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Selective Bactericidal Potential of Rice (*Oryza sativa* L. var. *japonica*) Hull Extract on *Microcystis* Strains in Comparison with Green Algae and Zooplankton

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Abstract We examined the selective inhibitory potential of rice hull extract on the toxic cyanobacterium *Microcystis aeruginosa*, in comparison with inhibitory effects on two green algae (*Ankistrodesmus convolutus* and *Scenedesmus quadricauda*) and a zooplankton (*Daphnia magna*) species. The inhibitory effect of rice hull extract, measured by algal growth or zooplankton survival using four different concentrations of extract (1, 10, 100 and 1000 μg L^{-1}), was highest on *Microcystis* strains (average: 98%, range: 95%–99%), followed by *D. magna* (average: 22%, range: 10%–47%), *A. convolutus* (average: 20%, range: 16%–24%), and *S. quadricauda* (average: 8%, range: 0%–15%). Rice hull extract had only a small effect on the growth of the green algae and *Daphnia*, particularly in the range 1–100 μg L^{-1}, and the inhibitory effect was somewhat diminished even at the 1,000 μg L^{-1} level, at the end of the experimental period, especially for *Daphnia*. Our study indicates that rice hull extract has a strong specific algicide potential when used to combat *M. aeruginosa*.

Keywords Rice hull extract · Selective control · *Microcystis aeruginosa* · Green alga · Zooplankton

The cyanobacterium *Microcystis aeruginosa* is the most common species of microalgae causing troublesome blooms in eutrophic water bodies worldwide (e.g., Oliver and Ganf 2000). Many strains of *Microcystis* are known to produce cyanobacterial hepatotoxins termed microcystins (Carmichael 2001). Thus, the control of microcystin-producing *Microcystis* is a very important issue with regard to improving water and ecosystem quality, and public health.

To date, chemical algicides (e.g., copper, chlorine, aluminum, and other chemicals) have been widely used in the control of harmful algal blooms including toxic *Microcystis* (Cooke et al. 2005). However, most chemical algicides are nonselective, and thus their use is limited because of general toxicity (Park et al. 2006a). Allelochemicals extracted from various plant species have shown specific effects and their use has been suggested to overcome the non-selectivity problem (Nakai et al. 2000; Ferrier et al. 2005; Kim et al. 2006). An increasing number of studies have shown that allelochemicals offer the advantages of biodegradability, economy, and environmental friendliness. Barley straw has been studied for algal control over many years since the 1980s (e.g., Welch et al. 1990; Ferrier et al. 2005).

Recently, allelochemicals from rice straw and hull have been applied to control *M. aeruginosa* and water weeds (Chung et al. 2002, 2007; Park et al. 2006a, 2009). Chung et al. (2002) identified nine phenolic compounds, including salicylic acid, from hull extracts of three rice cultivars. These compounds showed allelopathic activity on the weed barnyard grass (*Echinochloa crus-galli*). Amongst the nine phenolic compounds, salicylic acid most effectively inhibited the growth of *M. aeruginosa* (Park et al. 2006a). Moreover, allelochemicals from rice hull extracts, β-sitosterol-β-D-glucoside and dicyclohexanyl orizane, powerfully inhibited the growth of colonial *M. aeruginosa* (Park et al. 2009). However, the strong algicidal effect did not necessarily guarantee specific effects on all tested

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target species. A selective effect is important for useful applicability under in situ conditions.

This study aimed to determine whether rice hull extract exerts a selective inhibitory effect on strains of the toxic cyanobacterium *M. aeruginosa*, compared to effects on other non-target organisms (green algae and crustacean zooplankton).

Materials and Methods

The target cyanobacterium species (*M. aeruginosa*) and three non-target freshwater species (the green algae *Ankistrodesmus convolutus* and *Scenedesmus quadricauda*; the zooplankton *Daphnia magna*) were used. The cyanobacterium *M. aeruginosa* strains (NIES 298 and UTEX 2388) were obtained from the NIES (National Institute for Environmental Studies), Tsukuba, Japan, and UTEX (Culture Collection of Algae), at the University of Texas at Austin, respectively. The green algae *A. convolutus* (AG 10001) and *S. quadricauda* (AG 10003) were obtained from the KRIBB (Korea Research Institute of Bioscience and Biotechnology), Daejeon, Korea.

