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EVALUATION OF THE ABILITY OF SOME MEDICINAL PLANTS FOR INHIBITING THE GROWTH OF THE BLOOM-FORMING CYANOBACTERIUM *OSCILLATORIA BREVIS* (KÜTZ.)

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ABSTRACT

A short term study was conducted to evaluate the ability of the water extracts of two medicinal plants, *Hordeum vulgare* (L) straw and *Artemisia judaica*, to inhibit the growth of the bloom-forming cyanobacterium *Oscillatoria brevis* (Kütz.) which is known to produce off-flavor problems and toxins in water supply. *O. brevis* was collected from Ismailia freshwater canal during an algal bloom condition in February 2005 and was grown in BG-11 medium under laboratory conditions. Trichom's number was recorded using Sedgwick-rafter cell in a fixed period. The Trichom's number of the *O. brevis* was significantly decreased after the treatments with the two investigated plants either singly or in combination. The experiment results indicated the effectiveness of *H. vulgare* and *A. judaica* as an environment-friendly biomaterial for controlling the algal bloom of *O. brevis* in eutrophic water.

Keywords: Medicinal plants, *Oscillatoria brevis*, off-flavor, toxins, growth inhibition, freshwater canal

INTRODUCTION

Dense surface accumulation of phytoplankton (often blue green algae) is common in freshwater ponds and lakes (Lindenschmidt *et al*., 1998; Codd, 2004; Metcalf and Codd 2004). These blue green algal blooms are causing a variety of water quality problems including fish kills, aesthetic nuisance and production of toxins that release into the water following the senescence of the bloom (Carmichael, 1998; Codd *et al.*, 1999; Jacopy *et al*., 1999; Gomaa *et al*., 2000; Amin, 2001; Metcalf and Codd, 2004; Falconer, 2005).

Presently, the most common method to control cyanobacterial blooms is by dosing with copper based algicides. While this method destroys the blue-green algal cells, it is no longer considered an effective treatment method as it promotes blue-green tolerant species, destroys zooplankton, and releases toxins upon cell lysis (Codd *et al*., 1989; Kenefick *et al*., 1993; Jhingran, 1995). Traditional water treatment methods of chemical coagulation, flocculation, and sand filtration are often completely ineffective at removing cyanobacterial cells (Murphy *et al*., 1990; Edzwald, 1993).

Biological control of algal blooms is recommended as a safe method with low interpretations (Wium-Andersen, 1987). In a previous study, Schrader *et al*. (2000**)** identified several natural compounds that are selectively toxic towards *Oscillatoria perornata* (Skuja). One of these compounds is 9, 10-anthraquinone, which exists in plant tannin extracts (Robinson, 1967) and was found to induce a high degree of selective toxicity towards *Oscillatoria perornata*. Evidence has been accumulating since the late 1970's that barley straw can be used to control nuisance blooms of algae in freshwater systems (Welch *et al*., 1990; Barrett *et al*., 1994**;** Harriman *et al*. 1997; Caffrey and Monahan, 1999; Schrader *et al*., 2000).

The present study was conducted to evaluate the capabilities of some medicinal plants, *Hordeum vulgare* (L) straw and *Artemisia judaica* (L), to inhibit the growth of the bloom-forming cyanobacterium *Oscillatoria brevis* (Kütz.), which are known to produce off-flavor problems and toxins in water supply aiming to control the problems of algal blooms.

MATERIALS AND METHODS

Algal culture:

The cyanobacterium *O. brevis* was collected from Ismailia freshwater canal during algal bloom condition in February 2005. *O. brevis* was identified, isolated and purified using BG-11 medium which was solidified with 1% agar and incubated at 28°C (Allen, 1973 and Carmichael, 1985). Desired and unique trichoms were transferred and grown in liquid BG-11 medium as batch cultures under aseptic laboratory conditions with illumination intensity of about 5000 lux obtained from a set of fluorescent lambs. Dense algal growth was obtained after two weeks. The two weeks old cultures were mixed and

homogenized in automix (4000 r.p.m) for 5 minutes and diluted twice (Kobbia *et al*., 1991). The start inoculum of the cyanobacterium was adjusted to be 2000 trichoms l^{-1}. The trichoms's number was determined using Sedgwick-rafter counting cell, as recommended by (American Public Health Association, 1975).

