WALAILAK JOURNAL
http://wjst.wu.ac.th Agriculture Technology and Biological Sciences

Study of the Effect of Gypsum and Rice Straw Extract on the Control of Earthy-Musty Odor in Tilapia Culture Pond

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Received: 8 July 2015, Revised: 2 November 2015, Accepted: 20 December 2015

Abstract

The effect of gypsum and rice straw extract to reduce the levels of earthy-musty odor in tilapia earthen ponds was determined. Three treatments with 3 replicates in a completely randomized design were performed as follows: T1 - control; T2 - gypsum at 200 ppm, and T3 - rice straw tannin extract at 1 ppm. The results show that rice straw tannin extract treatment in tilapia ponds was the most effective method in reducing off-flavor in pond water. The use of 200 ppm gypsum in reducing off-flavor in ponds was also more effective than the control. Both rice straw extract and gypsum-treated ponds had increased fish biomass and %FCE. These methods have potential as alternative methods for the reduction of off-flavor, as well as the control of phytoplankton, turbidity, and TSS in tilapia ponds.

Keywords: Earthy-musty odor, geosmin, MIB, gypsum, rice straw extract, Nile tilapia

Introduction

Increasing freshwater fish consumption due to increased global population results in constant, growing demand in both domestic and export markets. Nile tilapia, *Oreochromis niloticus*, is a popular, fast growing freshwater fish that produce good fillets containing easily digestible protein and a host of nutrients. However, problems concerning earthy-musty odor and taste in fish hound the tilapia aquaculture industries, which affect marketability. Geosmin and 2-methylisoborneol (MIB) are the 2 main causative compounds of earthy-musty odor and taste (off-flavor) in freshwater-farmed fish. They are lipophilic terpenoid compounds, produced as secondary metabolites by certain species of cyanobacteria (planktonic and benthic), particularly filamentous forms such as *Anabaena circinalis* Kütz, *Lyngbya cryptovaginata* var. major BN Prasad, RK Mehrotra and Y Singh, *Oscillatoria* sp., *Phormidium* sp., and *Pseudanabaena* sp. in water [1] and actinomycetes that can modify water surface chemistry, with major socio-economic implications [2-5]. Cyanobacteria produce either MIB, geosmin, or both, during growth, store (intracellular form) these odorants within the cell, and release them (extracellular) in the water during cell lysis, depending on the growth phase, and also based on environmental factors [5].

In this study, gypsum was used, because it is a cheap and readily available source of calcium and sulfur that has been widely used in agriculture for the recovery of alkali soils and as a source of calcium and sulfate in fertilizer [6-8]. The calcium phosphate precipitation is a possible mechanism of gypsum in phosphate and phytoplankton reduction in water, because gypsum could be used to increase the concentration of calcium ions (Ca^{2+}) when added into the water. Boyd [9] suggested that gypsum could be used as a pond treatment in aquaculture for: (1) flocculation of clay particles, (2) increasing the concentration of calcium and total hardness, (3) precipitation of phosphate, and (4) reducing pH.

In aquaculture, barley and rice straw are alternative and sustainable materials derived from agricultural waste that can be used to economically control cyanobacteria. Several researches, for example, showed barley straw extract effectively inhibited the growth of several planktonic and filamentous cyanobacteria in the laboratory and reservoirs [10-12]. Barley straw (40 and 80 g m^{-2}) was capable of inhibiting the growth of *Microcystis*, *Anabaena*, and *Aphanizomenon* in ponds [13]. The utilization of rice straw offers another cheap and environmentally acceptable way of controlling cyanobacteria. Aside from barley straw, rice straw has received considerable attention as an algaecide, based on research done in many countries throughout the world, and has proved to have very successful side effects. Rotten rice straw successfully inhibited filamentous cyanobacteria in Suez Canal waters, due to the synergistic effects of various humic substances in the rice straw [14]. Similarly, several studies [12,15] reported that the growth of *Microcystis aeruginosa* was inhibited by rice straw extract, which was due to the synergistic effects of various phenolic compounds in the rice straw. Shahabuddin *et al*. [16] conducted an experiment to assess the effect of rice straw mat on the water quality parameters, plankton density, pond water bacteria, and mitigation of clay turbidity in fertilized fish ponds. They reported that rice straw mat effectively mitigates clay turbidity, and 25 kg per 200 m^2 pond can effectively reduce the population of phytoplankton and zooplankton.

