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## ENVIRONMENT-FRIENDLY BIOMATERIAL (RICE STRAW EXTRACT) FOR CONTROLLING THE CYANOBACTERIUM *MICROCYSTIS* *AERUGINOSA* IN NATURAL WATER IN ABBASSA AND RYAN WATER IN EGYPT

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### Abstract

Rice straws which are otherwise an agricultural waste product and the burning of straw causing the black cloud in Delta Egypt may be an environmentally friendly and sustainable source of a bioalgicide. The algicidal effects of the rice straw extract on the growth of *Microcystis aeruginosa* were investigated using cultured two isolate RYAN isolate and CLAR isolate in specific media and natural water (CLAR pond water, RYAN pond water and RYAN lake water ) the experiment carried out at plankton room temperature 26±2 with 14 h:10 h light dark photo period . The growth of *Microcystis aeruginosa* was inhibited by rice straw extract concentrations at 0.1, 0.5, 2.5, and 12.5 mg l<sup>-1</sup> in specific media and inhibited at concentration 0.1, and 0.5 in natural water. All four treatment concentrations of the rice straw extract, the number of *M. aeruginosa* cells was significantly reduced during the 10-day test period (P < 0.05). The growth inhibition of *M. aeruginosa* with 12.5 mg l<sup>-1</sup> of the rice straw extract was higher than that with the other extract concentrations in two isolate. No significant difference was found between the other treatment concentrations. Three natural water from two field ponds and the Rayan Lake were used to test the algal growth inhibition over a period of 10 days. The reduction growth of *Microcystis aeruginosa* was increase with time of experiment increase, it was more than 70% from control. The results suggest that the rice straw extract may serve as environmentally friendly agents for controlling the growth of toxic *Microcystis aeruginosa* in natural water.

**Keywords:** *Microcystis aeruginosa* ; Rice Straw extract ; natural water ; algicides .

### INTRODUCTION

In recent years, there has been an apparent increase in the occurrence of harmful algal blooms occurring in potable waters. *Microcystis aeruginosa* is the most common species of bloom causing cyanobacteria. Many strains of *Microcystis* are known to produce cyanobacterial hepatotoxins termed microcystins (Oh *et al.*, 2000; Yasuno *et al.*, 2000). These toxins, which are soluble peptides, damage the livers of higher animals (Codd and Poon, 1988; Watanabe *et al.*, 1989) and are lethal or harmful to many kinds of aquatic organisms (Penalosa *et al.*, 1990). Therefore, control of microcystin-producing *Microcystis* is an important environmental and public health issue. Various chemical or synthetic agents (e.g., copper, chlorine, aluminum, calcium, and potassium

