Image caption: Culture incubations with straw decomposition extract. Photo courtesy of Mary Savin.

Is Rice as Effective as Barley Straw or Hydrogen Peroxide in Inhibiting Cyanobacterial Blooms and Reducing Microcystin Concentrations?

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Abstract: Freshwater harmful algal blooms (HABs) are occurring more frequently and in new locations. The extract from decomposition of rice straw may serve as an effective algal inhibitor because of the production of inhibitory compounds during decomposition. Hydrogen peroxide may be the main inhibitory compound produced. We conducted a series of experiments to compare the effectiveness to inhibit algal growth of rice straw decomposition extract to hydrogen peroxide and barley straw decomposition extract. Hydrogen peroxide added at high concentrations showed immediate and lasting inhibition of algal growth throughout incubation of algal cultures. In *R. subcapitata* cultures after 28 days and in the absence of natural organic matter, decomposition extract filtered from 5.0 g/L rice straw showed 93.4% inhibition compared to the control, followed by 5.0 g/L barley straw (61.8%), whereas 2.5 g/L barley straw (25.2%) and 2.5 g/L rice straw extracts (11.2%) were not significantly different from the control. Phenolic concentrations were 2-3 orders of magnitude greater in hydrogen peroxide compared to straw extract treatments, likely resulting from the large input of hydrogen peroxide. Flavonoid concentrations were detected in straw extract cultures after 1 day, but there was a lag until after day 8 before concentrations increased in the hydrogen peroxide treatments and the control. In lake water, decomposition extract from 5.0 g/L but not 10 g/L rice straw showed delayed growth (at day 28) in *Microcystis aeruginosa* in the absence of bacteria; whereas, *Microcystis aeruginosa* grew in the control only at day 28 when bacteria were retained in the lake water. Results from controlled experiments do not support that inhibitory mechanisms from straw decomposition extracts is driven by hydrogen peroxide generation. Results do support that value can be added to rice straw decomposition extract as a product for algal growth inhibition with further research to specify mechanisms of action. Both the concentration of decomposition extract and the ecology within the lake water are important to understand in order to maximize effectiveness of the use of rice straw decomposition extract to inhibit algal growth in natural waters.

Key Points:

- Large concentrations of hydrogen peroxide showed immediate and lasting inhibition of algal growth throughout incubation of algal cultures and in lake water.
- Filtered extract from decomposition of aqueous 5 g/L rice straw showed greater inhibition of algae using measurements of chlorophyll-a and greater production of flavonoids and polyphenolics than filtered extract from 5 g/L barley straw decomposed under the same conditions.
- Experiments with two algal species and in lake water which has natural organic matter demonstrate the importance of the concentration of rice straw during decomposition in order for the decomposition extract to inhibit algal growth
- Ecology of the algae, and hence results of rice straw decomposition extract to inhibit algal growth, changed if bacteria are present
- Results indicate there is value to continue to investigate rice straw decomposition extract, and the role of polyphenolic compounds produced during decomposition, to inhibit harmful algal blooms in surface waters.

Introduction

Freshwater harmful algal blooms (HABs) are becoming increasingly more common and have been spreading into new locations in recent years. With global climate change, HABs are expected to continue to become more frequent and prevalent. Because cyanobacteria are adaptable organisms, the likelihood of events to predict and the ability to prevent them has been difficult (Pearl, 2017). As a local example, in 2019 the City of Fayetteville announced that there may have been harmful algae in Lake Fayetteville as water was sampled with microcystin concentration greater than 10 µg/L (e.g. http://www.fayetteville-ar.gov/CivicAlerts.aspx?AID=1793&ARC=3303). Arkansas has more than 127,000 farm ponds (Kelly, 2017). Reservoirs are important surface water impoundments in Arkansas. For example, Beaver Lake (encompassing 766,026 acres) is the first in a series of three U.S. Army Corps of Engineers reservoirs on the White River, operates at a capacity of 140 million gallons of water per day, and provides the drinking water for multiple municipalities covering one in seven Arkansans (Ouei and Daniels, 2017). Research continues for models that can accurately predict occurrences and restoration of watershed management approaches that prevent future outbreaks in the face of a changing climate. Meanwhile, use of a simple, ecofriendly approach to control both growth and toxin production of harmful cyanobacteria that does not persist or have non-target effects is imperative. Though Arkansas has not yet experienced catastrophic HABs, the potential for catastrophic HABs in local, freshwater systems makes the necessity of effective treatment approaches imperative.