However, little research has been conducted to investigate techniques to mitigate earthy-musty odor in fish ponds. Therefore, this study aimed to determine the effectiveness of gypsum and rice straw extract in removing earthy-musty odor in fish ponds.

Materials and methods

Experimental protocol

The study was carried out during a 4 month period (April to August 2014) at the Faculty of Fisheries, Technology and Aquatic Resources, Maejo University, Sansai, Chiang Mai, Thailand (18° 89' 81" N, 99° 01' 84" E). Nine experimental ponds, each with a 100 m^2 area and 0.8 m depth, were used. Three treatments with 3 replicates in a completely randomized design were performed as follows: T1 - control; T2 - gypsum at 200 ppm and T3 - rice straw tannin extract at 1 ppm. Tannin from rice straw extract was prepared by immersing rice straw in water (1 g in 1 L water) for 15 days to get an approximate tannin concentration of 5.4 ppm. The optimal concentrations used in this study had previously been tested in the laboratory.

Nile tilapias with an average initial weight of 115±2.5 g (55 days old) were transferred to the experimental ponds at a stocking density of 2 - 3 fish m^{-2} (**Table 1**). Samples for physico-chemical (water) and off-flavor (water and sediment) analyses were collected every 15 days. Data on fish off-flavor and growth were recorded every 30 days.

Table 1 Description of experimental treatments (T1 - control; T2 - 200 ppm gypsum, and T3 - 1 ppm rice straw tannin extract).

	Treatment		
	T1	**T2**	**T3**
Type	-	powder	aqueous extract
Method of application	-	broadcast	broadcast
The application frequency		Every 15 days	Every 15 days
Pond area (m^2)	100	100	100
Pond depth (m)	0.8	0.8	0.8
No. of Nile tilapia stocked	250	250	250
Feeding rate	Twice a day (3 - 5 % body weight per day)		

Sampling

All samples for physico-chemical (1.5 L) and earthy-musty odor analyses (120 mL) were collected directly from the water surface (20 - 40 cm depth) using plastic containers. Sediment samples (about 200 g) were collected using a hard plastic dipper attached to a PVC pole, by scraping the surface layer of the pond floor, and were placed in wide-mouthed 200 mL plastic bottles. Three pieces of Nile tilapia were collected from each pond using a cast net. Phytoplankton was sampled by filtering 5 L of composite pond water with plankton net of 10 µm mesh. Samples were concentrated in a 30 mL plastic bottle and preserved with 3 drops of Lugol's solution.

Extraction and analysis of geosmin and MIB

Geosmin and MIB were extracted from water (dissolved and particulate), sediment, and fish samples using solid phase microextraction (SPME). A 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (SUPELCO, USA) was extended into the headspace of the samples {10 mL unfiltered and filtered pond water; 5 g sediment with 10 mL deionized water; and 10 mL aqueous fish extract (5 g minced fish meat pre-extracted by microwave distillation (600 watt, 6 min) following Lloyd and Grimm [17] with modification}, and placed in a sealed 20 mL straight-sided vial containing sodium chloride (1.9 g) and a polytetrafluoroethylene (PTFE)-coated stirring bar. The sample was heated to 65 °C on a hotplate-stirrer and exposed to the SPME fiber for a 30 min adsorption period while undergoing vigorous agitation. After 30 min, the fiber was withdrawn from the sample and quantified with gas chromatography-mass spectrometry (GC/MS), according to Gutierrez *et al*. [18]. The fiber was desorbed in the GC/MS under a splitless mode at 230 °C for 5 min in the injection port of an HP 6890 N Network gas chromatograph equipped with a 5973 mass selective detector (Agilent Technologies, USA) operated in scanning mode. A Durabond HP-5 capillary column of 30 m length, 0.32 mm i.d., and 0.25 µm film thicknesses was used with helium carrier gas operated at a rate of 2.5 mL min^{-1}. The oven temperature was programmed at 60 °C for 1 min., then increased to 220 °C with a rate of 15 °C $min.^{-1}$ and maintained at 220 °C for 8 min. All analyses were performed as single measurements. Standard geosmin and MIB from Sigma were used for calibration [18]. The limit of detection was 0.01 µg L^{-1} or µg kg^{-1} for both geosmin and MIB.

