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Effect of Some Medicinal Plants Extracts on the Growth of the Alga *Microcystis Aeruginosa* Kuetz.

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Abstract

The aim of the present study was to investigate the effect of three aqueous extracts made from three medicinal plants; *Glycyrrhiza glabra* L, *Nigella sativa* L.and *Pimpinella anisum* L., in three graded concentrations as 3.5, 7.0 and 14.0 mg/ml of each extract on growth *of M.aeruginosa* (an alga belong to Cyanophyceae). The study criteria included total number of alga, growth rate and growth duplication time .The design of the experiment was CRD in three replications. Analysis of variance and the least significant difference (LSD) at 0.05% level utilized to detect treatment effects.

Results indicated that aqueous extracts had a significant effect on the study parameter of the experiment. Extract from *Glycyrrhiza glabra* L inhibited the growth of the alga (*M.aeruginosa*), in which highest GI(85%) was obtained at 14.0 mg/ml in 72 hours after the alga was exposed to the extract solution, while that of GI (71%) was at 70 mg/ml, 96 hours after exposure. Black seed (*Nigella sativa* L.) extract increase the growth rate of the test alga five times that of control treatment at concentration of 14.0 mg/ml and a reduction in multiplication time was noticed.

Extract of *Pimpinella anisum* L. in low concentration (3.5, 7.0) enhanced growth rate of the alga while high concentration of 14.0 mg/ml reduced it.

الخلاصة

هدفت الدراسة لمعرفة تأثير ثلاث مستخلصات مائية لنباتات طبية وهي عرق السوس والحبة السوداء واليانسون واستخدمت ثلاثة تراكيز مدرجة 3.5 و7.0 و14.0 ملغرام /سم^3 لكل مستخلص على نمو الطحلب ***Microcystis aeruginosa*** (العائد الى الطحالب الخضر المزرقة) واستخدم العدد الكلي للطحلب ومعدل النمو وزمن التضاعف كدلالة لمعرفة تاثير المستخلصات. أعتمد التصميم العشوائي الكامل (CRD) واخذ ثلاث مكررات وتم تحليل النتائج باستخدام اختبار تحليل التباين واقل فرق معنوي عند مستوى المعنوية (0.05%) لمعرفة تأثير المعاملات .

أشارت النتائج بان المستخلصات المائية لها تأثير معنوي على الدلائل المستخدمة في التجارب. أوضحت النتائج وجود فعالية تثبيطية لمستخلص عرق السوس حيث بلغ أعلى معدل للتثبيط 85% عند التركيز 14.0 ملغرام /سم^3 بعد 72 ساعة من التعريض في حين كان معدل التثبيط 71% عند تركيز 7.0 ملغرام /سم^3 بعد 96 ساعة من التعريض. لوحظ زيادة في نمو طحلب الاختبار عند استخدام مستخلص الحبة السوداء خمس مرات مقارنة بمعاملة السيطرة عند التركيز 14.0 ملغرام /سم^{3}ولوحظ اختزال في زمن التضاعف. التركيز المنخفض لمستخلص اليانسون عززت من نمو الطحلب في حين التركيز العالي عند 14.0ملغرام /سم^3 قد خفض من معدل النمو .

Introduction

The plant kingdom is well known to have, in the compestion of it's members, a natural substances that either enhance or retard growth and performance of other organism in their ecosystem (1). Several investigators studied the effects of plant extracts on growth of several organisms such as Bacteria and Animals (2,3).Others (4,5,6,7,8,9) studied the effect of medicinal plants containing chemical compounds having the botentials to modify physiological

activities of other organisms at different concentrations of it's extract.

Microcytic aeruginosa considered as one of the most important alga in fresh water all over the world because it produce toxic cyclic peptides called Microcystin, the excessive growth (blooming)of toxic cyanobacteria in drinking water sources is being increasingly recognized as a risk to the public health (10,11).

Studies on the effect of extractions obtained from medicinal plant on the performance and growth of algae especially *M.aeruginosa* is scare thus the purpose of this research is to find the effect of aqueous extractions of three medicinal plant on the word wide distributed alga *M.aeruginosa* in water that causes health hazards.