permanganate) are used to control nuisance phytoplankton and weeds in aquatic ecosystems. However, these algicides often induce secondary pollution such as the release of phytotoxins, which threaten drinking water supplies, persist in the environment, and are toxic to fish (Lam *et al.*, 1995; Karan *et al.*, 1998; Boyd and Massaut, 1999; Meepegala *et al.*, 2005). In recent efforts to control toxic bloom-forming *Microcystis*, algicides from natural biomaterials have received attention as alternatives to chemical agents. Such algicides are likely to be specific and biodegradable, and may therefore offer an environmentally friendly method for control of algal blooms (Park *et al.*, 2006a,b). Furthermore, the growth of *M. aeruginosa* is inhibited by L- 2-azetidinecarboxylic acid from *Polygonatum odoratum* var. *pluriflorum* (Kim *et al.*, 2006), salicylic acid from rice straw (Park *et al.*, 2006a), tannic acid from oak extracts (Park *et al.*, 2006b), and several compounds isolated from rice hulls (Chung *et al.*, 2007). However, these studies used unicellular *M. aeruginosa* strains. We think that it is important to test the effects of natural algicides on problematic colonial *M. aeruginosa*. Extracts from rice hulls have recently been found to have herbicidal and algicidal effects (Ahn, & Chung 2000; Chung *et al.*, 2007). Rice hull is the major by-product of Thus, rice hulls, which are otherwise an agricultural waste product, may be an environmentally friendly and sustainable source of a bioalgicide. The allelopathic activity of plants, such as rice straw, has already been documented, and phenolic compounds invariably identified as the allelopathic or phytotoxic substances (Rice 1984; Inderjit *et al.* 1995; Chung *et al.* 2001). Extensive research has also been conducted on the effect of allelopathic plants on microalgae, as regards inhibiting algal bloom in the field and laboratory (Gibson *et al.* 1990; Welch *et al.* 1990; Barrett *et al.* 1996; Ridge and Pillinger 1996; Nakai *et al.* 2000; Ball *et al.* 2001). For example, a barley straw extract was found to effectively inhibit the growth of several planktonic and filamentous algae in the laboratory and reservoirs (Gibson *et al.* 1990; Barrett *et al.* 1996). The allelochemicals released from barley and rice straw limit the germination, growth, photosynthesis, respiration and metabolism of other plants, and consist of phenolic compounds, such as p-hydroxybenzoic, p-coumaric, ferulic, vanillic, salicylic, syringic and benzoic acid (Rice 1984; Inderjit *et al.* 1995; Chung *et al.* 2001). Thus, the phenolic compounds in rice straw may inhibit the growth of the cyanobacterium *Microcystis aeruginosa*. Many kinds of chemical agent or synthetic compound, including copper, chlorine, aluminium, calcium and potassium permanganate, are currently used to control phytoplankton and aquatic weeds in lakes, reservoirs and ponds. However, chemical algicides such as copper sulfate exhibited toxic effects on fish (Karan *et al.* 1998). They also can induce secondary pollution, by releasing phytotoxins that increase potential health risks in drinking water supplies (Lam *et al.*



1995). Therefore, in the context of controlling algal blooms, the use of rice straw may be an alternative way to minimize economic costs and use sustainable material from agricultural waste (Chung *et al.* 2001).

## MATERIALS AND METHODS

### Algal strain and culture conditions

The cyanobacterium *M. aeruginosa* ((RAYAN isolate) and (CLAR isolate) were isolated in specific media (Allen medium) from Ryan lake water and the CLAR water pond at The Central Lab For Aquaculture Research in Abbassa. The algal isolate was grown in an Allen medium (Allen 1968), maintained at 28°C on a 14 h: 10 h LD cycle in a shaking incubator, and illuminated at 100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  by cool-white fluorescent lamps for 2 weeks. The composition of the Allen medium was (in mg) 1500 NaNO<sub>3</sub>, 39 K<sub>2</sub>HPO<sub>4</sub>, 75 MgSO<sub>4</sub>·7H<sub>2</sub>O, 21 Na<sub>2</sub>CO<sub>3</sub>, 27 CaCl<sub>2</sub>, 58 Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O, 1 EDTA, 6 citric acid, 6 ferric citrate, 2, 86 H<sub>3</sub>BO<sub>3</sub>, 1.81 MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.22 ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.39 Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.08 CuSO<sub>4</sub>·5H<sub>2</sub>O, and 0.05 Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O in 1000 ml of distilled water. The pH of the medium was adjusted to 7.8. For the seed culture, 10 ml of the culture was transferred to a 250-ml culture flask with a fresh 90 ml of the Allen medium.

### Extraction of rice straw

The rice straw were obtained from private farm in Wady Almolak Abou Hamad Sharkia, cut into uniform lengths (2 cm), and crushed. The extract was then obtained using the modified method of Suzuki *et al.* (1998). The straw strips (100 g) were mixed with methanol (1500 ml) and sonicated for 10 min at 50°C. The solution was then filtered through glass fiber paper (Whatman GF/C) and evaporated. The resulting extract was concentrated to 1% (dry weight basis) and stored in a freezer (-10°C) until required.