Preparation of medicinal plants:

Leaves and flowers from *A. judaica* and the straw of *H. vulgare* were dried on oven at 60°C for 12 hours (Hornok, 1992) then grinded well. The grinded materials of both plants were extracted according to the method recommended by (Weichtl, 1994; Weiss, 1988) by soaking the plant material in sterilized distilled cold water and steeped for 12 hours. The extracts were then filtered through cellulose filter paper. Serial dilutions were performed to select the sufficient concentrations causing algal inhibition.

For the primary investigations, 5000, 10000, 15000, and 20000 ppm were tested to inhibit the cyanobacterium growth. The ability of the assumed concentrations to inhibit the growth was obtained by recording the number of trichoms daily for seven days. From the water extracts of a concentration of 5000 ppm, 1, 2, 3 and 4 ml were taken using the micro-pipette which was introduced into a set of Erlenmeyer flasks with a capacity of 250 ml containing BG-11 medium with adjusted volumes of 239, 238, 237 and 236 ml of the medium respectively. The final concentrations of the plant material in each flask were calculated to be 20.83, 41.66, 62.5 and 83.3 ppm respectively. Finally, the flasks were inoculated with 10 ml of the previous homogenized and adjusted inoculums of *O. brevis*. The trichom's number was determined using Sedgwick-rafter in 3 days interval and continued for 42 days (six weeks) (Brownleel *et al.*, 2003).

RESULTS

In a primary test continued for 7 days, daily recorded number of cyanobacterial trichom's indicated that the growth of the cyanobacterium was greatly reduced in the presence of the extracts of the two plants in all chosen concentrations. Figure (1a-b) shows the results of the primary investigations during which 5000, 10000, 15000 and 20000 ppm of *H. vulgare* and *A. judaica* were applied and tested to inhibit the growth of *O. brevis* in relation to its growth in zero

concentration. Numbers of trichoms were remarkably higher with no additions of the extracts. In case of the application of the barley straw extract, the growth rate of the cultures was gradually suppressed and fall into 59%, 27%, 27% and 22.2% when 5000, 10000, 15000 and 20000 ppm were applied respectively (Table 1). The results indicated that both the second and the third concentrations have similar suppressive effect. The effect of the four concentrations of *A. judaica* on the growth of *O. brevis* was more pronounced. The growth was dramatically reduced within few days from the start of application of *A. judaica*. The rate of growth reduction was 16%, 21%, 28% and 5.3% at the concentrations of 5000, 10000, 15000 and 20000 ppm of *A. judaica* respectively**.**

Table (1): The growth rate reduction of the cyanobacterium *O. brevis* after each treatment with the investigated plants.

Conc. in ppm	*H. vulgare*	*A. judaica*	*H. vulgare* : *A. judaica* (1:1)	*H. vulgare* : *A. judaica* (3:1)
5000	59%	16%	not detected	not detected
10000	27%	21%	not detected	not detected
15000	27%	28%	not detected	not detected
20000	22.2%	5.3%	not detected	not detected
20.83	32.6%	20.5%	15.2%	10.60%
41.66	12%	12%	12%	7.4%
62.5	9%	10%	8%	8.6%
86.3	4.1%	8%	8%	7.2%