Materials and Methods

Local isolate of alga *Microcystis aeruginosa* kuetz (AUFRAC No.44) was obtained from algae unit in fish research in Iraqi Atomic Energy Commission. This isolate was maintained on Beijerinck medium table (1), for ten days inside illuminated incubator (Gallen Kamp) at 39.5 $\mu E.m^{-2}.s^{-1}$ and 25°C (13).

Table (1): Composition of the Bijerinck medium (12).

Stock No.	Salts	Concentration g/l
1. use 100 Ml/l	NH_4NO_3	1.5
	K_2HPO_4	0.2
	$MgSO_4$	0.2
	$CaCl_2.2H_2O$	0.0027
2. use 40 ml/l	KH_2PO_4	9.1
3. use 60 ml/l	K_2HPO_4	11.16
4. Micronut-rients use 1ml/l	H_3BO_3	1g/100 ml
	$CuSO_4.5H_2O$	0.15g/100 ml
	EDTA	5g/100 ml
	$MnCl_2.4H_2O$	0.5g/100 ml
	$FeSO_4.7H_2O$	0.5g/100 ml
	$(NH_4)_6MO_7O_{24}.4H_2O$	0.15g.100 ml

While the medicinal plants samples were collected from local markets in Baghdad and Al-Qadisiyah province (Iraq).These plants were classified by Babylon University herbarium (Table 2).

Table (2) Local name, scientific name and part used of medicinal plant uesd in study.

Local name	Scientific name	Part used
1-Liquorice (sus)	*Glycyrrhiza glabra* L	Woody roots
2-Black seed	*Nigella sativa* L	Seeds
3- Anise (Anise seed)	*Pimpinella anisum* L	Seeds

Aqueous extracts were prepared according to Shaheed *et al.* (7). Preliminary detection for active chemical compounds in medicinal plants was performed according to (14, 15).

The test alga culture treated with graded concentration 3.5, 7.0 and 14.0 mg/ml of each plant extracts for four days. Total cell number (TCN) counted with heamocytometer, growth rate (GR) and multiplication time (G) were obtained according to the equation of (16)

$$G = \frac{0.301}{K}$$

K= growth rate (GR).

These parameters were used to estimate the alga biomass. Growth inhibition rate (GI) and effective concentration (EC_{50}) were obtained for inhibitory concentration according to (17).Analysis of variance and least significant difference (L.S.A.) at 5% level utilized to detect treatment effects (18).

Results

The tentative identification of active chemical compounds in the extracts confirmed that licorice, gave positive results for alkaloids, saponins, tannins, glycosides, flavonoids and cumarins. Test for resines gave negative results. The pH value was 5.6. Black seeds had been contain all above tests and pH value was 5.5, while Anise extracts gave positive results for all

mentioned tests excepts alkaloids and cumarins and pH value was 4.6 table (3).

The Effect of Licorice Extract

The analysis of variance of the experiment revealed that the aqueous extract of licorice had an inhibitory effect on the test alga. This inhibitory affect was reflected in the decrease of TCN and GR and increase in multiplication time (G).

The high concentration of extract had less values of TCN and GR, and an increase of G (fig1, 2, 3). The growth inhibition rates (GI) were shown at the high concentration value (14.0 mg/ml) after 96 hours exposure, while less (GI) value was at low concentration (3.5 mg/ml) at 48 hours exposure (fig 4). On the other hand (EC_{50}) values at 72 and 96 hour exposure were 6.10 mg/ml and 4.5 mg/ml, respectively.

The effect of Black seeds extract

The results of black seed extract exposure on the test alga showed that extract had a stimulatory action on the growth indices of *M. aeruginosa* .These actions represented by increment of TCN, GR and decrease of (G).This increasing in growth parameters reached the highest level at concentration of 14.0 mg/ml after 96 (fig. 5,6,7,8) . The analysis of variance of the experiment revealed that the aqueous extract of black seed had significant difference between the treatment and control groups ($p < 0.05$; L.S.D = 0.0145) and there was positive correlation (r) between growth rate (GR) and concentration used (r=+0.97), While there was negative correlation between multiplication time (G) and concentration of extract (r = - 0 .74).

