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The Use of Barley Straw for Controlling of Cyanobacteria Under Field Application

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Abstract: This study was done to determine whether decomposing barley straw can be used to control the growth of cyanobacteria under field condition. Decomposing barley straw with two different amounts (40 and 80 kg m^{-2}) was placed as sausage forms into the experimental ponds and the quantity of cyanobacterial genera was measured as indicator of growth. The quantitative measurements over five months showed that the straw was effective for controlling overall growth of cyanobacteria compared to controls. Barley straw was capable for inhibiting the growth of *Microcystis*, *Anabaena* and *Aphanizomenon*. In contrast, decomposing barley straw stimulated the growth of *Oscillatoria*, whereas *Nostoc* grown wasn't affected by rotting barley straw. The results of this investigation indicated that barley straw could introduce an easy-to-use, practical and cost-effective method to assist water managers and, potentially, aquaculture ventures for managing the occurrence of cyanobacterial blooms in small freshwater basins.

Key words: Barley straw, cyanobacteria, control, ponds, decomposing, field

INTRODUCTION

Cyanobacteria (blue-green algae) are a prevalent group of algae inhabiting in all ecosystems (Fleming *et al*., 2002). Most of these organisms are present in freshwater bodies like lakes, slow-down flowing rivers and ponds throughout the world which several of them may become dominant (blooms) and form surface mats under favorable conditions (Messineoa *et al*., 2009). However, blooms of these photosynthetic prokaryotes can cause a number of problems such as musty taint and odor (Howgate, 2004), production of potent toxins (Codd *et al*., 2005) and in some instances human illness and animal poisoning mainly due to the widespread water eutrophication (Vasconcelos, 1999; Azevedo *et al*., 2002; Chen and Xie, 2005; Dittmann and Wiegand, 2006). Consequently, there is a raising attention for management of cyanobacteria populations in water supply reservoirs and recreational water systems.

Because of small size and rapid growth rate, the conventional methods for control of the other aquatic plants have not satisfactory effects on the control of cyanobacteria. The

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mechanical removal of filamentous cyanobacteria could help to reduce their problems. However, these methods are time consuming and impractical (Chen *et al*., 2004; Ghobrial *et al*., 2007). Chemical substances has been also used for the elimination of cyanobacteria, but their toxicity towards non-target organisms, short outcomes and persistence in environment are restricted their use (Ball *et al*., 2001; Barrett *et al*., 1999).

Application of barley straw as a biological compound to control of cyanobacterial has been promoted since 1980 in England (Welch *et al*., 1990). Different studies exhibit that decomposing barley straw produces chemical substances that effectively inhibit the growth of some nuisance cyanobacterial species (Gibson *et al*., 1990; Ridge and Pillinger, 1996; Barrett *et al*., 1999; Brownlee *et al*., 2003; Murray *et al*., 2009). The accurate chemicals of barley straw that control the cyanobacterial growth have not been clearly recognized, but limited research suggested that oxidized phenolics, or free radicals from their decomposition are the inhibitors (Pillinger *et al*., 1994; Choe and Jung, 2002; Waybright *et al*., 2008).

To date most of the works have been focused on the understanding the inhibitory effects of barley straw on cyanobacteria growth under laboratory conditions (Newman and Barrett, 1993; Caffrey and Monahan, 1999; Brownlee *et al*., 2003; Murray *et al*., 2009). Field experiments have been mainly conducted as management experimentations that involved a single pond, canal, or reservoir (Welch *et al*., 1990; Ridge and Barrett, 1992; Everall and Lees, 1997; Barrett *et al*., 1999; Caffrey and Monahan, 1999). Consequently, field studies with appropriate replications are needed to elucidate inhibitory activity of decomposing barley straw on the natural algal assemblages. The objective of this study was to consider the susceptibility of the cyanobacteria to the barley straw in a field trial. Furthermore, present study was conducted to determine the efficiency of different amount of barley straw on the growth of blue-green algae.

MATERIALS AND METHODS

Barley straw was collected from Qazvin Province, Iran during April and May 2009. The straw bales had broken up and loosely repacked by 2 m onion nets in sausage forms containing 2 kg barley straws. This shape of packing increased the diffusion of oxygen to the site of decomposition and speeded up the process.