The algal strains were grown in CB (NIES 298; Shirai et al. 1989) or Allen medium (AG 10001, AG 10003, and UTEX 2388; Allen 1968), maintained at 25°C with a 14 h light/10 h dark cycle in a shaking incubator, and illuminated at 100 μmol m^{-2} s^{-1} by cool-white fluorescent lamps for 2 weeks. For seeding, 10 mL of culture was transferred into a 250 mL culture flask with 90 mL fresh CB or Allen medium.

The zooplankton used in this study was *D. magna*, obtained from the K-Water Co. (Korea Water Resources Corporation), Hanam, Korea. Zooplankton were cultured in 20 L glass jars containing 15 L of tap water that received 24 h of prior aeration, maintained at 20°C with a 14-h light/10-h dark cycle, and illuminated at 30 μmol m^{-2} s^{-1} by cool-white fluorescent lamps. The *Daphnia* culture was fed a suspension of a green alga, *Chlorella vulgaris*, supplied by Daesang Co. (Seoul, Korea).

A cultivar of the plant *Oryza sativa* (termed *Ilpumbyeo* in Korean) was grown in an experimental plot of Konkuk University, Korea, and harvested in October 2002. A voucher specimen of hulls (reference code KKU 121, *Ilpumbyeo*) has been deposited in the herbarium of the Department of Applied Life Science, Konkuk University. Rice hulls from the harvested plants were separated on a milling machine and dried at room temperature (25°C) for 7 days. The dried rice hulls were pulverized using a Wiley mill and passed through a 40-mesh screen. The powdered rice hulls (10 kg) were immersed in methanol (60 L) for 1 week at room temperature and then the supernatant was concentrated under vacuum to yield 150 g of crude extract. This crude extract was stored in a freezer (−20°C) until required. The biocidal tests using rice hull extract were performed on five freshwater organisms, i.e., two cyanobacterium strains, two green algae, and one zooplankton. Table 1 summarizes the experimental design of the study.

To evaluate the effect of rice hull extract on microalgae, *Microcystis* strains (NIES 298 and UTEX 2388) and green algae (AG 10001 and AG 10003) were cultured in post-filtered (GF/C filter; pore size, ~0.7 μm) eutrophic lake water (from Lake Ilgam, Seoul, Korea; average total phosphorus 89 μg L^{-1} and average chlorophyll-*a* 800 μg L^{-1} during a cyanobacterial bloom lasting from August to October 2007), at room temperature (ca. 25°C), with a 14 h light/10 h dark cycle. Before adding rice extract to cultures, 1 mL aliquots were taken from each culture and fixed in Lugol's solution for cell enumeration. Rice hull extract was added to microalgal cultures at the concentrations of 1, 10, 100 and 1000 μg L^{-1}; the algal growth was monitored at 1 or 2 day intervals for 7 days, and algal cells of each culture were enumerated using a hemocytometer (Fuchs-Rosenthal instrument; Paul Marienfeld GmbH & Co.; Lauda-Königshofen, Germany) under a phase-contrast microscope (Axioplan instrument; Zeiss, Göttingen, Germany).

At the same time, the effect of rice hull extract on zooplankton was tested. Healthy-looking *D. magna* (10 individuals/jar) were transferred to 200 mL jars containing 100 mL of filtered eutrophic lake water, as described above. Rice hull extract was added to the jars at the same concentrations as used for the microalgal experiments. *Daphnia* swimming behavior was monitored every day for

Table 1 Experimental conditions on tested freshwater organisms

Tested organisms	Extracts ranges (μg L^{-1})	Inoculated concentrations	Observation periods (days)	Replications	Temperatures (°C)
M. aeruginosa (NIES)	1–1,000	1.8×10^6 cells mL^{-1}	7	Triplicate	25
M. aeruginosa (UTEX)	1–1,000	0.6×10^6 cells mL^{-1}	7	Triplicate	25
A. convolutus	1–1,000	2.4×10^6 cells mL^{-1}	7	Triplicate	25
S. quadricauda	1–1,000	1.9×10^6 cells mL^{-1}	7	Triplicate	25
D. magna	1–1,000	1 individual/10 mL	7	Triplicate	20

7 days. The death of *Daphnia* was determined when an organism ceased to swim and fell down to the bottom of the jar.