Cost-effective management approaches should control and prevent outbreaks with minimal risk to people, animals, and the environment. Barley straw has been shown in many lab and field experiments to inhibit algal growth, although experiments have contradicted these results and also shown no response or growth promotion (hUallacháin and Fenton, 2010; Islami and Filizadeh, 2011). Other cereal straws may be as or more effective, depending on specific environmental and biological conditions. Rice straw has been an effective algal inhibitor through the production of multiple inhibitory compounds during decomposition (Park et al., 2006). However, contradictory research has indicated that it may not be as effective an algal inhibitor as barley straw (Ma et al., 2015). Utilization of barley or rice straw depends on production of allelopathic compounds (e.g. phenolic compounds) that are released during aerobic aqueous decomposition of the straw.

Algal population inhibition needs confirmation under controlled laboratory conditions because a field study was inconclusive (Maris et al., 2019). We proposed a series of experiments investigating growth of populations of *Raphidocelis subcapitata* or *Microcystis aeruginosa* in the presence of hydrogen peroxide compared to aqueous extracts of decomposing barley and rice straw to evaluate effectiveness of rice straw extract as an inhibitor of green algae and/or harmful cyanobacteria.

Methods

This study consisted of a series of laboratory-based experiments. Rice straw was obtained from a farmer in central Arkansas and barley straw was purchased from a commercial source. Both straws were washed three times, cut into 2.5-cm pieces, dried (50°C), and stored dry until used in decomposition experiments. Straws (5 g/L) were decomposed aerobically for 30 days in an aquarium at 25°C with aeration controlled using an aquarium pump and light maintained on a 12 hr:12 hr light:dark cycle using cool, white fluorescent tubes. Extracts of decomposing straw were filter-sterilized through a series of filters ending with a 0.45-µm pore size filter. Commercial 3% solution of hydrogen peroxide was filter-sterilized through a 0.45-µm pore size filter and diluted for use in experiments. *Microcystis aeruginosa* and *Raphidocelis subcapitata* were purchased from culture collections and cultures were maintained in Bristol or blue-green (BG-11) and/or media with shaking at 110 rpm, 27°C, and under 12 hr:12 hr light:dark cycle at 100 µmol photons m^{-2} s^{-1} using cool, white fluorescent tubes. Experiments were conducted under these same conditions unless noted elsewhere.

Bioassays for objective 1 (see Table 1) utilized cultures of either *Raphidocelis subcapitata* or *Microcystis aeruginosa* inoculated into sterile culture medium (n = 3) at an initial density approximating 5.8 x 10^5 cells/mL (similar to Hua et al., 2018; USEPA, 2002). Bioassays for objectives 2 and 3 utilized a similar design with lake water collected from northwest Arkansas and filter-sterilized through a 0.45-µm pore-size filter for analysis of growth and toxin production without other organisms but in the presence of natural dissolved organic matter. Lake water filtered through a 5.0-µm pore-size filter for analysis of growth and toxin production in the presence of bacterioplankton was to understand the impact of treatments on both the harmful cyanobacteria in the presence of the bacterial community.

Samples were collected at time 0, 1, 4, and 24 hr for determination of hydrogen peroxide concentration as others have shown rapid decomposition of hydrogen peroxide despite prolonged effects on the algae (Weenink et al., 2015). Chlorophyll-a, pH, dissolved organic carbon (DOC), total phenolics and flavonoids were measured on samples collected on days 0, 1, 4, 8, 15, and 28.

Hydrogen peroxide was analyzed using a cerium sulfate method (Putt and Pugh, 2013). Chlorophyll-a was measured using a fluorometer (Turner Designs, Sunnyvale, CA). Growth inhibition for each algal population added to sterile media was calculated as a percentage of the control to indi-

Table 1: Each chemical (hydrogen peroxide or decomposing straw extract) treatment added to construct microcosms for algal bioassays.

