Efficiency of Glutinous Rice Straw Extracts (RD-Six) and Water Hyacinth in Inhibiting Algal Growth and Reducing Nutrients from a Hyper-eutrophic Pond

Wipawan Intasan[1], Ratcha Chaichana[1,2*], and Patana Anurakpongsatorn[1]

[1]*Department of Environmental Technology and Management, Faculty of Environment, Kasetsart University, Bangkok 10900, Thailand*
[2]*Research Group: Natural Environment in Forest and Freshwater Ecosystems, Faculty of Environment, Kasetsart University, Bangkok 10900, Thailand*

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*** Corresponding author:**
E-mail: fscircc@ku.ac.th

ABSTRACT

Eutrophication is one of the main water pollution problems, which occurs throughout Thailand. This research focused on the use of glutinous rice straw (RD-Six) extract and water hyacinth to reduce nutrients and to inhibit phytoplankton growth in hypereutrophic water. In this research, we used (1) control without glutinous rice straw extract and water hyacinth; and (2) experimental treatment with glutinous rice straw extract and water hyacinth. Experiments were set up for eight days in ten concrete circle pit blocks (0.80 m in diameter and 0.40 m in height) in year 2020 (five replicates for each control and experimental treatment). Water quality was analyzed physically, chemically and biologically, and the effect of glutinous rice straw extract on phytoplankton and the growth rate of water hyacinth were also determined. The result showed that values of water quality parameters (turbidity, DO, BOD, SS, TKN, NH_3-N, NO_2, TP, SRP, TN, and Chlorophyll a) were improved. The values of water quality were significantly lower ($p<0.05$) in experimental treatments than in controls. Glutinous rice straw extract had no effect on water hyacinth growth. The study of phytoplankton composition demonstrated that, prior to the experiment, Cyanophyta was the dominant group in both control and experimental treatments. After the experiment, Cyanophyta became less abundant in experimental treatments. Reduction of Cyanophyta could be caused by the direct effect of allelochemicals (phenolic compounds) from glutinous rice straw extract. Therefore, this combined method has displayed their effectiveness in reducing nutrients and inhibiting phytoplankton growth in eutrophic water.

1. INTRODUCTION

Human activities from rapid expansion of intense agricultural areas, industry and urban settlement have led to environmental problems and degradation. In particular, nutrient pollution, coming from both point sources and non-point sources is regarded as a serious environmental problem, especially in Thailand. Addition of excessive nutrients into lakes and rivers can cause a rapid algae bloom or eutrophication (Rodrigo et al., 2018). This problem can lead to water quality deterioration, reduction in diversity of flora and fauna, and human health problems posed by harmful algae blooms (HABs) of the cyanobacteria group (Usharani and Keerthi, 2020).

There are several physical and chemical methods that can be used to inhibit algal growth and to solve eutrophication problems. These methods include, for example, the use of chemical substances (e.g., copper sulphate, aluminium sulphate, Phoslock) (Jančula and Maršálek, 2011; Umphres et al., 2012) or ultrasonic treatment (Broekman et al., 2010). However, operation of these methods is often costly and may result in long-lasting chemical residues in the environment (Umphres et al., 2012). In recent years, rice straw extract has received considerable attention as an algaecide (Ella et al., 2007) and has been used in many countries. This method is environmentally friendly, effective, and low in operational cost. Most

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rice straw extracts are a group of phenolic compounds (coumaric, vanillic, ferulic, hydroxy benzoic, salicylic, syringic, and benzoic acids), which can inhibit phytoplankton growth and can cause abnormalities against photosynthesis processes of phytoplankton (Ferrier et al., 2005; Park et al., 2006). In particular, phenolic compounds selectively affect blue-green algae (e.g., *Microcystis aeruginosa*) (Hua et al., 2018) and phytoplankton communities then can shift from cyanobacteria to other groups such as diatoms (Islami and Filizadeh, 2011). Reduction of cyanobacteria, especially *Microcystis*, is beneficial since *Microcystis* produces microcystin that can cause toxicity to the liver and skin irritation in animals and humans.

Water hyacinth (*Eichhornia crassipes*), belonging to the Family Pontederiaceae, is a floating plant with large and thick leaves. Its roots are underwater, but the stems and leaves float above the water surface. It grows well in hot and humid climates with the optimum temperature between 25-35°C. Water hyacinth is propagated quickly and can be found everywhere in water bodies. It is more durable and grows better in deteriorated water than other plants. There are several research experiments that used water hyacinth such as; removal of heavy metals (Jayaweera et al., 2008; Mishra and Tripathi, 2009), reduction of concentrations of dyestuffs (Ekambaran and Perumal, 2017), and absorption of nutrients (Yi et al., 2009). From research, water hyacinth was highly effective in treating 89.4% nitrogen and 84.0% phosphorus (Lu et al., 2018) in eutrophic water restoration due to its strong nutrient absorption capacity (Gong et al., 2018; Rezania et al., 2015; Wang et al., 2012). The structure of its roots has a large surface area, which is suitable for the growth of different types of microbes that can decompose organic matter in wastewater into inorganic substances. It also enables the tracing of the elements that plants use to grow from the uptake which passed through its porous membranes and accumulates in leaves and roots (Chunkao et al., 2012; Gopal, 1987).

