instruments used:**

Washed and sterilized all-glass tools used in the media culture preparation and in the isolation, purification and cultivation of microalgae acid, hydrochloric acid HCl concentration (20%) then washed tools tap water and then distilled water was dried and sterilized using electric oven at a temperature of (105)°C for two hours were used flasks conical glass various sizes (250, 500) ml in culture experiments after closed at clean and sterile cotton, of the agar media have been used sterile plastic dishes .

##### **Sampling:**

Subsurface water samples were collected from the Different areas of the River Gharraf, the samples were collected by using clean polyethylene bottles, it installed to sample part using formalin concentration of 4% for the purpose of microscopic examination while leaving the other without installed for the purpose of of culture.

##### **Media Culture:**

Attended the media (Chu -10) and the modified from (Al- Aarajy, 1996) in Stock solutions Table (1) and use distilled water in the preparation and without sterilization while using it and save the media in the refrigerator at a temperature(4°C)in dark.

Then mixed equal amounts (1) mL of each Stock solutions and then complete the volume to 1 liter with distilled water then justice the pH between (7.4 - 7) by adding drops of sodium hydroxide solution NaOH concentration of 10 mg / L or acid Hydrochloric HCl concentration (10%) using pH - meter and infertility media in Autocleave under temperature (121) ° C and pressure (15) lbs / Ang for 20 minutes, then leave to cool degree lab temperature then phosphate salts added to it after sterilized by filtration using filtration paper their openings diameter (0.45) microns to prevent the deposition of phosphate on the walls of the glass bottle during sterilization, The culture media solid has attended the same liquid media components after the addition of Agar him by 15 g / liter, and sterility and left to cool then pour in the petri dishes dry and sterile near flame burner and preserved all the dishes after hardening in the refrigerator temperature (4) °C in the upside-down while in use.

**Table 1: The chemical composition of the media (Chu -10) modified by (Al – Aarajy , 1996).**

mg /l	Compound	mg/l	Compound
25	NaHCO <sub>3</sub>	53.3	NaNO <sub>3</sub>
0.045	MnCl <sub>2</sub> . 4H <sub>2</sub> O	10	K <sub>2</sub> HPO <sub>4</sub>
0.007	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> . 4H <sub>2</sub> O	25	MgSO <sub>4</sub> . 7H <sub>2</sub> O
0.056	ZnSO <sub>4</sub> .7H <sub>2</sub> O	40	CaCl <sub>2</sub> . 2H <sub>2</sub> O
0.02	CuSO <sub>4</sub> . 6H <sub>2</sub> O	1.46	FeCl <sub>3</sub> . 6H <sub>2</sub> O
0.72	H <sub>3</sub> BO <sub>3</sub>	6.2	Na <sub>2</sub> SiO <sub>3</sub> . 9H <sub>2</sub> O
0.01	CoCl <sub>2</sub> . 6H <sub>2</sub> O	31.8	Na <sub>2</sub> EDTA

### Isolation and purification of algae:

For order to obtain unialgal Culture Used dishes agar planning method and after a series of dilutions for a unialgal Culture (Stein, 1973).

The purification of unialgal Culture from bacteria, according to (Wilson and Demmig-Adams, 2007; Kyung and Lee, 2001) described detail in (Pereira *et al.*, 2006).

### Diagnosis species of algae :

The following sources adopted in the diagnosis of species of algae used in the study: (Gowda *et al.*, 2004; Kivanc and Kunduhoglu, 1997; Topal, 1989) Where it was isolated algae Classified and described below:

Division : Cyanophyta (Blue green algae)

Class: Cyanophyceae

Order: Chroococcales

Family: Chroococcaceae

Genus: *Chroococcus* sp., *Microcystis* sp.

### EXTRACTION PROCEDURE:

The plants used in this study were *Actinidia* (kiwi), The peels were collected from the local fruit juice shops. After collection, the peels were shade dried at room temperature (30 - 35°C). 20 gm of peels of kiwi were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers for further use.

