A Study on the Algicidal effects of Iraqi Indigenous plant extracts.

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Abstract

The Ethanolic extracts of different Iraqi indigenous plants which belong to different families showed algicidal activity against Anabaena, Nostoc and Myxosarcina species. Peganum harmala extract was the most potent in controlling the growth of tested Cyanobacterial species.

Introduction

Since the presence of Cyanobacterial, blooms cause problems in drinking water supplies(1); amenity waters(2); water used for industrial processes(3); in fishing waters where they cause deoxygenation and swimming pools(4). Researchs to find ways of controlling their growth have been encouraged. Mechanical control of Cyanobacteria in particular and algae in general is a laborious and costly operation. Biological control is considered as the most suitable way especially when compared with the chemical agents and their side effects and disadvantages. The use of plant-derived algicides in a country such as Iraq, with all the extremes of meterological, climatic and topographical features, and resting in varied and luxuriant flora of all types; is a feasible and suitable method of control(5). However, the aqueous extracts of the fruits of *Acacia nilotica* were evaluated and showed algicidal activity against several algal species(6) and the activity was due to tannins content in the plant.

From ancient times, *Peganum harmala* was claimed to be an important medical plant(7). It has been shown to have antibacterial ,antifungal and antiprotozoal activity(8).

In this study, an attempt to evaluate the algicidal properties of some Iraqi plants extracts was considered .

Materials and Methods

Euphorbia petiolata Banks et. Soland (Euphorbiaceae); Salsola *hierochuntica* Born. (Chenopodiaceae); *Salsola baryosma* (Sehult) Dandy (Chenopodiaceae); *Astragalus ancistrocarpus* Boiss (Leguminosae); *Melilotus officinalis* Desr. (Leguminosae); *Imperata cylindrica* (L.) P.Beauv. (Gramineae) and *Peganum harmala* L. (Zygophyllaceae) were collected from different environments of Iraq. These plants were identified and authenticated by the Iraqi National Herbarium (Baghdad, Abu-Ghraib). These plants were air dried at room temperature and grounded to a powder form.

Extraction

The powdered plant material (100g.) of each plant was extracted with 80% EtOH using Soxhlet apparatus until exhaustion. The cooled solution was evaporated to dryness under reduced pressure at 45^0C.

Testing of Cyanobacterial species and culture media

The Cyanobacterial species were collected from soils in Baghdad.

The isolates were purified and identified according to authentic keys of Geitler(9). These isolates where grown in Allens media(10) and stocked in the laboratory at 4^0C in an illuminated cooled incubator. An amount of 2% agar No.3 (Oxoid) was added to solidify the media if necessary. The Cyanobacterial species used in this study were *Anabaena cylindrica* Ghose and Randh; *Anabaena azollae* Strasburger; *Nostoc carneum* Ag.; *Nostoc muscorum* Ag.; *Nostoc rivulare* Kutz.; *Nostoc spongiaeforme* Ag. and *Myxosarcina spectabilis* Geitler.

Determination of algicidal activity

Allens medium with agar for each testing of Cyanobacterial species was made and allowed to cool down to approximately 45^0C with continuous swirling. Aliquots of known volume of homogenous Cyanobacterial suspension was then added and mixed rapidly before being poured into sterilized plates. These plates were kept at normal temperature for 2hrs. in order to allow the agar to solidify. Wells (9mm. In diameter) were made using the cork borer and vacuum to pull out the agar pellets. The extract was dissolved in 80% EtOH and aliquot of 0.2 ml of each plant extract (20 mg/ml) were added in triplicate, then incubated in an illuminated cooled incubator at 26 $\pm 1^0$C for 7 days. Ethanol (80%) was used as an experimental control; streptomycin sulphate (10%) was another standard in this study.

Results and Discussion

The percentage of EtOH extract yielded from *Peganum harmala* was the highest among the plants used in this study. However, the lowest percentage of the total extract was obtained from *Salsola baryosma* as indicated in table (1).