The Effect of Anise Extract

The results reveal that anise extract had an affect in manner different according to the concentration used. Low concentration (3.5 and 7.0 mg/ml) were shown stimulatory to the growth of the test alga, increase in TCN and GR and decrease in doubling time (G). While high concentration (14.0 mg/ml) was showed inhibitory to the growth of studied alga, decrease in TCN, GR and increase in (G) in a comparison to control group (fig 9, 10, 11). Correlation factor(r) showed negatively relation between concentration and growth rate (r = 0.45) and positive relation between concentration and multiplication time.

Discussion

The reduction in growth parameters (TCN, GR and G) with the use of licorice extract may be due to the presence of many naturally chemical compounds in this extract like saponin glycoside (glycyrrhizin) which had an inhibitory effect on algae (7). Also akaloidic compounds presence in licorice can suppress many physiological activities in plant cell (1, 15).

On the other hand tannic compounds which had been detected in this plant previously (14) had a strong inhibitory proprieties on algae. Many mechanisms proposed to explain this effect, it may be acting on cell wall proteins leading to form complexes and for this reason we can elucidate highly inhibitory proprieties for tannins (19).An other mechanism was inhibit alkaline phosphate enzyme (AP) which play a great role in phosphate uptake on algae, where it help algae to consume PO^{-4} from organic phosphor compounds in surrounding environment (20). Subsequent research has shown the combination of tannins with ferrous ions in the environment, this combination was strong when complex phenols (ploy phenols) react with Fe^{++} than simple phenols (19).

The present study results agreed with (7), when they found that watery extract of licorice inhibited the growth of algae, on the other hand, Mohamed *et al.* (8) find that licorice extract had a stimulatory effect on algae. This disagreement may be due to the different of concentration used and the nature of studied algae, they were studied the effect of liquorice extract on whole community of algae (mixed community) while we use axenic culture of unicellular blue green algae. The results exhibit a clear agreement between growth inhibition rates (GI) and EC_{50} where GI increase of with the increment of licorice extract concentration. This increase was followed by lowering in calculated LC_{50} this may be lead to conclude that liquorice extract had a strong. inhibitory effect, this agreed with (21) that blue green algae resistance against toxic substances reduced with the increase of used concentration and exposure periods (22).Aqueous extract of black seed had been contain all tested chemical compounds, this result completely agreed with many investigators who records that black seed contain alkaloidic compounds "Nigellicine" (14,23).

The presence of tannins and saponins was recorded by (23), while cumarins and flavonoids were noted by (14). Thus the aqueous extract of black seed for the studied alga referred that it was

a stimulatory to the growth of alga. This stimulation was represented by the increment of TCN, GR and decreasing in G, this results may be due to the presence of growth promotor substances (such as vitamins or auxins) in this plant (14). Many investigators reported that vitamins (B_{12}) had a stimulatory effect when it added to the culture media of algae (24).

The chemical analysis of anice seed showed that plant posses tannins, glycosides, saponins, resines and flavonoids in its constituent. These result coincided with (18, 25).

This study reveals that anise seed extract had a different effects on the *M. aeruginosa* depending on concentration used at the treatment of studied alga with 3.5 and 7.0 mg / ml lead to remarked increasing in growth parameters, the peak was at 72 hours exposure time, on the contrary high concentration (14.0 mg/ml) produce slightly decrease in growth of tested alga, these different effects may be due to the presence of volatile oils(such as Aniseketone, Anethol, Methylchavicol, Aldehydic compounds) and vitamins (25), which it may be responsible for the increasing of growth parameters in low concentration. Similar results recorded by (26), when they treated the green alga *Ulothrix subtilissma* with low concentration (3 mg/ml) of Indol Acetic Acid (IAA) which lead to increase the growth about 22 times in a compared with control group, while higher concentration were inhibitory for the same alga in this studies of allelopathic interactions, Rice (4) found that certain chemical compound may stimulate target organisms at very low concentration, but inhibit of higher concentration.