In Thailand, glutinous rice straw is one of the highest amount agricultural waste products as Thai people consume rice as a staple food. Farmers eliminate remaining glutinous rice straw by burning, and this can cause serious air pollution problems that are harmful to human health (Chen et al., 2017). Water hyacinth, an invasive alien species, was introduced to Thailand in 1901. It flourishes and is widespread in tropical countries and should be used for the benefit of society. The use of glutinous rice straw extract (Eladel et al., 2019; Kang et al., 2017; Iredale et al., 2012; Islami and Filizadeh, 2011) and water hyacinth should be an interesting option to control algal blooms. This combined technique is uncomplicated, has a low cost of operation and reduces the use of chemicals, and therefore is safe for humans, animals and the environment. The objectives of this study were to investigate the efficacy of glutinous rice straw extract application and water hyacinths to control phytoplankton growth and to improve eutrophic water quality, and to determine the impact of glutinous rice straw extracts on phytoplankton composition and assemblages. The data of this study may apply well in treating eutrophic waters in Thailand.

2. METHODOLOGY

2.1 Study site

This research was conducted at Lumpini Park (13.729932 N, 100.541309 E), which is a large public park located at the center of Bangkok, Thailand. Lumpini Park covers an area of 360 rai or approximately 57.6 ha. This park has a large pond (water surface area of 111,000 m^2, volume of 222,000 m^3 and average depth of 2 m) and a network of small ponds. A variety of fish and aquatic animals exists in the area (invertebrates, water birds, and water monitors). The park's pond has poor water circulation and receives public artificial bird and fish feeding. Therefore Lumpini Park represents eutrophic unban ponds that have severely suffered from frequent rapid growth of phytoplankton and should be urgently managed for a better ecological condition.

2.2 Experimental design and setup

In this study, ten concrete circle pit blocks (0.80 m in diameter and 0.40 m in height) were used (Figure 1) because they are available in the market, affordable and are heavy enough to be placed in the water without drifting. Moreover, concrete circle pit blocks are stronger and more durable in the water than other materials. 120 L of water were pumped into each concrete circle pit block from a large pond by an electrical pump. The experiments were divided into two sets; (1) control (five concrete circle pit blocks as replicates) without glutinous rice straw extract and water hyacinth, and (2) experimental treatment (five concrete circle pit blocks as replicates) with extracts of glutinous rice straw and water hyacinth (Figure 1).

Figure 1. Setup of experiment; (a) concrete circle pit blocks, (b) experimental treatment, and (c) control

In this study, RD-Six extracts from glutinous rice straw were used due to its high phenolic contents (146.92±6.12 mg/L) and our preliminary study revealed that rice straw extract at a concentration of 3.0 g/L showed high effect and resulted in the decline of chlorophyll a up to 81%. Extracts were prepared by cutting dried glutinous rice straw into small pieces (approximately two cm in length). Then, pieces of glutinous rice straw (30 g) were placed in a 1-L beaker with reverse osmosis water and were incubated at room temperature for five days. After five days, the aqueous extract was filtered with GF/C paper to get extracts at a concentration of 30 g/L. 12 L of aqueous extracts from glutinous rice straw were then applied into each concrete circle pit block of experimental treatments (about 10% by volume of the concrete circle pit block). The water level in each concrete circle pit block was about 30 cm. Young water hyacinths were selected and used in experimental treatment (approximate length of leaves: 15 cm). Fresh water hyacinths were weighed on digital scales prior to the experiment (by holding the water hyacinth in the air for 15 min until without water droplets) (Lu et al., 2018). After that, water hyacinths were put into a series of experimental concrete circle pit blocks (five replications), covering approximately 80% of the water surface.

Water samples were collected from all concrete circle pit blocks for physical and chemical water quality analysis prior to experiment. After water collection, glutinous rice straw extract and water hyacinth were applied to the concrete circle pit blocks. Water samples were collected daily for eight days in February, 2020, between 09.00-10.00 am. Water samples were stored in 1.5 L plastic bottles and preserved under the method of APHA et al. (1998).