Nine 300 m^2 earthen ponds (each 15×20 m) with a history of blue-green algae infestation in the past were selected in Ansari Breeding and Reproduction Center (ABRC), Guilan Province, Iran. The ponds had randomly distributed to 3 triplicate groups and treated with 40 and 80 g m^{-2} (12 and 24 kg for each pond) barley straws, respectively. The ponds were filled with water of same source and no fertilizer was used throughout the experiment.

On 5 May 2009, barley straw applied to the ponds according the guideline of Newman (1994). The packs were securely anchored 1 m from the bank to hold them in place during periods of high wind. The sausage packs placed around the pond in depth of 10 cm to keep the straw oxygenated and spread the chemicals equally throughout the ponds.

Water quality and cyanobacterial distribution of the treated and control ponds were monitored monthly over growth season (May to August) beginning on 20 May. Water temperature was determined by a mercury thermometer, while Dissolved Oxygen (DO) and pH were measured at day time with a digital multi-meter (WTW Multiline P4). The sampling of the pond water for cyanobacterial analysis was carried out by collecting 1 L pooled sample from three different points of each pond and filtering through plankton net with 25 μm mesh size. The pooled samples preserved in a polyethylene bottle by adding 1% Lugol's solution.

Cyanobacterial analyses were performed by transferring 1 mL of the pooled sample to the Sedgwick-Rafter counter and counting of 10 cells chosen randomly. The cell density was computed using the Stirling (1985) formula and the mean number of each genus was stated as unit mL^{-1} of pond water. The cyanobacteria were also identified up to the genus under a compound light microscope using keys and illustrations by Bellinger (1992) and Palmer (1989).

Treatments were organized in randomized complete block design with three replications. Differences in algal densities between control and each treated ponds were analyzed by split-plot analysis of variance followed by Duncan's multiple-range test. The results expressed as Mean±SD and a p-value less than 0.05 were considered statistically significant.

RESULTS

Microscopic considerations indicated present of 34 genera of cyanobacteria, dominated by *Microcystis*, *Anabaena*, *Aphanizomenon*, *Oscillatoria* and *Nostoc* in the experimental ponds. The overall counts showed that growth of cyanobacteria in control ponds increased subsequently during the period of experiment, with the maximum of 121.82×10^3 units mL^{-1} on August (Table 1). Cyanobacterial biomass of the ponds treated by 40 and 80 g m^{-2} exhibited similar amounts of total cell number by May, then declined during the remaining time. However, no significant difference was found between the straw treatments ($p<0.05$) (Table 1).

The results showed that biomass of *Microcystis*, *Anabaena* and *Aphanizomenon* were all significantly inhibited by barley straw (Table 2). Growth of *Microcystis* and *Anabaena* in the control ponds were increased, reaching to the maximum of 36.08×10^3 and 29.08×10^3 units mL^{-1}, respectively during the experiment. *Microcystis* grown completely inhibited in both treatments with the minimum cell density on August. Likewise, growth of *Anabaena* was declined in all straw treatments but only higher amount of straw showed significant reduction ($p<0.05$). *Aphanizomenon* cell density in the control ponds was also increased substantially over the growing season. In contrast, the growth of this cyanobacterium significantly ($p<0.05$) decreased in both straw treatments by July and remained in this amount until August.

Table 1: The Means±SE density of overall cyanobacteria (units $mL^{-1}\times10^3$) in different treatments for five months

Treatments	April	May	June	July	August
Control	32.32±3.24cdef	39.35±4.23cde	48.15±5.13c	73.30±6.24b	121.82±8.41a
40 (g m^{-2})	40.92±2.79cd	34.20±5.44cde	34.96±3.57cde	30.45±2.45cdef	24.42±2.08defg
80 (g m^{-2})	30.90±2.08cdef	42.17±5.71cd	32.60±2.37cdefg	24.00±1.98efg	19.50±1.47fg

Means with different letters within the treatments are significantly different ($p<0.05$)

Table 2: Mean±SE cyanobacterial density (units $mL^{-1}\times10^3$) of genera inhibited by barley straw in different treatments for five months