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Table (3) preliminary detection for active medical compounds in studied plants

Medicinal Plants / Active Group	Glycyrrhiza glabra L.	Nigella sativa L.	Pimpinella anisum L.
Tannins	+	+	+
Glycoside	+	+	+
Saponins	+	+	+
Alkaloids	+	+	-
Resins	-	+	+
Flavoids	+	+	+
Cumarins	+	+	-
PH	6.5	5.5	6.4

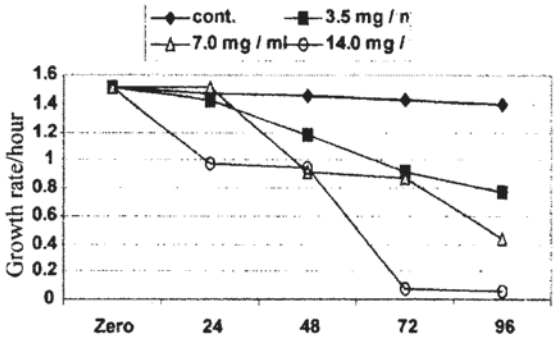

Fig (1) Effect of liquorice aqueous extract on total number of alga Microcystis aeruginosa (cell x 10^6 /ml).

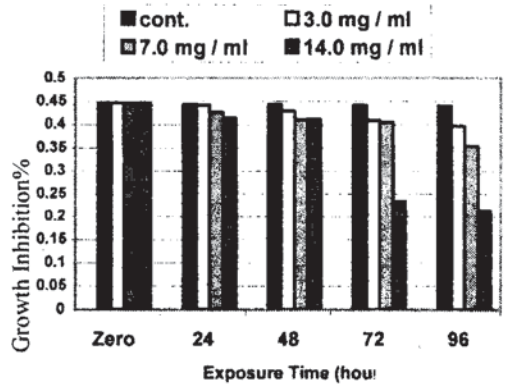

Fig: (2) The effect of licorice aqueous extract on growth rate (GR) of alga *Microcystis aeruginosa*.

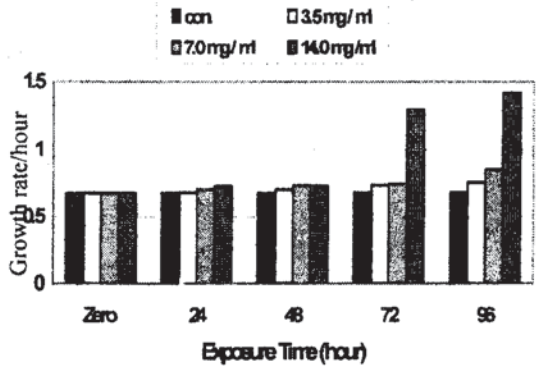

Fig (3) the effect of licorice aqueous extract on multiplication time (G) of alga *Microcystis aeruginosa*.

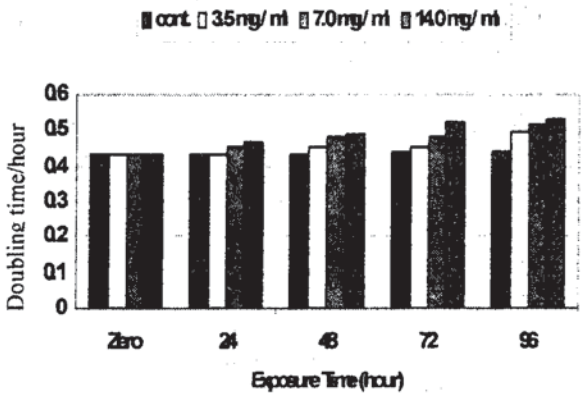

Fig (6) Effect of black seed aqueous extract on growth rate (GR) of alga *Microcystis aeruginosa*.

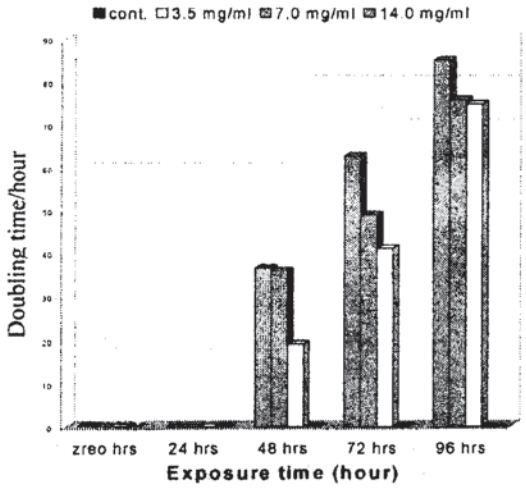

Fig (4) the effect of licorice aqueous extract on growth inhibition (GI) of alga *Microcystis aeruginosa*.