Water quality parameters were measured on site (temperature (°C), salinity (g/L), and conductivity (µs/cm) using the YSI 30 handheld meter). Other water quality parameters were measured in the laboratory, including pH (by pH meter (HACH, HQ411D)), turbidity (NTU, by turbidimeter (HACH, 2100AN)), dissolved oxygen (DO) (mg/L, by azide modification of the Winkler method), biological oxygen demand (BOD_5) (mg/L, by incubation), total suspended solid (SS) (mg/L, by dried at 103-105°C), total Kjeldahl nitrogen (TKN) (mg/L, by Semi-Micro-Kjeldahl (Velp,DKL)), ammonia nitrogen (NH_3-N) (mg/L, by titrimetric method (Velp, UDK139)), nitrate nitrogen, (NO_3-N) (mg/L, by Ultraviolet spectrophotometric method), nitrite (NO_2-N) (mg/L, by colorimetric-method (HACH, DR6000)), total phosphorus (TP), and soluble reactive phosphorus (SRP) (mg/L, by ascorbic acid-spectrophotometric method (HACH, DR6000), total nitrogen (TN as sum of TKN, NO_3-N, NO_2-N) (mg/L), and chlorophyll a (µg/L, by acetone extraction method).

The effects of glutinous rice straw extract on the density and composition of phytoplankton species was determined by filtering 10 L of water through a plankton net (mesh size: 20 µm). Samples of phytoplankton were preserved by adding 70% ethyl alcohol. Phytoplankton samples were then classified up to species level and counted in a Sedgewick Rafter counting chamber under a compound microscope. The growth rate of water hyacinths was also determined by comparing the weight at the beginning and the end of the experiment.

2.3 Statistical analysis

Mean±standard deviation is presented throughout this article, except algal composition that is presented as percentage. Independent samples T-test was used to examine the differences of physical, chemical and biological parameters between control and experimental treatment. Statistical significance was accepted at a level of $p<0.05$. Statistical analysis was performed by statistical analysis SPSS software version 23 (trial).

3. RESULTS

3.1 Water quality

The result of water quality is presented in Table 1. Overall, nutrient concentrations in both control and experimental treatment were exceptionally high. Trophic state can be classified as hypereutrophic condition as indicated by high TN and TP values. When comparing water quality values between control and experimental treatment, the results showed that temperature, pH, conductivity, salinity, NO_3-N, and SRP were not significantly different ($p>0.05$). In contrast, turbidity, SS, DO, BOD, TKN, NH_3-N, NO_2-N, TN, TP, and chlorophyll a were significantly different ($p<0.05$) between control and experimental treatment. At the end of the experiment, most values of nutrients and chlorophyll a were relatively lower in the experimental treatment than in control.

Table 1. Comparison of water quality parameters between control and experimental treatment at the end of experiment.

Parameters	Control	Experimental treatment
Temperature (°C)	$29±0^a$	$29±0^a$
pH	$7.3±0.1^a$	$7.3±0.0^a$
Conductivity (µs/cm)	$1707±15^a$	$1715±8^a$
Salinity (g/L)	$0.9±0.1^a$	$0.9±0.0^a$
Turbidity (NTU)	$28±1^a$	$15±2^b$
SS (mg/L)	$38±4^a$	$15±2^b$
DO (mg/L)	$6.0±0.3^a$	$3.4±0.2^b$
BOD (mg/L)	$12±0^a$	$7±1^b$
TKN (mg/L)	$5.04±0.00^a$	$3.02±0.31^b$
NH_3-N (mg/L)	$1.12±0.00^a$	$0.73±0.15^b$
NO_3-N (mg/L)	$1.10±0.07^a$	$1.03±0.01^a$
NO_2-N (mg/L)	$0.06±0.02^a$	$0.02±0.01^b$
TN (mg/L)	$6.20±0.07^a$	$4.07±0.29^b$
TP (mg/L)	$0.30±0.02^a$	$0.16±0.01^b$
SRP (mg/L)	$0.02±0.01^a$	$0.01±0.00^a$
Chlorophyll a (µg/L)	$306.93±48.71^a$	$91.56±10.18^b$

Remark: Values are mean±SD, different superscript letters in the same row indicates values with significant difference ($p<0.05$).

Table 2 shows removal efficiency of water quality parameters between control and experimental treatment. Overall, removal efficiency of water quality in experimental treatment was much higher than in control. In particular, removal efficiency of chlorophyll a in experimental treatment was up to 81.5% compared with that in control, of which the efficiency was only approximately 27.4%.