The dried and powdered peel materials (10 gm) were extracted with 200 ml of each solvent separately by using soxhlet extractor for 2 to 5 h at a temperature not exceeding the boiling point of the Solvent . The solvents used for the study were ethanol . The extracts were filtered and then concentrated to dryness. The extract were transferred to glass vials and kept at 4° C before use. The extracts were dissolved in 25% aqueous dimethyl sulfoxide (DMSO) to produce a stock solution of 100 mg/ml. Ladd *et al.* ( 1978)

It was prepared concentrations of alcoholic extract of the plant kiwi(*Actinidia*) was calculated by the following formula:

$$N1 * V1 = N2 * V2$$

Whereas:

C1: Treatment control concentration 0%

C2: Treatment of alcoholic extract concentration of the Kiwi plant 1% .

C3: Treatment of alcoholic extract concentration of the Kiwi plant 5% .

**Testing of extracts activity for (peel kiwi) *Actinidia* for growth rate of algae :**

Appoint isolates the addition of (0.10) from the farm pure liquid inoculums for each of the two algae to volumetric flasks (250 ml) container at media culture supported by alcoholic extract of pomegranate previous concentrations above, and using three replicates per concentration, incubter at a temperature (27 ± 2)° C with the level of lighting (50) Maekerooanstein / m<sup>2</sup> / s and system Lighting (16: 8) Lighting: darkness bearing in mind the continuous shaking of samples a day for the purpose of obtaining the desired growth (Tomaselli *et al.*, 1981), as well as the sample Culturing without adding alcoholic extract kiwi of her mind in order to control sample .

**Measuring the rate of growth:**

The growth rate of algae counted directly by Chamber Shidu(Coombs *et al.*,1986) .

**RESULTS:**

The results of the current study showed a decrease in the number of cells *Chroococcus* sp. and *Microcystis* sp. With increasing concentrations of alcoholic extract (peel kiwi ) and gradually during the period of incubation as in Figure (1.2) , and confirmed by the statistical analysis of the existence of a negative relationship between the number of cells and increasing concentrations of alcoholic extract (kiwi peel).

**Figure 1: Role of alcoholic extract (kiwi peel) *Actinidia*. in growth rate of *chroococcus* sp.**

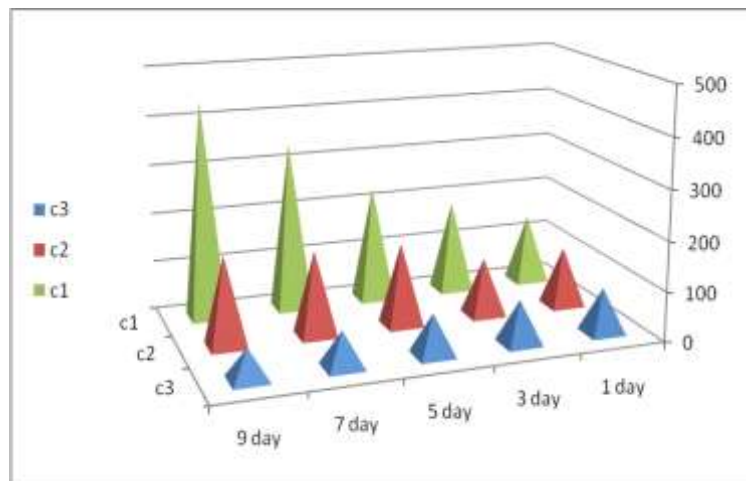
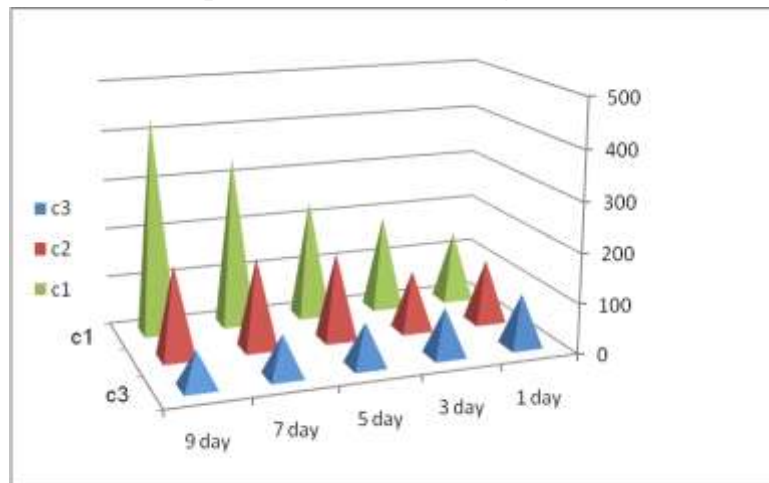