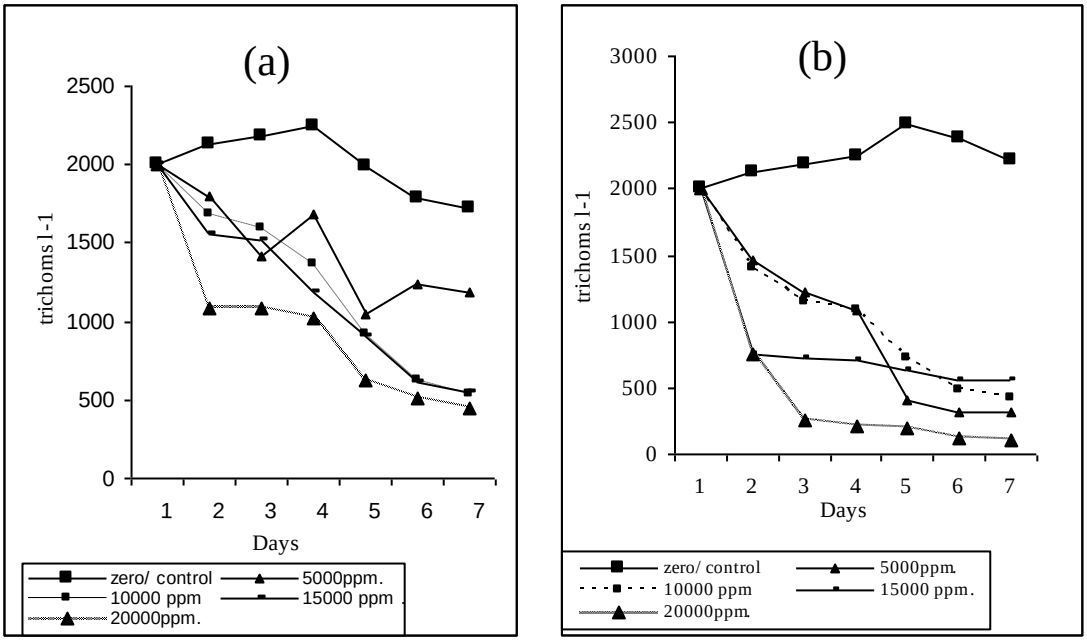

Figure (1a-b): Primary investigations for the effect of different concentrations of *H. vulgare* **(a)**, *A. judaica* **(b)**, on *O. brevis* in relation to control (Zero) during a period of one week