### Growth inhibition by Rice Straw extract

All the *M. aeruginosa* growth inhibition experiments were carried out at room temperature (26 ± 2 °C) with a 14 h: 10 h LD photo period. To examine the effect of different concentrations of the rice straw extract on the cyanobacterium, *M. aeruginosa* was cultivated in specific media, Allen media treated with 0.1, 0.5, 2.5 and 12.5 mg l<sup>-1</sup> of the rice straw extract compared with control (without rice straw). The number of *M. aeruginosa* cells was enumerated at 2-day intervals using Quantitative estimation of *M. aeruginosa* cells, 1 ml was drawn and placed into Sedgwick-Rafter cell, and then it was microscopically examined for counting the colony and count the average number of cells in the colony, APHA (19 85). The results were then expressed as cell counts ml<sup>-1</sup>, to determine any changes in the cell density over 10 days. Meanwhile, to understand the effect of

rice straw extract inhibition on the cyanobacterium, *M. aeruginosa* (CLAR and RYAN isolate) were cultured in three kinds of natural water from two ponds: one from CLAR pond and other from Ryan pond naturally infected by *Microcystis aeruginosa* and the third from the Ryan lake water) and its growth compared when using 0.1 and 0.5 mg l<sup>-1</sup>, four replicates for each treatment compared with the control from three kinds of naturally

#### Sterilization of fish pond water

Sterilized fish natural water, naturally infected with *Microcystis* by adding 3 ml/l Chlorox (common household bleach, 5.25% chlorine) to the water for 24 hour can be neutralized by adding sodium thiosulfate 1% concentration (use 1.1 ml/10L.) Connell, G.F. (1996). Analyses of pH, N, P, alkalinity, hardness, and Ammonia were carried at the start and end of experiments.

LC<sub>50</sub> for tested the toxicity of *Microcystis* by serial dilution from *Microcystis* 10<sup>1</sup>, 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup>, 10<sup>6</sup>, 10<sup>8</sup>, 10<sup>10</sup> cells/ml ten fish *Oreochromis niloticus* average size 2.51 ± 0.26 in aquarium contain 50 liters sterilized water fish pond for 96 hours.

#### Experimental design

Twenty Flasks were filled with 0.25 L of sterilized Allen media, and four Flasks were assigned for each treatment. Rice straw extract was added from the stock solution at four concentrations 0.1, 0.5, 2.5 and 12.5 mg/l supplemented by *Microcystis aeruginosa* (10<sup>4</sup> cells/ml).

The second experiment

Twelve carboys were filled with 10 l. from natural water from CLAR pond water and four carboy were assigned for each treatment (0.1, 0.5 mg/l-1 rice straw extract) and four as control (without rice straw extract) all carboys supplemented by *Microcystis aeruginosa* (10<sup>4</sup> cells/ml) and repeated this experiment with the two natural water (RYAN pond water and RYAN Lake water)

#### Data analysis

The reduction growth was calculated using the modified equation

$$\text{Reduction growth \%} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

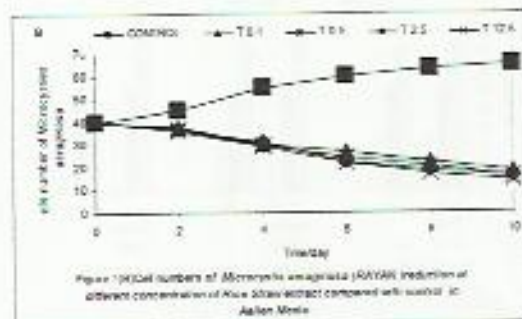
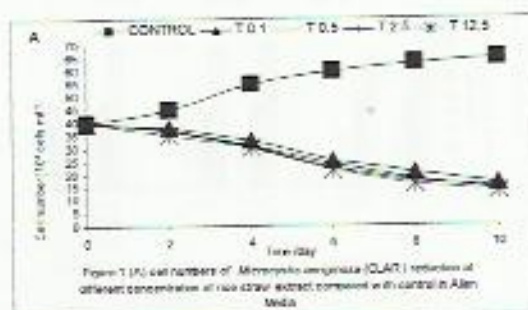
The difference in the cell densities between the treated and control culture was analyzed using an analysis of variance (ANOVA) and the data compared using linear contrasts. A P-value of < 0.05 was considered significant. All statistics were run on the computer, using the SAS program (SAS, 2003)