The inhibitory effects of rice hull extract on growth of the freshwater organisms were evaluated with the following formula:

$$\text{Growth inhibition}\,(\%) = [(\text{control} - \text{treatment})/\text{control}] \times 100.$$

The differences in cell or individual densities between control and treatment cultures were analyzed by an analysis of variance (ANOVA), and data were compared using linear contrasts. A p value of <0.05 was considered significant.

Results and Discussion

Allelochemicals produced by higher plants either inhibit or stimulate the growth of aquatic microorganisms in freshwater ecosystems (Pillinger et al. 1994; Everall and Lees 1997; Nakai et al. 2000; Park et al. 2006b). Previous reports suggest that higher plants can selectively control some algal species. Figure 1 shows the effects of rice hull extract at different concentrations (1, 10, 100 and 1000 μg L^{-1}) on the growth of the cyanobacterium *M. aeruginosa*, and the green algae *A. convolutus* and *S. quadricauda*. The degree of algal growth inhibition at the tested concentrations of rice hull extract was similar for both *M. aeruginosa* strains (NIES 298 and UTEX 2388). However, the rice hull extract showed a much higher inhibitory effect on *Microcystis* than on green algae. The extract decreased the cell density of two *Microcystis* strains by >95%, and that of two green algae by <24%, over 7 days. Thus, rice hull extract, which contains various allelochemicals (Chung et al. 2002, 2007; Park et al. 2009), selectively inhibited the growth of *M. aeruginosa*. Similarly, previous studies have reported that extracts of some terrestrial plants (e.g., birch, elm, hazel, and a tree in the genus *Polygonatum*) showed higher inhibitory effects on the growth of cyanobacteria than on green algae (Pillinger et al. 1995; Kim et al. 2006). A submerged macrophyte *Myriophyllum spicatum* also exhibited stronger inhibition of the growth of cyanobacteria than of green algae or a diatom (Körner and Nicklisch 2002). This difference in the sensitivity of different phytoplankton taxonomic groups to allelochemicals may influence the competitive balance between cyanobacteria and other algae in freshwater ecosystems (van Donk and van de Bund 2002).

It is not surprising that colony formation by some microalgae may be a common defense strategy against environmental stresses caused by biotic and abiotic factors such as predation, chemicals, temperature, salinity, light intensity, and nutrient deprivation, in aquatic ecosystems (Jang et al. 2003). We also observed that colony formation of the green algae tested (AG 10001 and AG 10003) occurred even on addition of rice hull extract (data not shown). In our experiments, the cell number per colony in the controls was always 2–4, whereas the cell number per colony in the treatment groups increased from 2–4 to 8, and the settling