Table 2. Removal efficiency between control and experimental treatment

Parameters	Control			Experimental treatment		
	Day 0	Day 7	Removal rate (%)	Day 0	Day 7	Removal rate (%)
Turbidity	66±8.00	28±1.00	57.6	64±4.00	15±2.00	76.6
SS	60±6.00	38±4.00	36.7	55±5.00	15±2.00	72.7
BOD	19±3.00	12±0.00	39.2	19±1.00	7±1.00	63.5
TKN	5.82±0.31	5.04±0.00	13.4	5.15±0.47	3.02±0.31	41.4
NH_3-N	1.01±0.15	1.12±0.00	-10.9	0.95±0.15	0.73±0.15	23.2
NO_3-N	1.14±0.03	1.10±0.07	3.5	1.15±0.05	1.03±0.01	10.4
NO_2-N	0.03±0.01	0.06±0.02	-100	0.03±0.01	0.02±0.01	33.3
TN	6.99±0.33	6.20±0.07	11.3	6.33±0.48	4.07±0.29	35.7
TP	0.32±0.07	0.30±0.02	6.3	0.31±0.07	0.16±0.01	48.4
SRP	0.02±0.01	0.02±0.01	0.0	0.02±0.01	0.01±0.00	50.0
Chlorophyll a	422.82±63.28	306.93±48.71	27.4	494.96±69.29	91.56±10.18	81.5

Figure 2 shows the tendency of water quality values between the control and experimental treatments. Values of turbidity and SS showed a tendency to decrease from the beginning toward the end of the experiment. In the experimental treatment, BOD increased after application of glutinous rice straw extract and then started to decline toward the end of the experiment. In contrast, DO dropped after the addition of glutinous rice straw extract and subsequently increased and remained constant until the end of experiment.

Figure 3 shows the tendency of nutrient concentrations between the control and experimental treatments. In the experimental treatment, nitrogen contents (ammonia, nitrate, and TN) appeared to increase right after application of glutinous rice straw extract. Then, concentrations of nitrogen contents started to decline toward the end of experiment. TP and SRP values in experimental treatment were relatively lower than in control and gradually declined until the end of experiment.