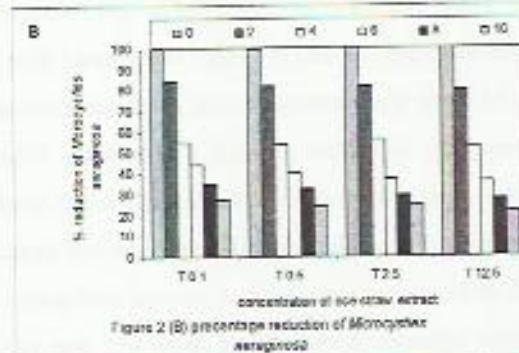
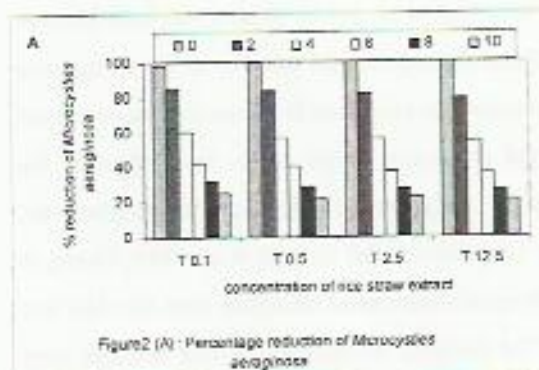
## RESULTS

Growth inhibition of *M. aeruginosa* by rice straw extract

The inhibitory effect of the rice straw extract was quite distinct compared with the control Fig. 1 (A&B).

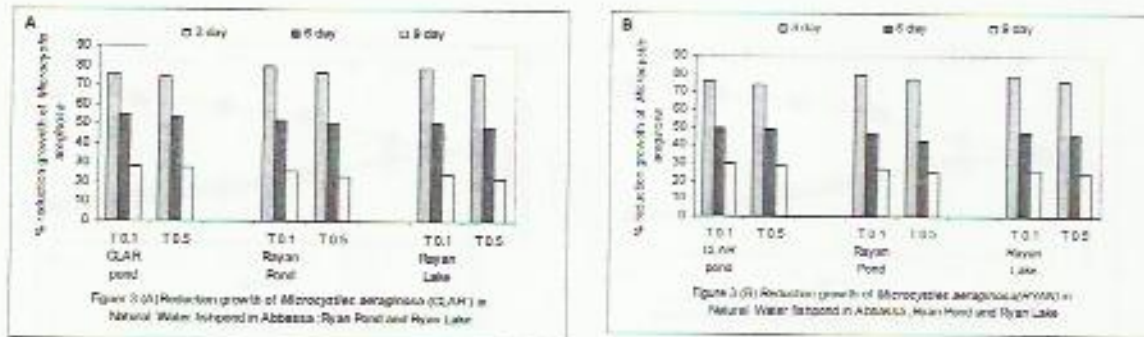


For all four treatment concentrations of the rice straw extract, the number of *M. aeruginosa* cells was significantly reduced during the 10-day test period ( $P < 0.05$ ). The growth inhibition of *M. aeruginosa* with 12.5 mg/l of the rice straw extract was higher than that with the other extract concentrations in two isolates (RYAN and CLAR). No significant difference was found between the other treatment concentrations. Three natural water from two field ponds and the Rayan Lake were used to test the algal growth inhibition over a period of 10 days. In the medium from CLAR pond ( $\text{NO}_3^- \text{-N}$ ,  $0.16 \pm 0.02$  mg/l; P,  $1.2 \pm 0.3$  mg/l, pH,  $8.2 \pm 0.3$ , hardness  $226 \pm 14$  mg/l, Alkalinity  $220 \pm 12$  mg/l cells with 0.5 mg/l of extracts were similar to those with 0.1 mg/l of the regular extract until day 7, at which point, reduced cell numbers were noted until day 10 Fig. 2 (A&B).