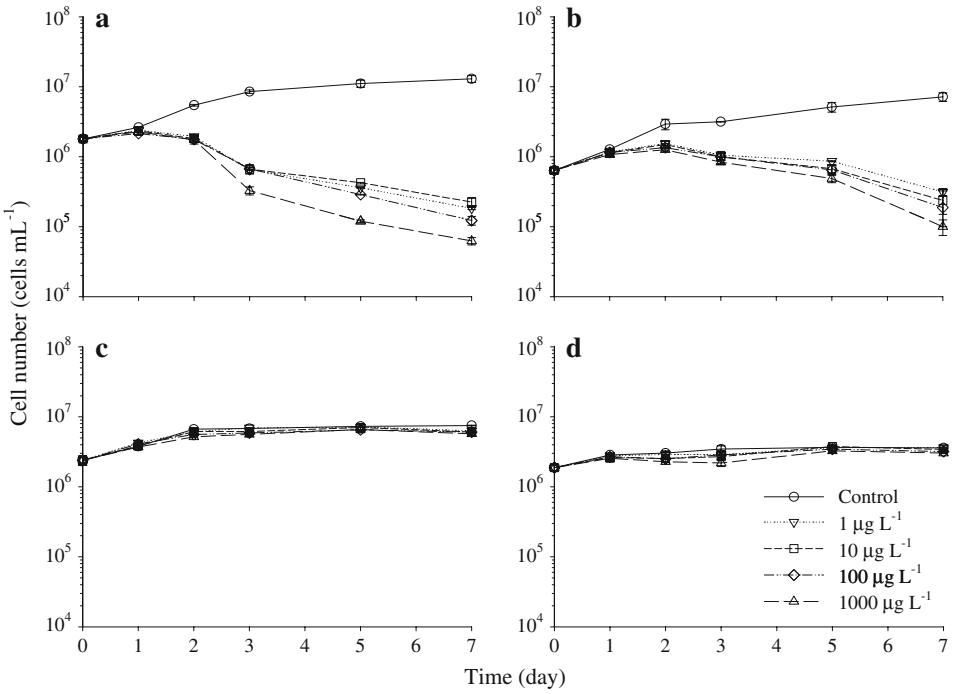

Fig. 1 Effects of rice hull extract on the growth of *Microcystis aeruginosa*, *Ankistrodesmus convolutus*, and *Scenedesmus quadricauda* at different extract concentrations. Data are the averages with SEs (*bars*) of triplicate experiments. **a** *M. aeruginosa* (NIES), **b** *M. aeruginosa* (UTEX), **c** *A. convolutus*, **d** *S. quadricauda*

Table 2 Survival rates of *Daphnia magna* in the presence of rice hull extract at different concentrations

Time (day)	Survival rate (%)				
	Control	1 μg L^{-1}	10 μg L^{-1}	100 μg L^{-1}	1,000 μg L^{-1}
1	100.0 ± 0.0	100.0 ± 0.0	96.7 ± 3.3	96.7 ± 3.3	93.3 ± 6.7
2	100.0 ± 0.0	96.7 ± 3.3	93.3 ± 6.7	96.7 ± 3.3	86.7 ± 6.7
3	100.0 ± 0.0	90.0 ± 5.8	83.3 ± 8.8	93.3 ± 6.7	80.0 ± 5.8
4	100.0 ± 0.0	90.0 ± 5.8	83.3 ± 8.8	90.0 ± 5.8	80.0 ± 5.8
5	100.0 ± 0.0	90.0 ± 5.8	83.3 ± 8.8	90.0 ± 5.8	80.0 ± 5.8
6	100.0 ± 0.0	90.0 ± 5.8	83.3 ± 8.8	90.0 ± 5.8	63.3 ± 3.3*
7	100.0 ± 0.0	90.0 ± 5.8	83.3 ± 8.8	86.7 ± 6.7	53.3 ± 3.3*

Data are the averages with SEs of triplicate experiments

* A significant difference from the results using rice hull extract at 1, 10, and 100 μg L^{-1} ($p < 0.05$)

of colonies was also enhanced. However, colonization did not occur in the cultures of cyanobacterium *M. aeruginosa* (NIES 298 and UTEX 2388) under any treatment condition, including the control treatment, in our experiments. Men et al. (2007) reported that the allelochemical EMA (ethyl 2-methylacetoacetate) extracted from a reed (*Phragmitis australis* Trin) increased colony formation, with subsequent growth stimulation, of a green alga *Scenedesmus obliquus*. Lürling (2006) also reported that surfactant FFD-6 at concentrations of 1–10 mg L^{-1} induced colonization of *Scenedesmus*. In addition, some green algae are capable of degrading allelochemicals such as phenolic compounds (Pinto et al. 2002). Taken together, the ability to form colonies and possible biodegradation abilities of the green algae *A. convolutus* and *S. quadricauda* tested seem to be survival strategies against allelochemicals contained in rice hull, resulting in the selective control by rice hull extract of strains of the cyanobacterium *Microcystis*.