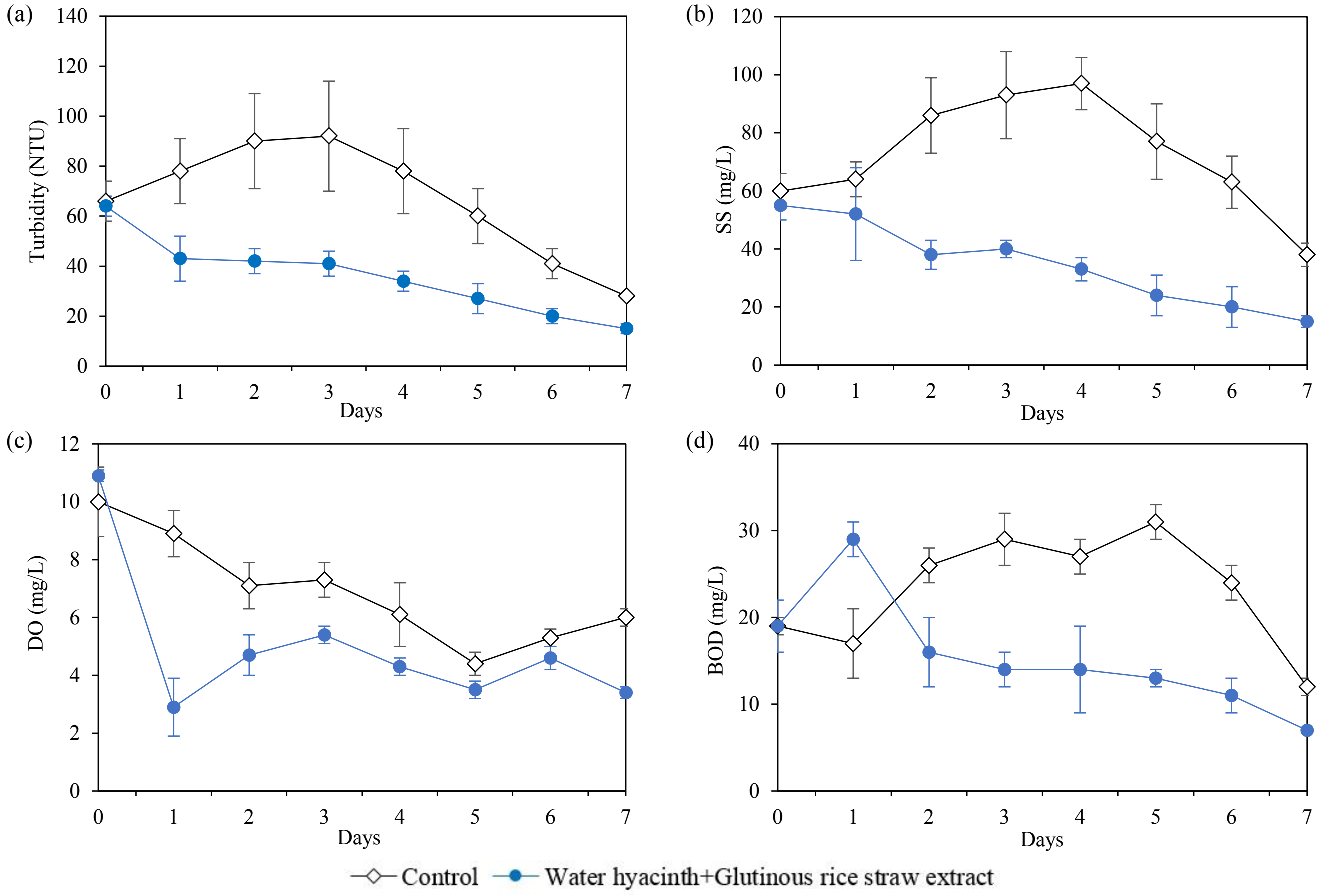

Figure 2. Physical-chemical parameters variation between control and experimental treatments (a) turbidity, (b) SS (c) DO, and (d) BOD

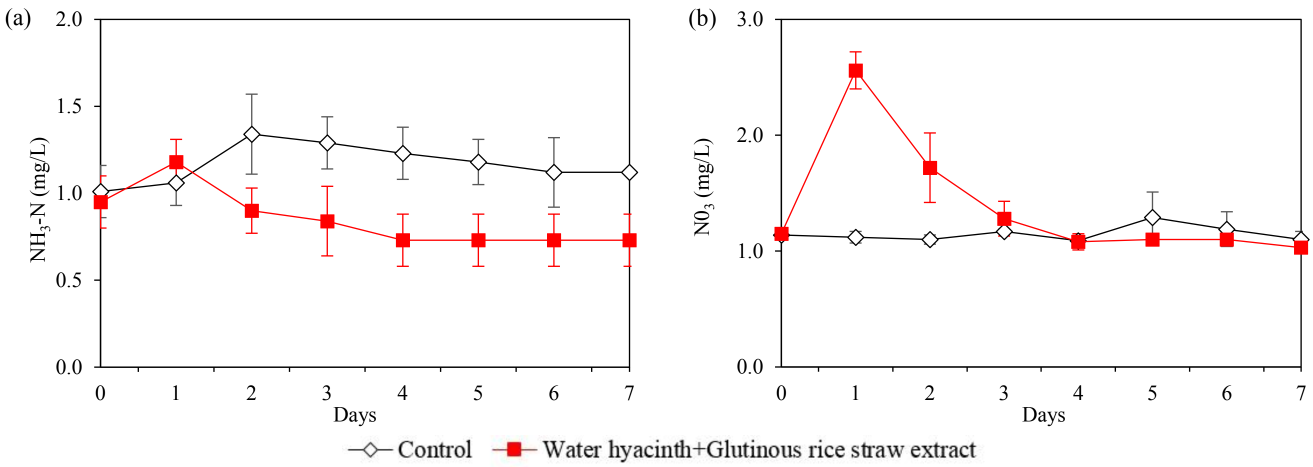

Figure 3. Comparison of nutrient concentration between control and experimental treatment; (a) NH_3-N, (b) NO_3, (c) TN, (d) TP, and (e) SRP.