No difference was found between the groups treated with the two concentration of rice extracts., while a relatively higher reduction was observed in Ryan pond ( $\text{NO}_3^- \text{-N}$ ,  $0.12 \pm 0.03$  mg/l ; P,  $0.11 \pm 0.03$  mg/l pH,  $8.5 \pm 0.3$ , hardness  $320 \pm 30$  mg/l, Alkalinity  $200 \pm 12$  mg/, and Ryan lake water ( $\text{NO}_3^-$

-N,  $0.01 \pm 0.002$  mg/l ; P,  $0.03 \pm 0.01$  mg/l, pH 8.5, hardness 340mg/l, Alkalinity 220 mg/l media.  
 Fig. 3 (A&B).



The growth inhibition effect of the rice straw extract was much higher on day 9 than on day 3 and 6. Therefore, it seemed that the combination of N and P concentrations in water and the period of treatment had an effect on the allelopathic activity of the rice straw extract on *M. aeruginosa*.

## DISCUSSION

It was clearly demonstrated that the rice straw extract effectively inhibited the growth of *M. aeruginosa* in specific media and natural water. The number of *M. aeruginosa* cells significantly decreased with all treatments relative to the control. The results obtained were similar to those previously found when using barley straw for various test microalgae (Gibson *et al.* 1990; Welch *et al.* 1990; Barrett *et al.* 1996; Ridge and Pillinger 1996; Ball *et al.* 2001). In addition, both the two concentration 0.5 and 0.1 mg/l rice extract also effectively reduced the number of *M. aeruginosa* cells in the three natural media, suggesting that allelochemicals released from the rice straw extract were able to diffuse through a membrane filter and minimally destroyed by heat. Most of the allelochemicals in rice straw are well known as phenolic compounds, i.e. ferulic, p-hydroxybenzoic, p-coumaric, vanillic, salicylic, syringic and benzoic acid (Rice 1984; Inderjit *et al.* 1995; Chung *et al.* 2001). In previous studies, several allelopathic chemicals have been identified from rice leaf and straw extracts, decomposing straw and rice soil. The phenolic compounds in rice are also very similar to those in barley (Inderjit *et al.* 1995). The release of phenolic compounds, such as ferulic acid and p-hydroxybenzoic acid from the decomposition of barley straw cell walls and low molecular weight aromatic compounds from the incomplete decomposition of lignin, has already been found to play a crucial role in the inhibition of algae (Inderjit *et al.* 1995; Ridge and Pillinger



1996). However, in the present study, the growth responses of *M. aeruginosa* to the allelochemicals included both inhibition and stimulation. Growth was significantly inhibited with the addition of salicylic acid, while the other six allelochemicals were able to inhibit or weakly stimulate the algal growth depending on their concentration. Tillberg (1970) found that salicylic acid at concentrations of  $10^{-6}$ – $10^{-3}$  mol  $\Gamma^{-1}$  (0.138–138 mg  $\Gamma^{-1}$ ) decreased the phosphorus uptake of the alga *Scenedesmus*. Meanwhile, Scharff and Perry (1976) reported that under anaerobic conditions, at a low pH and 30°C, yeast lost  $K^+$  ions in the presence of salicylic acid and glucose utilization was inhibited. Thus, they concluded that a fundamental action of this compound in many organisms was to reduce the  $K^+$  content in the cells. The growth of *M. aeruginosa* was decreased significantly by 70% and 80% relative to the control with 0.1 and 0.5 mg  $\Gamma^{-1}$  of the rice straw extract, respectively ( $P < 0.05$ ). However, in the previous studies each individual allelochemical only exhibited a low growth inhibition (0–26%) compared with the rice straw extract when added at concentrations of 0.01 and 0.1 mg  $\Gamma^{-1}$ . Dedonder and Van Sumere (1971) previously demonstrated that almost 40 phenolics effectively inhibited the growth of the green alga *Chlorella vulgaris*, due to the enhanced respiration of phenolics, including the uptake of oxygen. Meanwhile, Rice *et al.* (1980) demonstrated that five phenolic inhibitors (ferulic, p-hydroxybenzoic, p-coumaric, o-hydroxyphenylacetic and vanillic acid) from the decomposition of rice straw inhibited the growth and nitrogen fixation of *Anabaena cylindrica*. In particular, they reported that a combination of all five compounds was more effective in inhibiting the growth and nitrogen fixation, indicating a synergistic effect. This is especially important, as the five compounds always occur together in rice residue decomposition (Rice 1984). In addition, many researchers showed that a combination of three phenolics (p-coumaric, salicylic and benzoic acid) at a final concentration of 0.01 mg  $\Gamma^{-1}$  produced a higher growth inhibition (66%) than each individual phenolic at the same concentration, indicating that the presence of many allelochemicals at low concentrations in rice straw probably promotes a high synergistic, agree with our results where the reduction growth were more than 75% from the control.