Cyanobacterial genera	April	May	June	July	August
Microcystis					
Control	9.42cde	11.80±1.35def	12.35±3.11ef	21.37±5.70g	36.15±6.24h
40 (g m^{-2})	11.76±1.21def	10.32±0.95def	4.27±0.98b	1.54±0.04a	0.84±0.09a
80 (g m^{-2})	8.74±0.87cde	7.26±0.82cd	3.81±0.77a	1.09±0.08a	0.57±0.10a
Anabaena					
Control	8.08±0.64de	9.83±0.74def	11.35±1.12ef	18.73±2.14g	29.08±4.71h
40 (g m^{-2})	9.43±1.13def	7.16±0.52cde	6.78±0.52bc	5.92±1.26bc	5.74±0.85bc
80 (g m^{-2})	7.69±0.74cde	5.481±0.43bc	3.12±0.41ab	2.48±0.18a	1.50±0.09a
Aphanizomenon					
Control	4.32±1.02cd	5.42±1.05de	7.22±0.58ef	10.49±1.35g	21.64±3.58h
40 (g m^{-2})	4.41±0.92cd	3.42±095cd	2.97±0.21bc	1.08±0.14ab	0.87±052ab
80 (g m^{-2})	4.53±0.83cd	3.27±0.84cd	2.14±0.14bc	0.79±0.20ab	0.45±0.10ab

Means with different letters within the treatments of each algal genus are significantly different ($p<0.05$)

Table 3: Mean±SE cyanobacterial density (units $mL^{-1}\times10^3$) of genera unaffected by barley straw in different treatments for five months

Cyanobacterial genera	April	May	June	July	August
Oscillatoria					
Control	4.57±0.94a	4.92±0.95a	5.28±1.58ab	8.47±1.74c	8.22±2.57c
40 (g m^{-2})	5.64±1.24a	5.41±1.26a	7.24±2.73bc	11.14±2.41d	12.25±1.85d
80 (g m^{-2})	4.89±0.95a	5.02±1.63a	6.97±2.08ab	9.85±1.91cd	11.47±2.19d
Nostoc					
Control	2.07±0.58a	2.54±0.74a	2.72±0.45ab	3.28±0.74bc	4.87±1.35cd
40 (g m^{-2})	1.68±0.68a	2.13±0.61a	2.50±0.27ab	3.53±0.47bc	4.4±1.59cd
80 (g m^{-2})	1.97±0.23a	1.75±0.08ab	2.48±0.63bc	3.12±0.67bc	3.93±1.08cd

Means with different letters within the treatments of each algal genus are significantly different ($p<0.05$).

Table 4: Physicochemical parameters of the experimental ponds for five month

	Control		40 (g m^{-2})		80 (g m^{-2})	
Parameters	Mean	Range	Mean	Range	Mean	Range
Temperature (°C)	24.39a±0.27	18.25-32	24.51a±0.24	18.5-31.25	24.97a±0.22	18.83-31.2
pH	7.93a ±0.04	7.64-8.24	7.84a ±0.04	7.54-8.19	7.71a±0.04	7.18-8.23
DO (mg L^{-1})	3.47a±0.19	2.45-7.87	5.34b±0.25	3.78-8.05	5.72b±0.43	3.91-7.87

Means in the same row with different letters are significantly different ($p<0.05$)

The average unit counts of *Oscillatoria* and *Nostoc* in the control ponds increased rapidly after the initial algal sampling by June and remained steady until August. *Oscillatoria* grown was showed relatively similar units from April to June in 40 g m^{-2} straw treatment, but stimulated and reached to final cell count of 12.25×10^3 units mL^{-1} by August. In contrast, *Nostoc* grown was not significantly affected by the both amounts of barley straw ($p<0.05$), although 80 g m^{-2} straw treatment slightly suppressed its growth when the results compared to the control ponds (Table 3).

The comparison of the physicochemical variables in experimental ponds is shown in Table 4. The results demonstrated no significant differences of measured parameters between the ponds except for dissolved oxygen ($p<0.05$). The average DO value of control ponds (3.47 mg L^{-1}) was significantly ($p<0.05$) lower than both straw treatments (5.34 and 5.72 mg L^{-1}, respectively). Besides, 40 g m^{-2} straw treatment exhibited wider fluctuation of DO than 80 g m^{-2} straw treatment.

DISCUSSION

The results of this study show that decomposing barley straw effectively controlled the overall growth of cyanobacteria under field application. However, no significant difference was found between the straw treatments. This is contrary to the report of Ferrier *et al.* (2005) which were not able to demonstrate that barley straw retarded algal growth in small ponds. The present study corroborates further reports in field applications of barley straw that exhibited an overall decline in algal biomass (Ridge and Barrett, 1992; Barrett *et al.*, 1996; Everall and Lees, 1997; Caffrey and Monahan, 1999).