Objective 1 Treatments[1]	Objective 2, 3 Treatments
Control	Pond water control
2.5 g/L H_2O_2	Bacteria control
5.0 g/L H_2O_2	25 mg/L H_2O_2, no bacteria
2.5 g/L BS extract	25 mg/L H_2O_2 with bacteria
5 g/L BS extract	50 mg/L H_2O_2, no bacteria
2.5 g/L RS extract	50 mg/L H_2O_2 with bacteria
5 g/L RS extract	100 mg/L H_2O_2, no bacteria
	100 mg/L H_2O_2, with bacteria
	5 g/L RS extract, no bacteria
	5 g/L RS extract, with bacteria
	10 g/L RS extract, no bacteria
	10 g/L RS extract, with bacteria

[1]BS is barley straw, RS is rice straw, H_2O_2 is hydrogen peroxide

cate inhibition in the presence of each straw or hydrogen peroxide concentration over time. Sample pH was measured using an electrode and calibrated pH meter. Dissolved organic carbon was analyzed on a TOC-V PC-controlled organic C analyzer (Shimadzu, Columbia, MD). Total phenolics (Margraf et al., 2015) and total flavonoid content (Hatamnia et al., 2014) were analyzed by microplate methods. Mean and standard errors were calculated and data were analyzed by repeated measures analysis of variance (ANOVA, $P < 0.05$), with post hoc tests to separate means where appropriate.

Results and Discussion

The main objective of this research was to determine the efficacy of barley and rice straw as algal inhibitors through evaluation of polyphenolic compounds released during decomposition as compared to H_2O_2. Different concentrations of H_2O_2 and aqueous extracts after 30 days of decomposition of rice and barley straw were added to green algae *Raphidocelis subcapitata* and cyanobacterial *Microcystis aeruginosa* cultures. The cultures were sampled for H_2O_2, chlorophyll-a, pH, DOC, and total phenolics and flavonoids. In *R. subcapitata* cultures after 28 days and in the absence of natural organic matter, decomposition extract filtered from 5.0 g/L rice straw showed an inhibitory effect of 93.4%, followed by 5.0 g/L barley straw at 61.8% inhibition (Figure 1). In contrast, decomposition extract filtered from 2.5 g/L barley straw (25.2%), and 2.5 g/L rice straw (11.2%) were not different from the control. Decomposition extract filtered from 2.5 g/L and 5.0 g/L rice straw were effective at inhibiting *Microcystis aeruginosa* growth for 28 days when grown in media (Figure 2). In lake water, decomposition extract from 5.0 g/L but not 10 g/L rice straw showed delayed growth (at day 28) in *Microcystis aeruginosa* in the absence of bacteria; whereas, *Microcystis aeruginosa* grew in the control only at day 28 when bacteria were retained in the lake water (Figure 3). Concentrations initially greater than 5 g/L may be especially important in inhibition of different algal populations in surface waters. The ecology within a lake will also be important to understand better in order for algal control to be achieved.

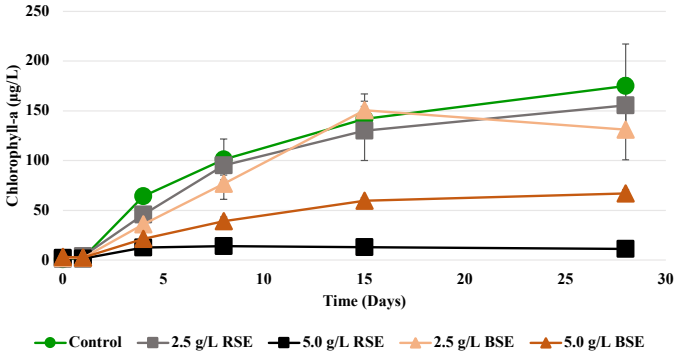

Figure 1: Chlorophyll-a (µg/L) in media based *Raphidocelis subcapitata* cultures for 0 to 28 days following treatment with rice straw extract (RSE), barley straw extract (BSE), or no treatment (control, C) (n=3).