**In conclusion,** *M. aeruginosa* was effectively inhibited with a low concentration (0.1 mg  $\Gamma^{-1}$ ) of a rice straw extract. Moreover, the inhibitory activity of the rice straw extract on *M. aeruginosa* was likely due to the synergistic effect of various phenolic compounds in previous studies. Consequently, these results indicating that a rice straw extract can inhibit the growth of the microcystin-producing cyanobacterium *M. aeruginosa* have an additional significance as a factor that could reduce the health threat posed by cyanobacterial blooms. Thus, future studies will test the anti-algal effect of the rice straw extract in the field and other organisms, like zooplankton and fish.



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## مستخلص قش الأرز كمادة عضوية صديقة للبيئة يثبط نمو طحلب الميكروسيستس أريجينوزا في المياه الطبيعية بالعباسة والريان بمصر

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<sup>١</sup> - قسم الليبولوجى المعمل المركزي لبحوث الثروة السمكية مركز البحوث الزراعية

<sup>٢</sup> - المركز الدولى لبحوث الأسماك مركز أقليمي لأفريقيا وغرب آسيا العباسة أبو حماد شرقية جمهورية مصر العربية

أستهدفت هذه الدراسة معرفة التأثير المثبط لمستخلص قش الأرز كمادة عضوية صديقة للبيئة على نمو طحلب الميكروسيستس أريجينوزا في المياه الطبيعية بالعباسة والريان بمصر كمبيد طحلبى طبيعى . فقد درست فعالية هذا المستخلص على نمو طحلب الميكروسيستس أريجينوزا المعزول من منطقة الريان (RYAN) والعباسة (CLAR) في وسط بيئى متخصص وكذلك المياه الطبيعية ومن الدراسة أتضح أن المستخلص كان له تأثير محد لنمو الطحلب في الأربع معاملات عند تركيز او ، ٥٠ ، ١٠٠ ، ٢٠٠ ، ٤٠٠ ، ٨٠٠ ملليجرام/لتر في الوسط البيئى المتخصص وكان مثبط لنمو الطحلب في المياه الطبيعية ( مياه أحواض العباسة وأحواض الريان وبحيرة الريان) عند تركيز او ، ٥٠ ، ١٠٠ ، ٢٠٠ ، ٤٠٠ ، ٨٠٠ ملليجرام/لتر على كلا من العزلتين فوجد تناقص ملحوظ في أعداد الطحلب يزداد بمرور زمن التجربة ولم يوجد أختلافا معنويا بين المعاملات فقد سجل هذا التناقص في الأعداد أكثر من ٧٠% في المعاملات اذا قورن بالمجموعة الضابطة من هذه الدراسة يمكن الاستنتاج انه يمكن استخدام مستخلص قش الأرز كمادة عضوية صديقة للبيئة كمثبط لنمو طحلب الميكروسيستس أريجينوزا في المياه الطبيعية بالعباسة والريان بمصر عند تركيز او ، ٥٠ ، ١٠٠ ملليجرام/لتر .