Water quality and nutrient analysis

Physico-chemical parameters (pH temperature, dissolved oxygen (DO), and turbidity) were measured *in situ* using a multimeter (TOA-DKK WQC-22A Model, Japan). Standard methods [19] were used for the analysis of total ammonia-nitrogen (TAN), nitrate-nitrogen, nitrite-nitrogen, orthophosphate-phosphorus, and total suspended solids (TSS) in the laboratory. Alkalinity and total hardness were analyzed by the method as described by Boyd and Tucker [20].

Tannin analysis

Total tannin concentration in rice straw extract samples was determined using the modified Folin Ciocalteu reagent (FC reagent) assay [21]. Five milliliters of diluted FC reagent (10× dilution) was added to a test tube containing 1 mL filtered rice straw extract sample or 1 mL distilled water (blank). Four milliliters of 7.5 % sodium carbonate solution was added to the test mixture and was incubated for 2 h in subdued light at room temperature. The absorbance was read at 740 nm using a spectrophotometer. Three replicates of each extract were analyzed. Total tannin concentrations were expressed as tannic acid equivalents.

Hydro-biological analysis

Chlorophyll-*a* in water samples was extracted with 10 mL hot methanol (60 °C in water bath) and quantified with a spectrophotometer (Hach D9000, USA) [19]. Chlorophyll-*a* concentration in the extract was calculated as described by Wintermans and de Mots [22] and Saijo [23]. Phytoplankton identification was carried out using a picture database and related texts, such as Peerapornpisal [24]. Phytoplankton genera/species and numbers were determined using a light microscope (Olympus BH2, Japan).

Isolation and screening of earthy-musty odor-producing streptomycetes in sediments

Ten grams of sediment samples from tilapia ponds were mixed with 90 mL of 0.85 % NaCl solution. The suspension was shaken at 150 rpm for 30 min at room temperature and serially diluted tenfold to 10^{-5}. Soil suspensions (100 μL) from 10^{-3}, 10^{-4}, and 10^{-5} dilutions were spread onto the surface of starch casein agar (SCA) plates, and incubated at 30 °C for 7 - 14 days. Actinomycetes were assigned into streptomycetes (filamentous and fungus-like sporulation structures) and non-streptomycetes groups. Colonies from both groups were counted and recorded as CFU g^{-1}. Earthy-musty odor-producing streptomycetes were purified by re-streaking on SCA, sealed with paraffin film, and incubated at 30 °C for 7 - 14 days or until complete sporulation was observed [25].

Growth performances of Nile tilapia

The growth parameters were calculated following the method described by Bagenal [26] as follows:

Weight gain = Final weight-initial weight

$$\text{Average Daily Gain (ADG)} = \frac{\text{Final weight-Initial weight}}{\text{Days}}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Total feed (g)}}{\text{Weight gain (g)}}$$

$$\text{Feed Conversion Efficiency (FCE)} = \frac{\text{Weight gain (g)}}{\text{Total feed (g)}} \times 100$$

$$\text{Survival Rate (\%)} = \frac{\text{No. of animals survived (fish)}}{\text{No. of animals leased (fish)}} \times 100$$

Data analysis

Analysis of variance (ANOVA) was used to test for difference between means of observed parameters and each treatment. Tukey's multiple range tests, at 95 % confidence level, were used for treatment comparison. Relationships between water quality variables, phytoplankton, and earthy-musty compounds were analyzed using Pearson correlation analysis. Significant correlation was assumed when $p < 0.05$ in either positive or negative correlations.

Results and discussion

The results of water quality analysis in 9 experimental ponds are shown in **Table 2**. No significant differences ($p > 0.05$) in pH, temperature, DO, total ammonia, nitrite, nitrate, and orthophosphate were found, while alkalinity, turbidity, total hardness, and TSS showed significant differences ($p < 0.05$) among the treatments. The gypsum–treated ponds had lower alkalinity, turbidity, and TSS, but had higher total hardness than the other treatments. Boyd [9] reported that gypsum could be used as a pond treatment in aquaculture for the flocculation of clay particles, and that it also increases the concentration of calcium and total hardness. In this study, it was suggested that the nitrogen nutrients (total ammonia, nitrite, and nitrate) were rather low when compared with other treatments.