Table 2 shows the effects of rice hull extract on the survival of the zooplankton *D. magna*. Low concentrations of rice hull extract showed little inhibitory effect on the survival of *D. magna* for the 7 days of the experimental period; survival figures were 90%, 83% and 87% at the concentrations of 1, 10 and 100 μg L^{-1}, respectively. The highest tested level 1,000 μg L^{-1}, afforded 53% survival of *D. magna*. Allelopathic effects of rice hull on the zooplankton *D. magna* have not been reported elsewhere, although other studies have documented such effects by some terrestrial plants. Jančula et al. (2007) reported that three terrestrial plant taxa (*Chelidonium majus*, *Macleaya microcarpa*, and *Stylophorum lasiocarpum*) exerted significantly lower toxicity against *D. magna* than against cyanobacteria (*Microcystis aeruginosa* and *Synechococcus leopoliensis*) and green algae (*Pseudokirchneriella subcapitata* and *Scenedesmus quadricauda*). Especially, *C. majus* showed the highest bactericidal effect against *M. aeruginosa*, whereas the toxic effect was low against

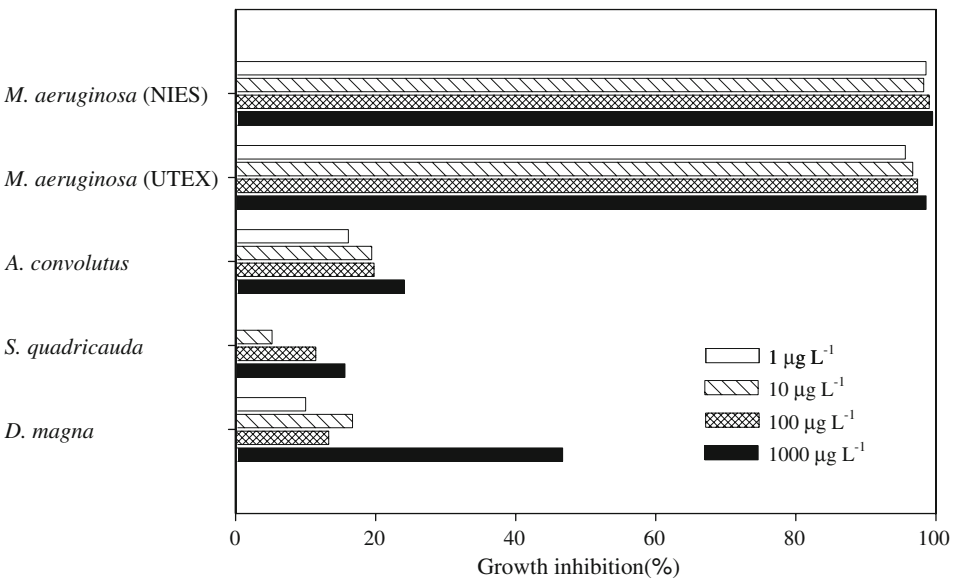

Fig. 2 Growth inhibition of selected freshwater organisms by rice hull extract after 7 days of treatment

the zooplankton *D. magna*. Similarly, rice hull extract did not show a significant inhibitory effect on *Daphnia* survival in our study, but, at high concentration, some inhibition was evident.

The effect of growth inhibition by rice hull extract on some freshwater organisms tested in this study is summarized as follows: *Microcystis* strains (95% to 99% inhibition), *A. convolutus* (16% to 24%), *S. quadricauda* (0% to 15%), and *D. magna* (10% to 47%; Fig. 2). The much higher inhibition of target organisms (*Microcystis* strains) than non-target organisms (green algae and zooplankton) indicates that rice hull extract is useful to selectively control harmful *Microcystis aeruginosa*. Further studies will test the selective inhibitory effect of rice hull extract in the field, and on various other organisms, and will determine suitable rice hull concentrations for field application to examine whether rice hull has field potential.

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