Figure 2: Role of alcoholic extract (peel) *Actinidia*.(kiwi) in growth rate of *Microcystis* sp.



The results of the present study shown in figure (1,2). The result showed the first day a significant Resistant ( $p < 0.05$ ) in growth of rate of *Microcystis* sp of alcoholic extract (Kiwi peel) more than *Chroococcus* sp , Where the rate of growth at calculation found that the highest number of algae cells *Chroococcus* sp. When treatment to differing concentrations of alcoholic extract (Kiwi peel) (439cells / ml when the treatment C1 (0%) in the ninth day of treatment and the lowest number was (60) cells / ml when the treatment C3 (5%) on the same day , either *Microcystis* sp. has reached a higher number of cells (435) cells / ml when the treatment C1 (0%) in the ninth day also and the lowest number (72) cells / ml when the treatment C3 (5%) on the ninth day showed the results of the statistical analysis and the presence of significant differences ( $P \leq 0.05$ ) between treatments and between periods of incubation.

## DISCUSSION:

Results showed isolation from the local environment and the spread of the species and genus of algae, mostly of blue-green algae and diatoms, and between these genres is more *Chroococcus* sp. A unicellular algae assembles in colonies and characterized cells form spherical and be the contents of the cell to an area distinct dark and light area.

The blue-green alga *Microcystis* sp. It is also present in our environment, but it will be longer growth rate than growth rate *Chroococcus* sp. And present in the colonies also because the colony be either regular or elongated or circular or irregular, a toxic algae because it produces toxic compound Microcystin a polypeptide as mg per kg body causing death (al-Saadi & Sulaiman, 2006) .

Widespread use of the two genus in the our environment and local bluegreenalgae from being encouraged to isolate and purify and study the effect of alcoholic extract *Actinidia* (Kiwi peel).

Indicate the negative relationship between the concentrations of alcoholic extract of (Kiwi peel) and the rate of growth two algae to influence the inhibitory extract may be due to the effectiveness of *Actinidia* against two algae to fit on a number of effective compounds against microorganism such as compounds alkaloid, flavonoid, glycosides , polyphenol , tannin (Al-Brahim 2008; Lu *et al.* 2002; Ahmad & Beg. 2001)

The resistance may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Abu-Shanab *et al.*,2004).

Another reason may be due to the fact that these two algae belongs to a class of blue- green algae which resemble bacteria in many of the character of so called Cyanobacteria (Saadi Sulaiman, 2006), they are to walk the path of the bacteria in the fact that the alcoholic extract inhibitor of Kiwi is very effective against bacteria (Ghalibi ,2013) .

Plant also contains a number of phenolic compounds such as Caffeic acid( Lu et al., 2002) which has shown to be effective against bacterial and against fungal (Cowan, 1999). The effectiveness of the alcoholic extract was due to the synergistic action of a group of chemical compounds such as phenols, flavonoids and alkaloids present in the extract and a different side chains, giving it the flexibility to work on several targets from microscopic cell (Hugo & Russell, 1987) has pointed Reed (1995) to the ability of these compounds on the deposition of proteins and so by the composition of hydrogen bonds between the hydroxide cyclic groups and proteins, thus inhibiting an enzyme necessary for the metabolism microorganism. Algae differ among themselves in terms of resistance to him for alcoholic extract *Actinidia* (Kiwi peel), as expressed *Microcystis* sp Resistant more than the other algae in response to different concentrations of the alcoholic extract *Actinidia* (Kiwi peel) reason may be due to the presence of the genes of the virulent organism poison gives him recipe the direction of the resistance effects.