Newman (1994) expressed that greater dosages of barely straw could cause more effects on cyanobacterial growth, but the results of this investigation did not certify it. Both treatments controlled the cyanobacterial growth, but no significant difference was found between the treatments. Removing the heavy and water-logged packed straw from the pond and deposing of them at the end of study have also needed a considerable effort. Therefore, lower amount of barley straw (40 g m^{-2}) seems to be more preferable for application in reservoirs like fishponds.

The findings presented in Table 2 support earlier field considerations and laboratory results that showed *Aphanizomenon* was susceptible to the rotting barley straw (Everall and Lees, 1996; Martin and Ridge, 1999). Both amounts of decomposing barley straw completely inhibited the growth of this alga which is implicated in undesirable taste and odor problems as well as toxin production in water supplies (De Figueiredo *et al*., 2004; Yamamoto and Nakahara, 2005; Preussel *et al*., 2006).

Microcystis is one of the most important cyanobacterium that finds in freshwaters worldwide (Ozaki *et al*., 2008). Because of toxin production that threaten public health (Geng *et al*., 2006; Wu *et al*., 2007), control of this blue-green alga has been the subject of expanding researches over the last decades (Vincent, 1987; Sivonen, 1996; Schrader, 2003; Choi *et al*., 2005; Ke *et al*., 2007). The present investigation confirmed the previous laboratory and field studies that showed growth of *Microcystis* is constantly inhibited by barley straw.

Responses of cyanobacteria to the decomposing barley straw in this study were often different from those obtained previously. For example, the growth of *Anabaena* was inhibited in the present study but found to be stimulated by rotting straw in earlier researches (Martin and Ridge, 1999; Ferrier *et al*., 2005). This alga is one of the problematic cyanobacterium that proliferates in vast numbers and could produce intracellular toxins and release them to the surrounding waters, especially when they are in a senescent growth phase or when an algaecide has been applied (Tsujimura, 2004; Spoof *et al*., 2006; Osswalda *et al*., 2007). Therefore, barley straw could be recommended as alternative method to control of this toxic cyanobacterium bloom.

The response of *Oscillatoria* grown to components released from decomposing barley straw has also differed broadly. *Oscillatoria* is a bloom forming cyanobacterium that frequently found in shallow eutrophic lakes (Venter *et al*., 2003) and often dominates in early spring. Everall and Lees (1997) demonstrated that barley straw was decreased the growth of this blue-green alga under field application but the present study doesn't support it. Present results reiterate the findings of Martin and Ridge (1999) that expressed the growth of *Oscillatoria* was stimulated by the presence of barley straw. This stimulatory effect may be related to the reduction of the other cyanobacterial genera that take more space, nutrient and opportunity for resistant cyanobacteria.

This is the first study on the growth responses of *Nostoc* to the barley straw. The result showed that growth of this alga was not significantly affected by barley straw; however the higher amount of barley straw slightly reduced its growth. It seems that inhibitors released from barley straw had just algistatic effect on the growth of *Nostoc* and, probably, more amounts of barley straw required for constant control of this toxic cyanobacterium (Hirata *et al*., 2003; Smith *et al*., 2008).

Present findings were reinforced the previous reports that expressed barley straw is more effective in water bodies with sufficient amount of oxygen where the rotting of the straw is not interrupted (Nicholls *et al*., 1995; Boylan and Morris, 2003). The DO value over the experiment was always higher than 2.45 mg L^{-1} that secured oxidative decomposition of barley straw. Furthermore, treated ponds had higher average oxygen level that could reflect their more healthy condition compared to the control ponds.

The results present here and those previously reports (Ridge *et al*., 1999; Choe and Jung, 2002), showed that rotting barley straw produces and releases many compounds to the water, which one or more of them may control cyanobacteria populations. The chemical compounds don't eliminate existing cyanobacterial counts but interfere with prevent the growth of new cyanobacterial cells (Ferrier *et al*., 2005). As old blue-green algae cells

naturally die off, few new cells are produced and the cyanobacterial population is controlled as long as the compound is being produced.

This investigation indicates that barley straw could introduce an easy-to-use, practical and cost-effective method to assist water managers and, potentially, aquaculture ventures for managing the occurrence of cyanobacterial blooms in small freshwater basins. Nonetheless, the resistance of some genus to the barley straw and expectancy in the change of cyanobacterial dominancy may have important implications for water management. Further studies, however, are needed to evaluate the behavior of cyanobacteria in the presence of barley straw. Accurate identification of inhibitors responsible for algistatic activity could also help to improve the efficiency of barley straw application.

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