Table 2 Physico-chemical and biological characteristics of fish ponds in the study (mean±SE)*.

Parameter	Treatment		
	Control	**200 ppm gypsum**	**1 ppm rice straw tannin extract**
pH	7.07±0.59	6.79±0.57	7.10±0.61
Temperature (°C)	30.6±0.74	30.7±0.73	30.7±0.73
DO (mgL^{-1})	4.30±0.88	4.00±0.91	4.61±0.99
Alkalinity (mgL^{-1})	74.84±15.33^b	51.07±15.85^a	70.96±18.87^b
Turbidity (NTU)	122.5±40.6^b	52.5±29.5^a	157.4±73.0^b
Total hardness (mgL^{-1} as $CaCO_3$)	64.6±16.20^a	251.0±69.48^b	70.1±18.43^a
Total ammonia-N (mgL^{-1})	0.553±0.30	0.441±0.15	0.343±0.10
Nitrite-N (mgL^{-1})	0.012±0.011	0.003±0.001	0.008±0.003
Nitrate-N (mgL^{-1})	0.016±0.008	0.016±0.009	0.015±0.008
Orthophosphate-P (mgL^{-1})	0.048±0.02	0.035±0.01	0.059±0.02
TSS (mgL^{-1})	124.5±54.21^b	41.7±16.14^a	125.2±51.29^b

*Means followed by different letters in the same row were significantly different, according to Tukey's multiple range tests at $p < 0.05$.

Table 3 Growth performances of Nile tilapia; initial weight, final weight, weight gain, average daily gain (ADG), feed conversion ratio (FCR), feed conversion efficiency (FCE), survival rate (%), and biomass of fish (mean±SE)*.

Growth Performance	Treatment		
	Control	**200 ppm gypsum**	**1 ppm rice straw tannin extract**
Initial weight (g $fish^{-1}$)	114.7 ± 2.6^a	115.0 ± 2.3^a	115.3 ± 2.6^a
Final weight (g $fish^{-1}$)	374.0 ± 11.7^a	413.3 ± 20.2^a	385.3 ± 17.5^a
Weight gain (g $fish^{-1}$)	259.3 ± 9.1^a	298.3 ± 18.7^a	270.0 ± 15.0^a
ADG (g day^{-1})	2.16 ± 0.08^a	2.49 ± 0.16^a	2.25 ± 0.13^a
FCR	1.53 ± 0.13^b	1.16 ± 0.06^a	1.16 ± 0.04^a
FCE (%)	66.37 ± 5.79^a	87.02 ± 4.32^b	86.58 ± 2.67^b
Survival rate (%)	71.20 ± 6.80^a	84.53 ± 5.41^a	90.40 ± 6.11^a
Biomass of fish (kg $pond^{-1}$)	66.37 ± 5.79^a	87.02 ± 4.32^b	86.58 ± 2.67^b

*Means followed by different letters in the same row were significantly different, according to Tukey's multiple range tests at $p < 0.05$.

The growth performances of Nile tilapia are shown in **Table 3**. The highest survival rate (90 %) of tilapia was recorded in rice straw extract-treated ponds, while the control ponds gave the lowest (71 %). No significant difference ($p > 0.05$) was observed in growth parameters (final weight, weight gain, ADG, FCR, FCE, and biomass) of fish in both gypsum and rice straw extract-treated ponds, although it was higher in the gypsum-treated (200 ppm) group, which might be due to the binding of cations of calcium from gypsum on anions of phytoplankton to form complex macro-aggregates (biofloc) serving as additional feed sources for tilapia in these ponds. The consumption of biofloc by shrimp or fish has demonstrated numerous benefits, such as improvement of growth rate [27] and decrease of FCR and associated cost in feed [28]. In tilapia, Hari *et al*. [29] estimated that feed utilization is higher in biofloc, at a rate of 20 % less than in a conventional water-exchange system.