It became clear from this study that alcoholic extract *Actinidia* (Kiwi peel). a broad impact on microbiology (algae) and different algae with each other in terms of him resistance to extract alcoholic *Actinidia* (Kiwi peel), where showed *Microcystis* sp Resistant more than the other algae response to different concentrations of the extract alcoholic *Actinidia* (Kiwi peel). We recommend further studies on *Actinidia* (Kiwi peel). and extract effective materials and isolate them to increase their impact on the algae, especially poisonous ones.

## REFERENCES :

- [1] Abu-Shanab, B.; Adwan, G.; Abu-Safiya, D.; Jarrar, N; and Adwan, K. (2004). Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. Truk. J. Biol. 28:99-102.
- [2] Ahmad M.M., Salim-ur-Rehman, Iqbal Z., Anjum F.M. and Sultan J.I. Genetic variability to essential oil composition in four citrus fruit species. Pak. J. Bot., 38(2): pp.319-324(2006).
- [3] Ahmad, L. and Beg, A. (2001) . Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistance human pathogens. J. Ethnopharmacol. 74: 113-123.
- [4] Al- Aarajy, M. (1996). Studies on the mass culture of some microalgae as food for fish larvae. Ph. D. Thesis, Univ. Basrah - Iraq, P : p 107.
- [5] Al- Brahim, J.S.R. (2008) . Effect of pomegranate ( *punica granatum* ) juice on the inhibition of wound bacterial infection. Ass.Univ. Bull. Environ. Res.11(2).
- [6] Al-Saadi, Hussein Ali and Sulaiman, Nidal Idris. (2006). Science algae phycology. Dar Yazouri scientific publication and distribution. Amman / Jordan from 0.16 to 17.
- [7] Aronson J.K., Nature Publishing Group. Retrieved from:
- [8] Claus, E.P. ( 1956 ) . Gathercoal & wirth pharmacognosy, Henry kimpton, Pennsylvania,p.432.
- [9] Cowan, M.M. (1999) . Plant products as antimicrobial agents. Clinical microbiology Reviews. 12(4): 564-582.