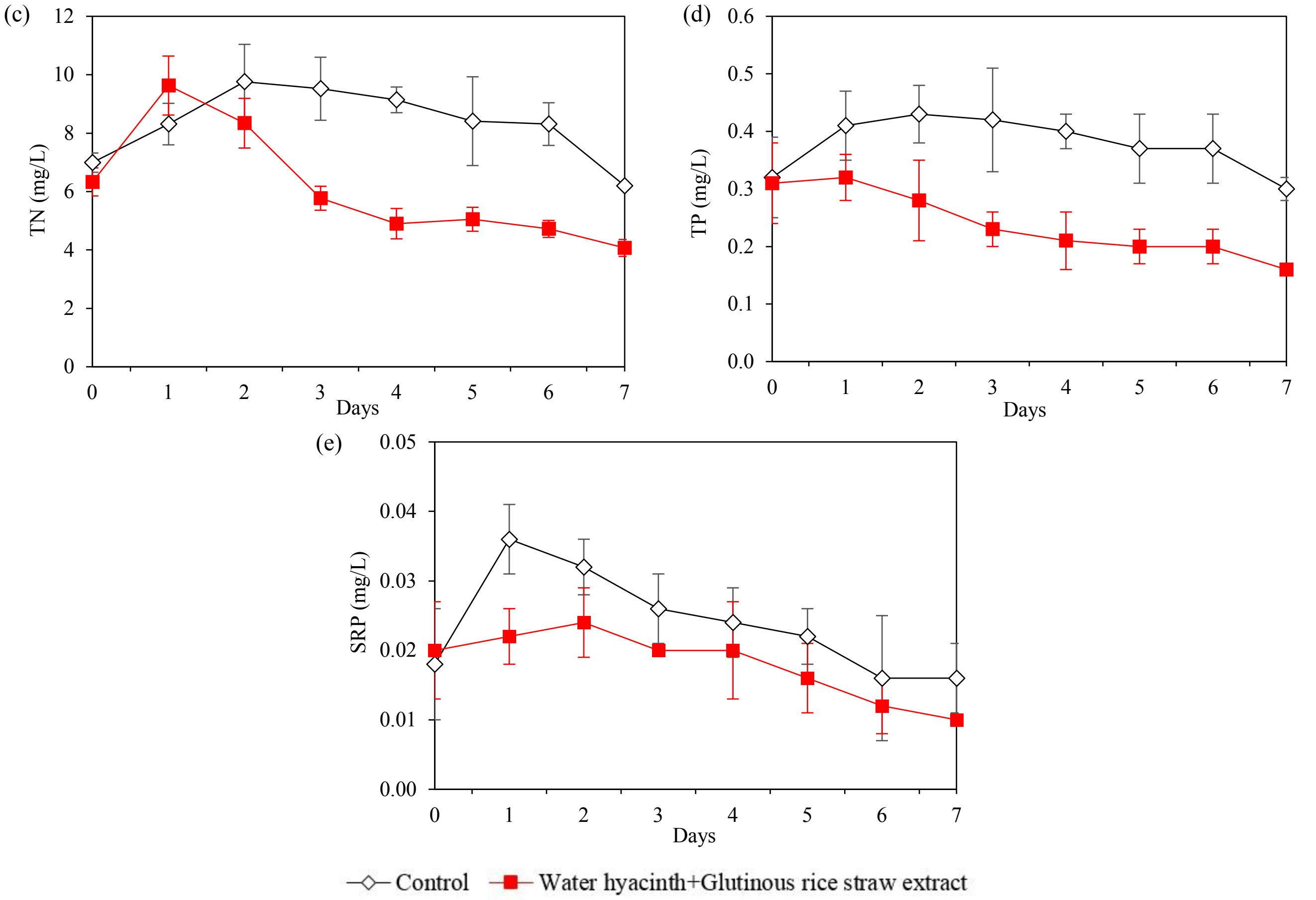

Figure 3. Comparison of nutrient concentration between control and experimental treatment; (a) NH_3-N, (b) NO_3, (c) TN, (d) TP, and (e) SRP (cont.).

3.2 Effect of glutinous rice straw extract on phytoplankton

This study investigated the effect of glutinous rice straw extract on chlorophyll a. It was revealed that, in the control, chlorophyll a concentration increased in the middle of the study and was relatively higher than in experimental treatment (Figure 4). In contrast, chlorophyll a concentration in the experimental treatment started to decrease from day one until the end of the experiment.

The result of phytoplankton composition showed that there were three divisions of phytoplankton belonging to; (1) Cyanophyta, (2) Chlorophyta, and (3) Chromophyta. In total, there were 34 species of phytoplankton (Table 3). *Cylindrospermopsis raciborskii* was the dominant species in both control and experimental treatment. At the beginning of the experiment, Cyanophyta was the most abundant group (69.8%) in the control, followed by Chromophyta (23.2%) and Chlorophyta (7.0%) respectively. In the experimental treatment, Cyanophyta was also the main phytoplankton group (88.8%), followed by Chromophyta (8.0%) and Chlorophyta (3.2%).

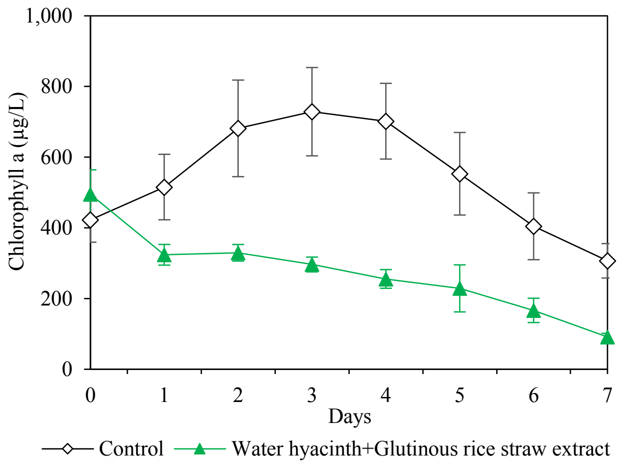

Figure 4. Tendency of chlorophyll a content between control and experimental treatment.