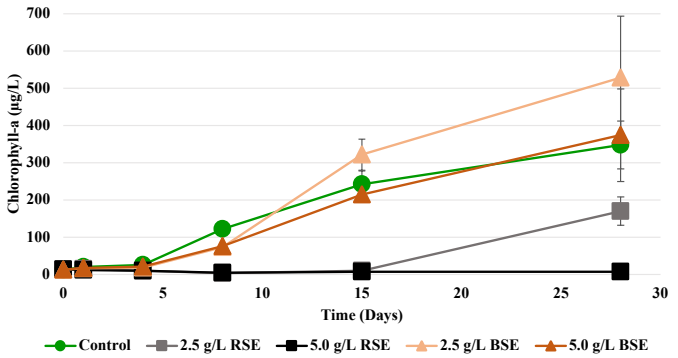

Figure 2: Chlorophyll-a concentrations (µg/L) in media based *Microcystis aeruginosa* cultures for 0 to 28 days following treatment with rice straw extract (RSE), barley straw extract (BSE), or no treatment (control) (n = 3).

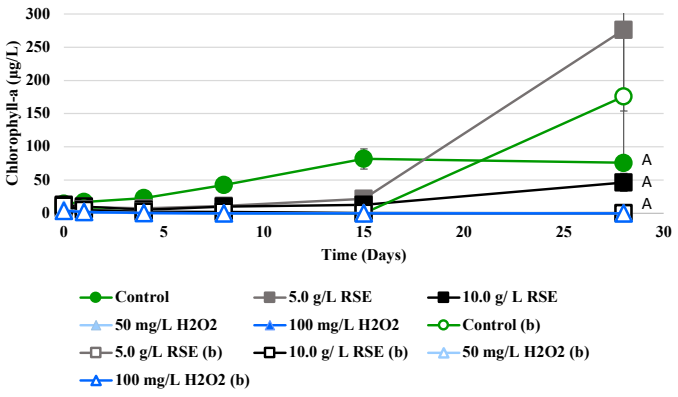

Figure 3: Chlorophyll-a concentrations (µg/L) in lake water based *Microcystis aeruginosa* cultures for 0 to 28 days following treatment with rice straw extract (RSE), hydrogen peroxide (H_2O_2), or no treatment (control) and with bacteria (b) or without bacteria (n = 3). Similar letters represent lack of significant differences between the treatments ($P < 0.05$).

Previous research has indicated that hydrogen peroxide is an effective inhibitory compound and results obtained in these experiments support that conclusion. However, if adding value to rice straw as a product to prevent or inhibit algal blooms is a potential market, there are economic and environmental reasons to continue pursuing mechanistic understanding to explain the inhibitory effect on algae. Results from the rice and barley straw extract treatments do not show appreciable concentrations of hydrogen peroxide produced within 24 hours. Furthermore, the production of polyphenolic compounds is orders of magnitude less than when high concentrations of hydrogen peroxide are applied to water. Thus, it seems that either specific compounds could be produced having an effect on algae, compounds that are produced react chemically before being measured by the experimental analysis, or there are other mechanisms - perhaps more diverse mechanisms – unaccounted for in these experiments. Continuing measurements of the phenolics, flavonoids, DOC, nutrients, and toxin concentrations in the presence of a known lake community composition should provide more insight into potential mechanistic differences contributing to algal inhibition from the decomposition extract of rice straw.

Conclusions

Managers of small aquatic systems may purchase barley straw, guided by the belief that it will prevent HABs (M. Lankford, personal communication). Experimentation on the practical application of using rice straw extract for algal control in Arkansas or the Midsouth is lacking. Experiments utilizing populations in culture media maintained known, controlled conditions to optimize growth while experiments utilizing pond water focus on algal growth under conditions more likely to be present in environmental waters. Results from controlled experiments do not support that inhibitory mechanisms from straw decomposition extracts are driven by hydrogen peroxide generation. Results do support that value can be added to rice straw as an environmental product for algal growth inhibition with further research to specify mechanisms of action. Both the concentration of straw used to produce the decomposition extract and the ecology within the lake water are important variables in order to maximize effectiveness of the use of rice straw decomposition extract to inhibit algal growth in natural waters.

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