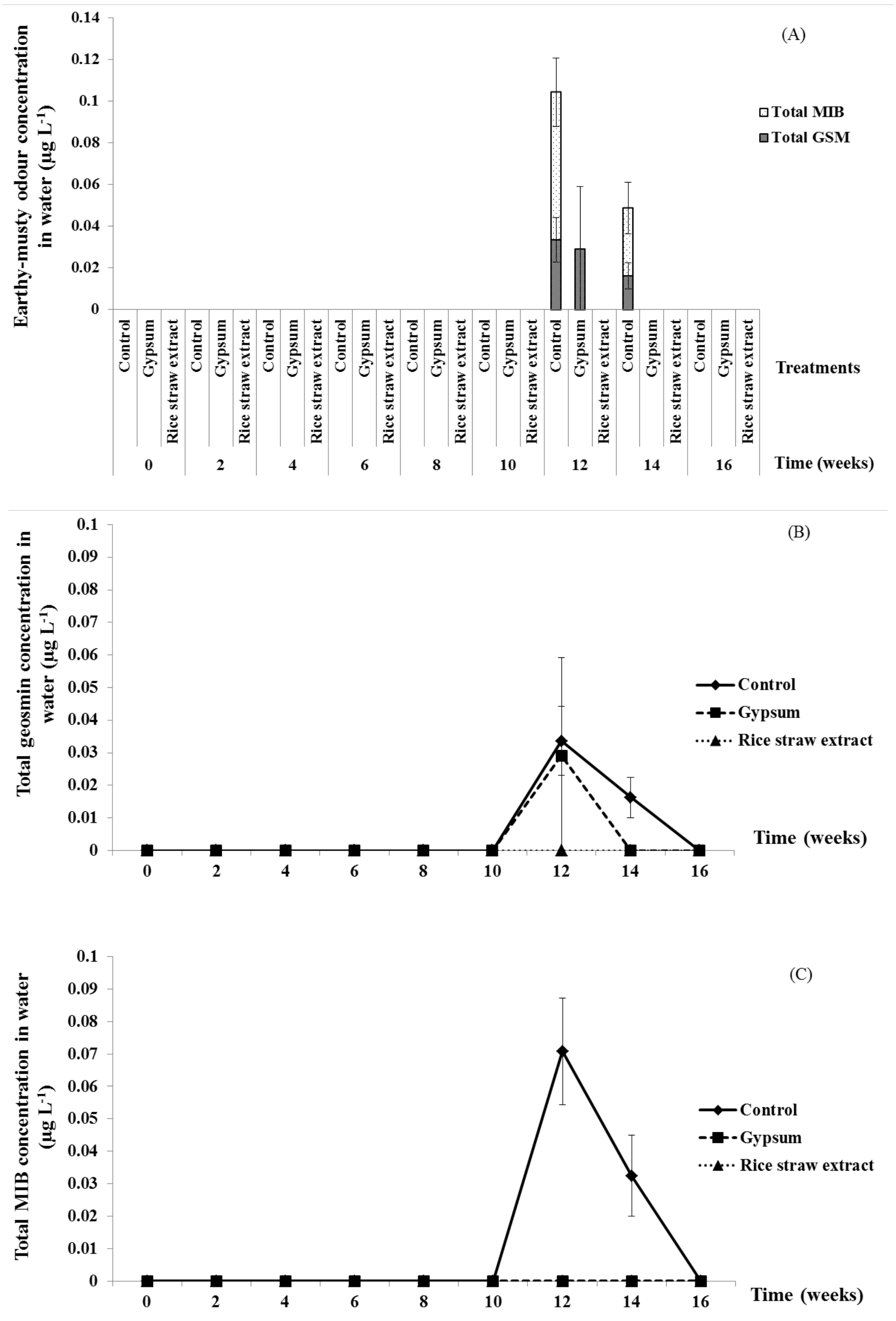


Figure 1 Concentrations of earthy-musty odor in pond water; (A) total geosmin and MIB, (B) total geosmin and (C) total MIB. Error bars represent the standard error of 3 replicates (control; 200 ppm gypsum, and 1 ppm rice straw tannin extract).

Earthy-musty odor in fish pond

Total geosmin and MIB concentrations in pond water are shown in **Figure 1**. No water off-flavor taint was detected in all treatments, except in the control and gypsum-treated ponds in week 12 and 14. Maximum concentration (0.104 μg L^{-1}) occurred in the control ponds in the 12^{th} week (**Figure 1A**) and was significantly different than in gypsum-treated ponds. Geosmin was detected in both the control and gypsum-treated ponds (**Figure 1B**) whilst MIB was detected only in the control ponds (**Figure 1C**). No traces of off-flavor compounds were found in water in all ponds treated with rice straw extract throughout the culture period.