At the end of the experiment, the most common phytoplankton in the control was still Cyanophyta (83.3%), followed by Chromophyta (12.3%) and Chlorophyta (4.3%), respectively. In contrast, the abundance of phytoplankton groups in the experimental treatment shifted to Cyanophyta (45.7%), Chromophyta (45.2%), and Chlorophyta (9.0%), respectively.

Table 3. Impact of glutinous rice straw extract and water hyacinth on algal composition in treatments.

	Control		Treatment	
	Before	After	Before	After
Cyanophyta				
Chroococcus sp.	11,134	-	7,524	-
Cylindrospermopsis raciborskii	2,672,552	4,099,762	7,302,232	335,502
Merismopedia minima	102,988	147,552	121,076	14,341
Oscillatoria sp. (1)	67,412	24,680	30,164	7,676
Oscillatoria sp. (2)	16,720	-	-	-
Spirulina sp.	6,853	19,141	2,886	-
%	69.8	83.3	88.8	45.7
Chlorophyta				
Closteriopsis sp.	-	19,051	11,742	3,952
Coelastrum microsporum	7,258	7,271	3,762	5,181
Crucigeniella sp.	3,382	9,912	9,595	3,762
Monoraphidium contortum	72,816	75,703	32,179	14,051
Monoraphidium sp.	22,255	14,089	9,746	-
Pediastrum duplex	3,838	-	3,762	3,838
Pediastrum angulosum	-	-	3,914	-
Scenedesmus armatus	37,962	23,780	30,874	11,993
Scenedesmus acuminatus	34,899	28,733	9,428	7,879
Scenedesmus sp.	6,688	-	-	-
Selenastrum quadricanda	48,317	11,102	96,525	3,965
Tetraedron trigonum	-	7,448	-	3,800
Tetraedron gracile	3,838	-	3,762	-
Euglena sp.	24,746	22,630	40,050	3,927
Euglena acus	-	3,601	-	4,180
Phacus acuminatus	-	-	15,656	-
Phacus helikoides	-	-	-	4,180
Phacus ranula	3,838	-	-	-
Lepocinclis sp.	20,064	-	-	-
%	7.0	4.3	3.2	9.0
Chromophyta				
Aulacoseira granulata	-	-	1,955	-
Cocconesis sp.	5,035	7,448	-	4,180
Cyclotella sp.	815,108	424,149	565,007	283,845
Fragilaria sp.	3,382	-	-	-
Navicula sp.	10,526	10,602	7,091	10,298
Nitzschia sp.	115,657	171,920	93,518	49,157
Nitzschia closterium	5,976	14,610	2,083	6,061
Surirella sp.	-	-	186	-
Peridinium sp. (1)	-	5,504	3,724	-
%	23.2	12.3	8.0	45.2

3.3 Effect of glutinous rice straw extract on water hyacinth

During the experiment, water hyacinths grew well and produced new sprouts. At the end of the experiment, leaf size increased, and stems and roots were also longer and larger. Figure 5 shows fresh weight of water hyacinths before (552.54±1.93 g) and after (1,362.38±77.38 g) the experiment. The growth rate of water hyacinth was at 59.4%.

4. DISCUSSION

4.1 Water quality

The results of water quality analysis showed that the values of most parameters in the experimental treatment were lower than in the control, with significant differences ($p<0.05$). Water quality from the lake indicated that the lake was hypereutrophic with algal blooms. Treatment of eutrophic water by glutinous rice straw extract and water hyacinth showed remarkable results. Turbidity and suspended solids content decreased in the experimental treatment. This may be linked to filtration done by the roots of water hyacinths. Some of the suspended solid content may be adsorbed by the surface of the roots, and then slowly settled down (Lu et al., 2018) at the bottom of the concrete circle pit blocks. Leaves and stems of the water hyacinth may also help reduce the wind speed, thereby reducing the diffusion of sediment in the water. The decrease of dissolved oxygen in the experimental treatment is possibly due to the decrease of phytoplankton. Water hyacinth obstructed sunlight, causing the decrease of oxygen produced by the photosynthesis process of phytoplankton (Di Luca et al., 2019).

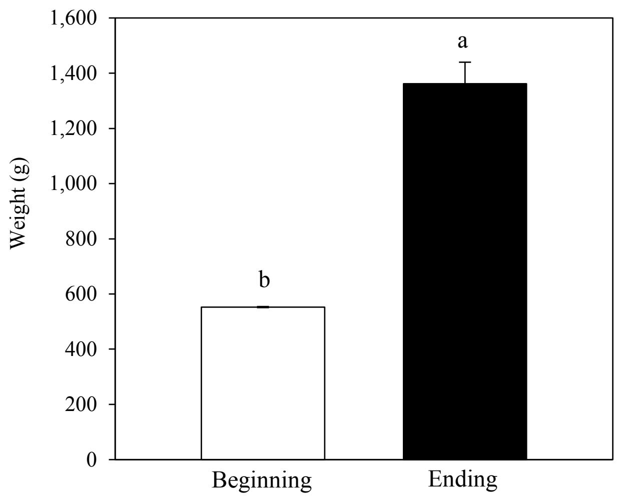

Figure 5. Comparison of wet weight (g) between the beginning and the end of experiment. Different letters on bars indicate significant difference of water hyacinth weight ($p<0.05$).