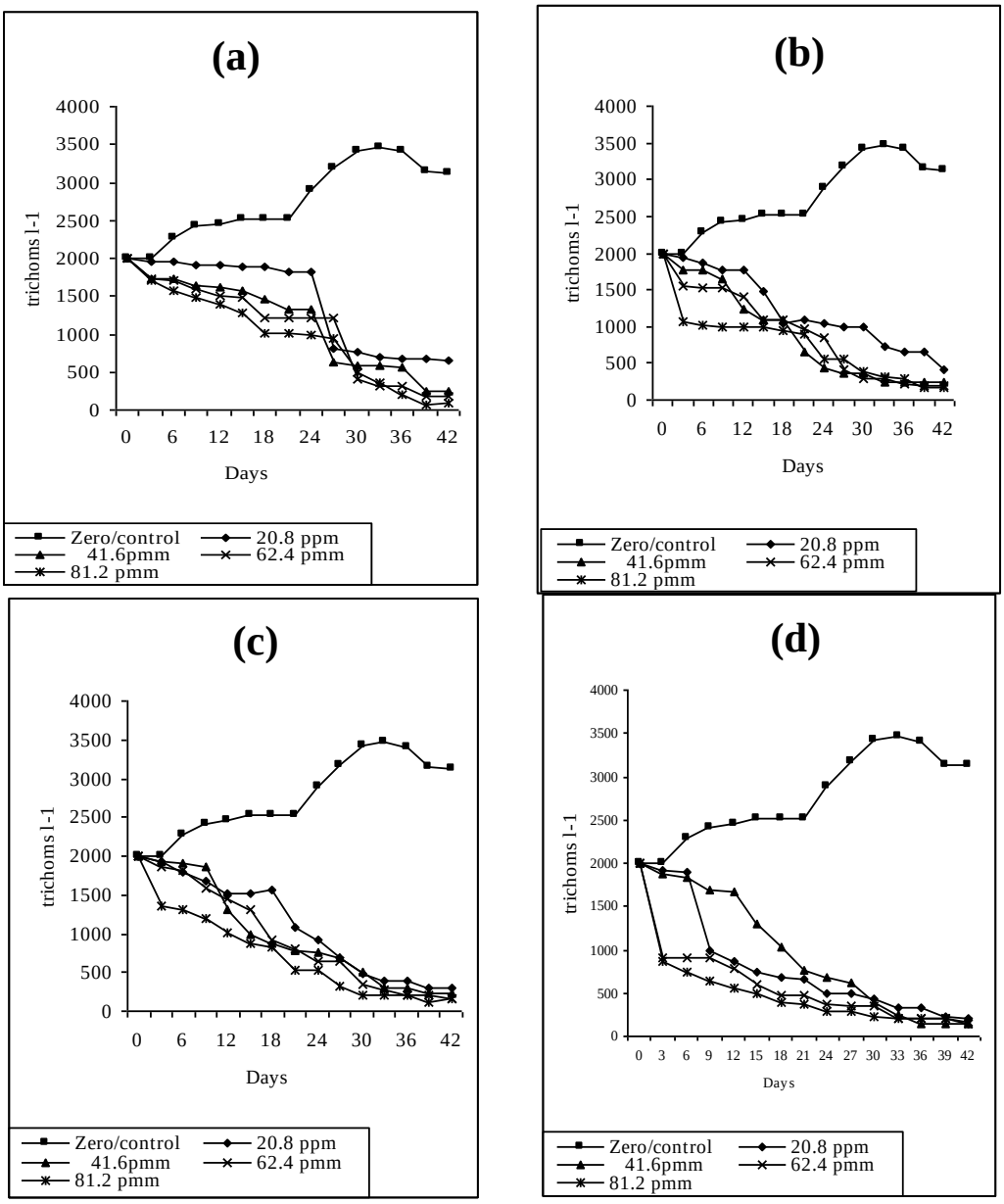

Figure (2a-d): Results of applying different concentrations of extract of *H. vulgare* **(a)**, *A. judaica* **(b)**, 1 *H. vulgare* : 1 *A. judaica* **(c)** *and* 1 *A. judaica* : 3 *H. vulgare* **(d)** on *O. brevis* in relation to control (Zero) during an experiment continued for 6 weeks.

The second experiment was carried out in the same pattern of the previous one. This experiment was conducted for about six weeks, during which the extracts were applied as 20.8, 41.6, 62.4 and 81.2 ppm into the cultures in order to test the inhibitory effect of the lower doses of both plants. Generally, the yields of the cultures were greatly suppressed after additions of the extracts (Table 1). The results of the secondary concentrations were illustrated in figure (2, a-d). In case of the application of barley straw extract the yield suppression ratio (YSR) were calculated to be 32.6 %, 12 %, 9%, and 4.1% when 20.8, 41.6, 62.4 and 81.2 ppm were applied to the cultures respectively. When the same concentrations were applied from *A. judaica* the suppression rate was found to be 20.5%, 12%, 10 % and 8%. Also, the combination between the two plants had a clear impact in the growth of the cyanobacterium. Assessing the effect of the combinations of both plants indicated that there were pronounced decreases in the number of trichoms throughout the experiment. The recorded suppression ratios were 15.2 %, 12%, 8 % and 8 % when *H. vulgare* and *A. judaica* were combined together in 1:1 ratio and added in the same previous concentrations. The trichom's numbers were more affected due to the combinations of *H. vulgare* and *A. judaica* in a ratio of 3:1 that caused a higher reduction ratio equal to 10.6 %, 7.4 %, 8.6 % and 7.2%. This may suppose that *A. judaica* acting synergistically with *H. vulgare* against the investigated cyanobacterium.

DISCUSSION

The present study provides further evidence to support the use of *H. vulgare* in controlling undesirable algal bloom in freshwater. The growth of *O. brevis* (Kütz.) was inhibited when exposed to 20.8 ppm of *H. vulgare* and *A. judaica* extracts, which was found to be the least concentration causing cyanobacterial inhibition during the experiment. Above ground parts of *A. judaica* (L) (leaves and flowers) were emphasized to have medicinal properties for treating liver problems, joint pain, digestive discomfort, loss of appetite, insomnia, epilepsy and menstrual problems. These parts often used as an insect repellent in the form of a spray to deter pests in organic gardening. (Juteau *et al.*, 2003).