Both cell-bound and dissolved geosmin and MIB concentrations were detected in pond water samples and observed to be mostly present intracellularly (cell-bound) or in particulate fraction (**Figures 2** and **3**). This could also be the reason why no off-flavor was detected in all Nile tilapia sampled during the period. Less extracellular geosmin dissolved in pond water, and more intracellular form stored in the cells, could mean uptake of geosmin through the gills is less. Geosmin and MIB have low octanol/water partition coefficients (Kow) and, thus, uptake is overwhelmingly through the gills [30] and not by ingestion of cyanobacterial cells. Hence, the magnitude of fish contamination may depend on the concentration excreted into the water by cyanobacteria and actinomycetes [18].

Both intracellular and extracellular geosmin were detected in the gypsum treatments after 12 weeks (only 1 out of 3 ponds), which coincided with the time when *Phormidium* sp. was found. Because *Phormidium* sp. is a well known producer of odorous compounds [1], it may have contributed to both particulate-bound and dissolved geosmin fractions in water in the gypsum-treated ponds. Although very small amounts of cyanobacterial biomass would be sufficient to explain the particulate-bound geosmin in ponds, other cyanobacteria should not be ruled out, and may have contributed to the particulate-bound geosmin fraction of the pond water.

The non-detection of both geosmin and MIB in water in ponds treated with rice straw extract might be due to the inhibitory effects of tannin or tannic acid compounds from rice straw extract on earthy-musty odor-causing cyanobacteria in the pond. Decomposing rice straw can release phenolic compounds that have a synergistic effect on the growth of plankton production [16]. Apparently, this supports the recorded low biomass of earthy-musty odor-producing cyanobacteria, such as *Anabaena circinalis*, *Phormidium* sp., and *Pseudanabaena* sp., in ponds treated with rice straw extract, as compared with the 2 other treatments (**Table 4**). Several studies reported that rice straw can inhibit the growth of some cyanobacteria, such as *Microcystis* sp., *Anabaena* sp., and *Oscillatoria* sp. [12,16]. Although decomposing rice straw in this study had some inhibitory effects on odor-producing cyanobacteria, the growth of *Microcystis* sp, however, appeared to have not been inhibited by the rice straw extracts relative to other treatments, as shown in **Table 4**. Other cellulosic materials, like decomposing barley straw, similarly produce chemical substances that effectively inhibit the growth of some nuisance cyanobacterial species under laboratory conditions [31,32]. The inhibitory effect of barley straw might be due to chemical compounds, such as oxidized phenolics and hydrogen peroxide, which occur during the decomposition process [33,34]. The inhibitory effect of decomposing rice straw towards cyanobacteria is deemed to follow the same mechanism as in barley straw.

Earthy-musty odor levels in pond sediments are presented in **Figure 4**. The results indicated that geosmin was mainly responsible for the off-flavor episodes in pond sediments (**Figure 4A**). The concentration of geosmin in fish pond sediments ranged between ND - 0.384 μg L^{-1}. The highest concentration of geosmin was observed in the control group at 12^{th} week culture period. Culture time affected geosmin concentration, as geosmin was normally detectable in the later part of the culture (after 12 weeks in this study), but not earlier than 6 weeks, which is consistent with the observation of Pilmorat *et al*. [35]. In the present study, high geosmin occurrence was observed in the control group, whilst ponds treated with rice straw extract contained lower geosmin than the control and gypsum-treated ponds, except in the 16^{th} week, where geosmin levels in gypsum-treated ponds tended to have lower levels than both the control and rice straw extract treatments (**Figure 4B**).