- [10] Desikashary.T. (1959). Cyanophyta . India council of agriculture Reaserch , New Delhi, 517 p.
- [11] Droop,M.(1967).A producer for routin purification of algae culture with antibiotics .Br.Phycol.Bull.3:295-297.
- [12] Freeman , F . and Kodera, Y. ,(1995)."Garlic: stability of S-(2-propenyl)-2- propene -1 sulfinothioat callicin )in blood , solvent s , and simulated physiological fluids". J. Agric. food chem.43: 2332-2338.
- [13] Ghalibi, Iqbal Aziz Amin. (2013). Inhibitory effect of extracts of fruit peels in the growth of some bacteria positive and negative for the gram stain. publication accepted.
- [14] Good-Win, T.W.; and Mercer, E.I. (1986). Introduction to plant Biochemistry. Pergamon Press Oxford, New York. 3.rd. Ed.
- [15] Gowda, N. K. S.; Malathi, V. and Suganthi, R. U. (2004). " Effect of some chemical and herbal compounds on growth of *Aspergillus parasiticus* and aflatoxin production" .Animal Feed Science and Technology 116(3-4): 281-291.
- [16] Gross, E.M. (1999). Allelopathy in benethic and Littoral Areas: case studies on Allelochemicals from benethic Cyanobacteria and Submersed Macrophytes. In Principles and Practice in plant Ecology. pp. (179-199).
- [17] Gross, E.M.; and Sutfeld.R. (1994). Polyphenols with algicidalin the submerged macrophyte *Myriophyllum spicatum*. L. Acta. Hortic . 381:710-716.
- [18] <http://medicine.nature.com>. (2001)
- [19] Hugo, W.B. and Russell, A.D. (1987 ). Pharma ccutical microbiology. Backwell scientific publication oxford London. 511p.
- [20] Jayaprakasha G.K., Girenavar B. and Patil B.S. Radical scavenging activities of Rio red grape fruits and sour orange fruit extracts in different in vitromodel systems. Bioresour. Technol., 99(10): 4484-94 (2008).
- [21] Kawaii, S., Yasuhiko T., Eriko K., Kazunori O., Masamichi Y., Meisaku K., ChihiroIto and Hiroshi F. Quantitative study of flavonoids in leaves of Citrus plants. J. Agric. Food Chem., 48: 3865-3871 (2000).
- [22] Klug, J. (2003). Effects of variation in nitrogen and phosphorus ratios and concentrations on phytoplankton communities of the Housatonic River, Eco., 81: 387-398.
- [23] Kruse, M.V. & Mahan, L.K. ( 1984 ). Food, nutrition and diet therapy, A textbook of nutrition care, 7th ed. W.B. Saunders Co. Philadelphia. P. 850-977.
- [24] Ladd, J.L. ; Jacobson, M. and Buriff, C.R. ( 1978 ). Japanese beetle extracts from neem tree as feeding deterrents. J. E com. Entomol. (71) : 810-813.
- [25] Lawson, L.D. and Hughes, B.G. ,(1992)."Characterization of the formation of allicin and other thiosulfinates from garlic" planta med. 58: 345–350.
- [26] Lu, E.P. ; Gokmen, V. andArtik, N. (2002) . Organic acides and phenolic compound in pomegranate ( *Punica granatum* L.) grown in Turkey. J. Food composition and analysis. 15(5): 567-575.
- [27] Mohammed,A.A.; Hassan, F.M.; Mohammed, B.T.(1999).Effects of aqueous extracts of licorice (*Glycyrrhiza glabra* .L ) & cinnamomes (*Cinnamomum zylanicum*) on growth characteristics of algae . J. of Babylon University.(4) 3: 724-728.



- [28] Mouloud, Bahram Khidr Suleiman, Nidal Idris and Bassam Ibrahim Tawfiq (1990). Algae and Alorkikonat. The Ministry of Higher Education and Scientific Research. Baghdad University
- [29] Nadkarni, A.K. (2000) . Dr.K.M. Nadkarnis Indian Materia Media. 3rd ed. Popular prakashan private Limited. Vol.1.
- [30] Precott ,G.(1975).Algae of the western Greet take areas. Ellion C. Brown Co. Phd, Iowa. Pp.977.
- [31] Reed, J.D. (1995 ). Nutritional toxicology of tannins and related polyphenolsin forage legumes. J. animal socity, 73 : 516-528.
- [32] Robinson,N.J.(1989). Algal metallothioneins : ssecondary metabolites and proteins .J.app.phycol.1:5-18.
- [33] Shahnah, S.M., Ali S., Ansari H and BagriP. New sequiterpene derivative from fruit peel of citrus limon (Linn) Burn. F. Sci. Pharm., 75: 165-170 (2007).
- [34] Sprue, Haider Hamoudi (1992). Algae and water pollution. Omar University Almokhtar-Libya.
- [35] Stein, J. R. (1973). Hand book of phycological methods. Cambridge Unv. Press. Cambridge, UK. W.M.C. Comp. Publisher, Dubuque Iowa , pp: 977.
- [36] Tomaselli, L.; Giovannetti, L. and margheri, M. (1981). The mechanism of trichome breakage in Spirulina platensis and Spirulina maxima. Ann. Microbiol., 31: 27 - 33.
- [37] Watt, J.M. & Breyer – Brandwijk, M.G. ( 1962 ). The medicinal and poisons plants of southern and eastern Africa. E. and S. Livingston Ltd. Edinburgh and London. pp 875- 876.
- [38] Weidman .V.;Walne , P. and Tinor ,F.(1984).A new technique for obtaining axenic culture of algae .Can. J.Bot.42:958-959.