The greatest suppression ratio was obtained from the application of the *H. vulgare* in a concentration of 81.2 ppm throughout the six

weeks. Susceptible populations of field *Aphanizomenon flos-aquae* (a cyanobacterium) were found in the Press Top reservoir in 1994 following treatment with 50 ppm *H. vulgare*. The straw eliminated the cyanobacterium even though it had occurred as a summer dominant during the previous three years, when no straw treatment had been used (Everall and Lees, 1997). Martin and Ridge (1999) also noted susceptibility of *Oscillatoria redekei*, *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* and *Synechococcus* to *H. vulgare* but in levels higher than those used in the present study.

Chlorophycean species were proved to need higher concentration of the straw than that required for cyanobacteria (Everall and Lees, 1997). Brownleel *et al.* (2003) observed an obvious reduction in biomass when *Ankistrodesmus falcatus*, *Chlorella capsulata*, and *Isochrysis* sp. exposed to aged *H. vulgare* slurry at levels of 312.5 – 1250 ppm.

Evidences have been accumulated since the late 1970's that *H. vulgare* can be used to control nuisance blooms of algae in freshwater systems. The most extensive use of this treatment has been in the British Isles, where it has been used in lakes of varying sizes, potable water reservoirs, canals and streams (Welch *et al*., 1990; Harriman *et al*., 1997; Barrett *et al*., 1999; Caffrey and Monahan, 1999). Many of published accounts report consistent success of *H. vulgare* in preventing nuisance algal blooms. No problems of taste or odor were reported in drinking water supplies treated with *H. vulgare* (Everall and Lees 1997; Barrett *et al*., 1999). During the present study the lower concentrations of *H. vulgare* was found to be more effective to inhibit the growth of *Oscillatoria* than in case of the application of some higher concentrations of *H. vulgare*. More evidence and measurements such as proteins, carbohydrates etc. are recommended to emphasize the kinetics of the inhibitory effects of both *H. vulgare* and *A. judaica.* These findings suggest the potentiality of these herbs as an environment-friendly biomaterial for controlling the algal bloom in eutrophic water.

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تقييم لقدرة بعض النباتات الطبية على تثبيط نمو السيانوبكتر اسلاتوريا بريفيز المسبب لظاهرة الازدهارات الطحلبية

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تم دراسة وتقييم قدرة بعض النباتات الطبية على تثبيط نمو طحلب السيانوباكتر الأوسلاتوريا بريفيز وكان اختيار هذا الطحلب لما يسببه من مشاكل بيئية وصحية وبيولوجية عديدة مثل انبعاث الروائح الغير مقبولة وتغيير طعم مياه الشرب بالإضافة إلى إفراز السموم في المسطحات المائية. و قد تضمن البحث اختبار المستخلصات المائية لاثنين من النباتات الطبية هي قش الشعير والشيح، حيث بدأت الدراسة بعزل الاسلاتوريا بريفيز من مياه ترعة الإسماعيلية في أثناء حدوث هذه الظاهرة في فبراير 2005 وذلك بتعريفة ثم عزله واستزراعه في وسط مغذى تحت ظروف معملية مناسبة. كما تم تجهيز سلسلة من التركيزات التدريجية للمستخلصات المائية عن طريق النقع لاختيار التركيز المناسب لتثبيط نمو السيانوبكتر. وأثبتت الدراسة عن طريق عد الخيوط بصورة دورية قدرة كل من قش الشعير والشيح على الحد من نمو السيانوباكتر. وتعتبر هذه الدراسة تعزيز لإثبات قدرة قش الشعير في القضاء على بعض أنواع الطحالب الغير مرغوب فيها بالإضافة إلى اكتشاف قدره نبات الشيح على كبح نمو الاسلاتوريا بريفيز في المياه الغنية بالمواد المغذية.