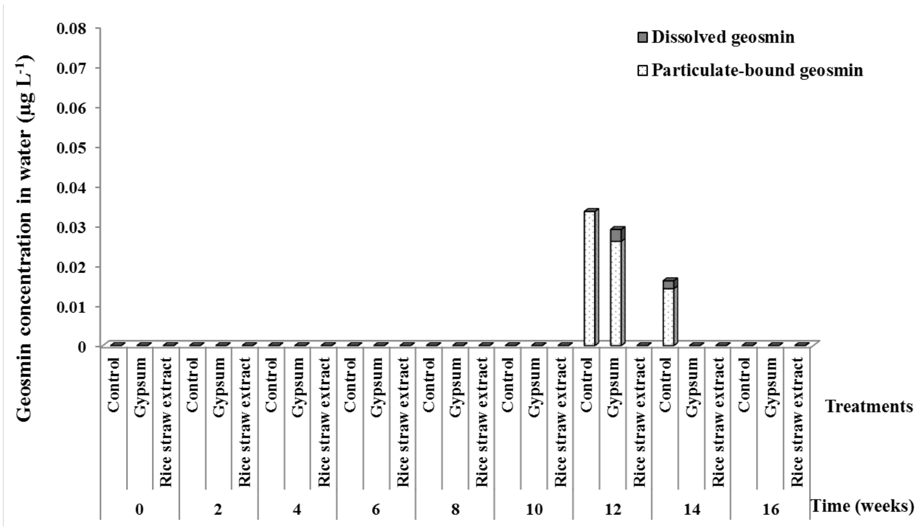


Figure 2 Concentrations of geosmin (particulate-bound and dissolved) in pond water (control; 200 ppm gypsum, and 1 ppm rice straw tannin extract).

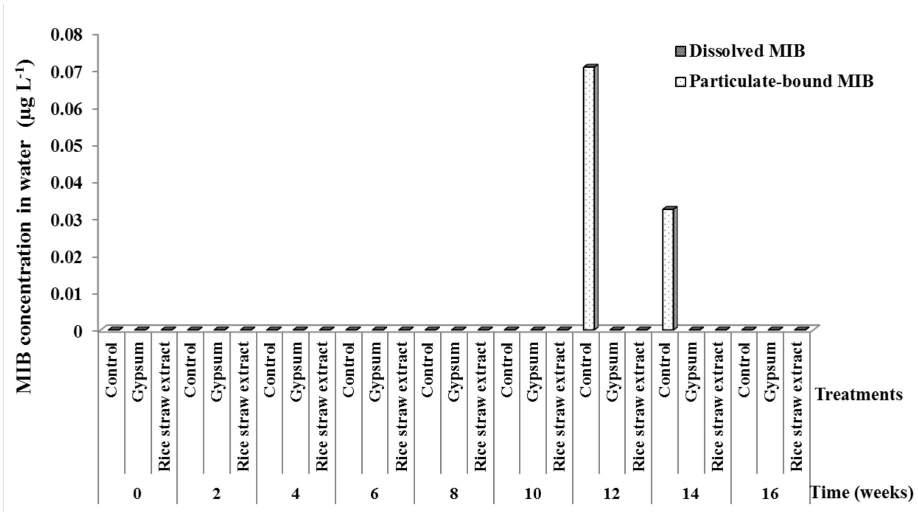
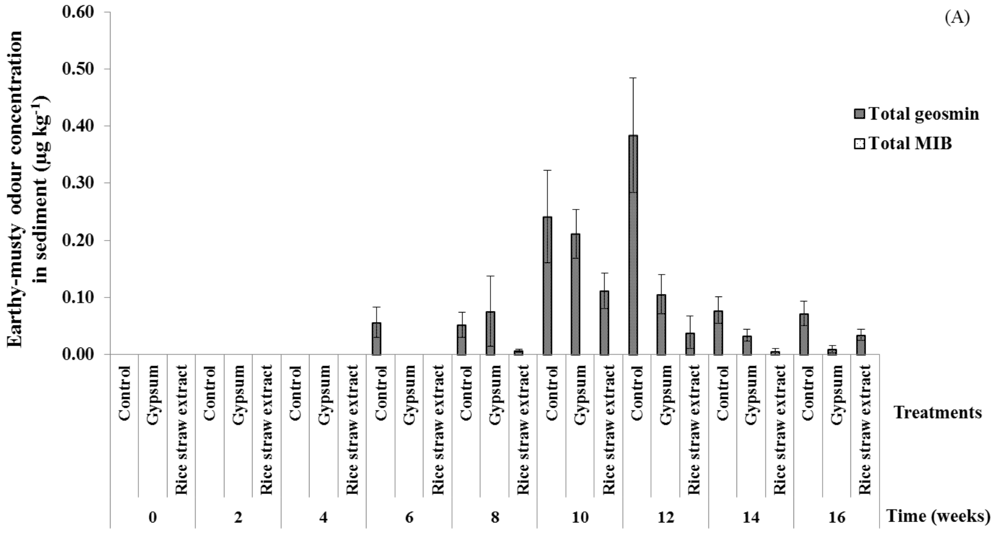