The anticyanobacterial potency of 80% EtOH plant extracts (20mg/ml) were observed after 3-7 days of incubation, and visible clear inhibition zones were noted around the holes. The inhibition zone diameters were increased dramatically after 3days and the highest zones of inhibition were determined after 7 days. Table (1) shows the anticyanobacterials activity of the different plant extracts used in this study. *Peganum harmala* was the most potent plant which exhibited the major and highest anticyanobacterial activity against all tested species.

Furthermore, *Salsola baryosma* and Melilotus *officinalis* showed activity against all tested species but they were less active than the *Peganum harmala* extract. However, the other plant extracts (*Imperata cylindrica, Astragalus ancistrocarpus, Salsola hierochuntica and Euphorbia petiolata)* were the less active and showed activity against some tested Cyanobacterial species.

The Cyanobacterial filaments in the zone of inhibition were first discoloured and later on they were no longer visible. The 80% EtOH which was used as a control has not given any significant antagonstic activity. However, streptomycin sulphate (10%) exhibited activity against all Cyanobacterial species which was similar to the results obtained from the *Peganum harmala* extract. This result indicates that some plant extracts in this study were very significant in comparison with streptomycin sulphate in the biological control of Cyanobacterial blooms.

Harmalin, harmalol and harmin were the major constituents present in *Peganum harmala* extract(11). However, saponin compounds were proved to be the major constituents in *Euphorbia petiolata, Salsola species* and *Imperata cylindrica*. Tannin compounds isolated from *Acacia nilotica* fruits showed algicidal activity(12). The potency of the plant extracts, however, could be due to the presence of these compounds in the extracts. Therefore, our future work will be the isolation of these major constituents and testing their efficiency in controlling algal blooms.

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Table (1) Anticyanobacterial properties of different plant extracts after 7 days of incubation at 26 ± 1^{0}C.

Plant species	Plant family	%Yield	Cyanobacterial species						
			A.a.	A.c.	N.m.	N.c.	N.r.	N.s.	M.s.
Euphorbia petiolata	Euphorbiaceae	14.1	+++	—	+	—	—	—	+
Salsola hierochuntica	Chenopodiaceae	10.6	++	—	—	+	+	—	+
Salsola baryosma	Chenopodiaceae	7.5	+	+++	+	+	+	+	++
Astragalus ancistrocarpus	Leguminosae	11.6	+++	—	—	+	+	—	+
Melilotus officinalis	Leguminosae	15.2	+	+	+	+	+	+	++
Imperata cylindrica	Gramineae	9.3	++	+++	+	—	+	+	+
Peganum harmala	Zygophyllaceae	27.9	+++	+++	+	++	+	+++	++
Ethanol 80%	----------	----	—	—	—	—	—	—	—
Streptomycin sulphate (10%)	----------	----	++	+++	+++	++	++	++	++

-=No inhibitory action, + =Inhibitory zone of inhibition between 12-16 mm., ++ = Inhibitory zone of inhibition between 17-22 mm.,+++ = Inhibitory zone of inhibition between 23-30 mm., A.a =Anabaena azollae

A.C=Anabaena cylindrica, N.m=Nostoc muscorum, N.c.=Nostoc carneum,N.r=Nostoc rivulare ,N.s. =Nostoc spongniaeforme,M.s=Myxosarcina spectabilis.

مجلة ابن الهيثم للعلوم الصرفة والتطبيقية المجلد 18 (3) 2005

دراسة تأثير مستخلصات نباتات عراقية كمبيدات للطحالب

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الخلاصة

أظهرت المستخلصات الكحولية لبعض النباتات العراقية والتي تعود إلى عوائل نباتية متنوعة فعالية مضادة لأنواع من الطحالب تعود لاجناس *Myxosarcina,Nostoc, Anabaena*. ومن بين المستخلصات النباتية المدروسة اظهر الحرمل(*Peganum harmala*) تأثير على كل أنواع الطحالب المستخدمة في دراستنا هذه من ناحية كما أظهر أعلى فعالية على تلك الطحالب المستخدمة في التجربة من ناحية أخرى.