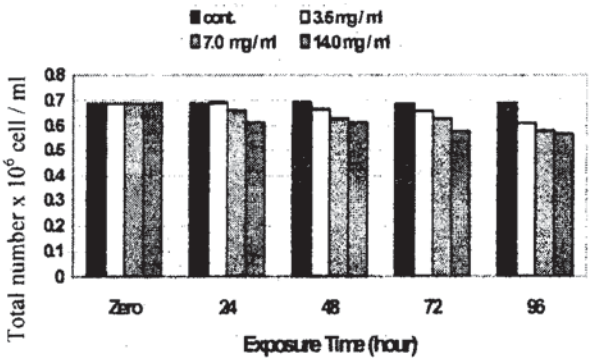

Fig (7) effect of black seed aqueous extract on multiplication time of alga *Microcystis aeruginosa*.

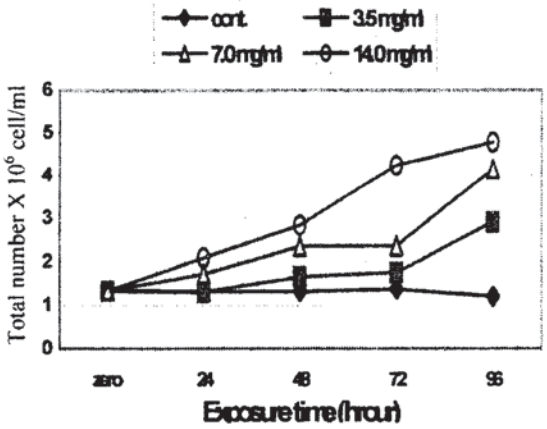

Fig (5) Effect of Black seed extracts on total number of alga *Microcystis aeruginosa*.

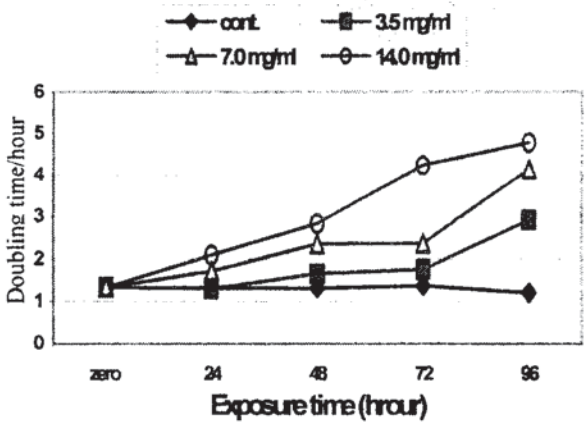

Fig (8) The effect of anise aqueous extract on the total number of alga *Microcystis aeruginosa*.

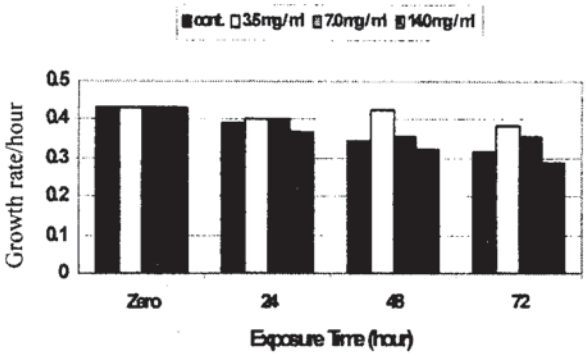

Fig (9) The effect of Anise aqueous extract on growth rate (GR) of alga *Microcystis aeruginosa*.

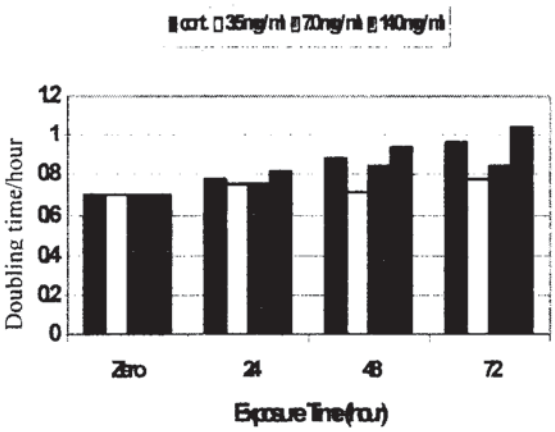

Fig (10) The effect of Anise aqueous extract on doubling time of alga *Microcystis aeruginosa*.

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