Nitrogen contents at the beginning of the experimental treatment were high after addition of glutinous rice straw extract (Duan et al., 2017; Eladel et al., 2019). Toward the end of experiment, nitrogen slightly declined, which may be explained by microbes living around the roots of the water hyacinth that transformed organic into inorganic substances and absorbed them through the root cells (Gopal, 1987). BOD value in the control remained higher than in the experimental treatment. This might attribute to the fact that in the absence of plant rhizomes, the rate of impurity elimination by microbes became slower (Valipour et al., 2015). In contrast, BOD reduction in the experimental treatment was faster and could result from decomposition by microbes associated with water hyacinth. Water quality results showed that water hyacinth was effective in reducing the amount of nitrogen and phosphorus in the water through plant absorption (Lu et al., 2018).

4.2 Effect of glutinous rice straw extract on phytoplankton

The use of glutinous rice straw extract, together with water hyacinth, effectively reduced the amount of phytoplankton. The experimental treatment, with the addition of glutinous rice straw extract, had lower levels of chlorophyll a than in the control with significant differences statistically ($p<0.05$). Glutinous rice straw extract inhibited the growth of phytoplankton as its phenolic compounds caused the abnormalities against photosynthesis and cell processes of the algae (Hua et al., 2018). This study is consistent with the study in a fish pond (*Oreochromis niloticus*) as it showed that content of chlorophyll a in the pond containing extracts from rice straws were 50% less than those without addition of rice straw extract (Eladel et al., 2019). Shading from water hyacinth may have also limited the growth rates of phytoplankton in experimental treatment.

In addition, glutinous rice straw extract affected the composition of phytoplankton. Phytoplankton composition shifted from Cyanobacteria to Chromophyta in the experimental treatment. It is believed that phenolic compounds released from glutinous rice straw selectively influenced Cyanobacteria more than other phytoplankton groups (Ridge and Pillinger, 1996). The results of this study corresponded with Islami and Filizadeh (2011) who used rice straw to control algal species in water. Rice straw extract is effective in controlling algae, especially cyanobacterial phytoplankton (*Microcystis aeruginosa, Scenedesmus subspicatus,* and *Anabaena flos-aquae*), without inhibiting diatom species (*Hydrodictyon reticulatum* and *Oscillatoria tenuis*) (Islami and Filizadeh, 2011). Further research is still needed to understand mechanisms of glutinous rice straw extracts involved in cyanobacteria growth inhibition. Photosynthetic activities, maximum quantum yield (Fv/Fm) and growth inhibition efficiency of cyanobacteria should be further compared and investigated.

4.3 Effect of glutinous rice straw extract on water hyacinth

This study also evaluated the effect of glutinous rice straw extract on growth rate of water hyacinths in the experimental treatment. The results showed that water hyacinth grew well in the concrete circle pit blocks with glutinous rice straw extract. The weight of water hyacinth increased by 59.3% compared to its weight prior to the experiment. Phenolic compounds released from glutinous rice straw seem to have no effect on water hyacinth but have negative effects only on phytoplankton (Eladel et al., 2019). The growth of water hyacinth in the experiment may also be linked to various environmental factors such as high nutrient contents in the water, optimum water temperature (27-30°C), pH (7.2-7.5) and salinity (0.8-0.9 g/L). These environmental factors are consistent with the previous studies showing that water hyacinth is resistant to a wide range of environment. Water hyacinth can grow in water with temperature between 10-40°C and pH 6-8 (Rezania et al., 2015).

5. CONCLUSION

Glutinous rice straw extracts and water hyacinth proved an effective control method for nuisance algal blooms and high nutrient contents in a fresh-water environment. Glutinous rice straw extract application reduced phytoplankton and shifted species composition, especially Cyanophyta. Phenolic compounds released directly from glutinous rice straw extract, appeared to be the main inhibitory effect on phytoplankton. However, the extracts from glutinous rice straw did not affect the growth of water hyacinth. Water hyacinth plays a synergistic role to compete for nutrients with phytoplankton. Water hyacinth, along with other microorganisms associated to it, significantly reduced the level of organic and inorganic substances. Therefore, this combined method, which is simple, uncomplicated and environmentally friendly, should be promoted.

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