Figure 3 Concentrations of MIB (particulate-bound and dissolved) in pond water (control; 200 ppm gypsum, and 1 ppm rice straw tannin extract).



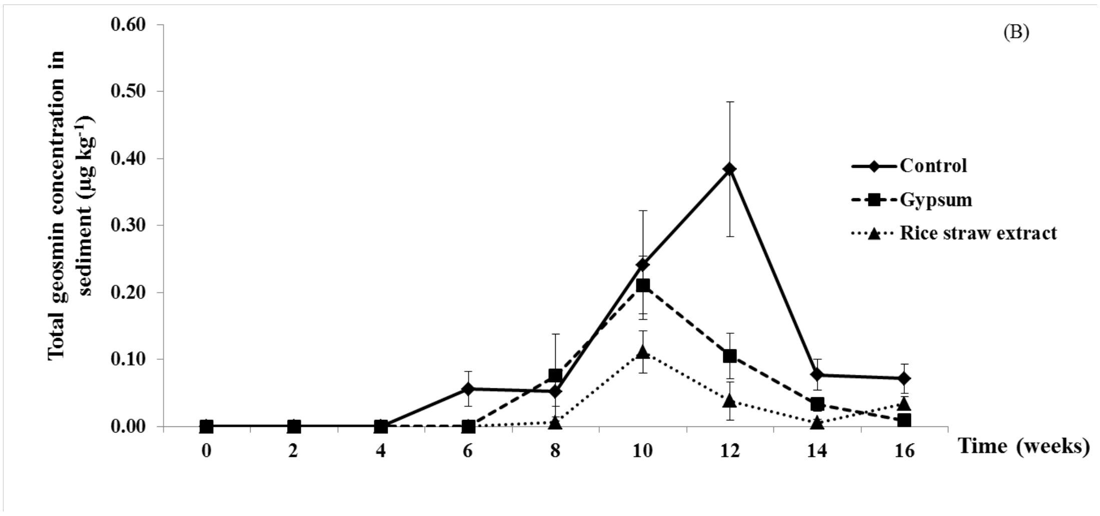


Figure 4 Concentrations of earthy-musty odor in pond sediments; (A) total geosmin and MIB, (B) total geosmin. Error bars represent the standard error of 3 replicates (control; 200 ppm gypsum, and 1 ppm rice straw tannin extract).

Geosmin in the pond sediments might have been produced by actinomycetes. Actinomycetes are another systematic group that has been shown to produce MIB and geosmin [36]. Actinomycetes produce many compounds, including 2-MIB and geosmin, and have long been recognized as sources of severe earthy-musty tastes and odors in drinking water [37]. In this study, the number of actinomycetes spp. in sediment samples were found to range between 0.72 - 7.90 × 10^4 $CFUg^{-1}$. The highest number of actinomycetes was found in gypsum-treated ponds at the 6^{th} week culture period, while the lowest was detected in the rice straw extract-treated ponds at the 10^{th} week culture period (**Figure 5**). The genus *Streptomyces* spp., which is widely reported to produce odorous compounds, was detected in the sediments after 12 weeks of the culture period (**Figure 6**). Significant levels of *Streptomyces* spp. in sediments were observed in both control and gypsum-treated ponds, which coincided with the detection of geosmin and MIB in pond water. The results suggest that *Streptomyces* spp. might also be the biological origin of geosmin in these tilapia ponds, along with *Phormidium* sp., and possibly *Pseudanabaena* sp. and *Anabaena* sp. Klausen *et al*. [38] attributed the occurrence of geosmin and MIB in freshwater environments to *Actinobacteria,* most of them belonging to the genus *Streptomyces* [36,37,39,40].

The concentrations of geosmin and MIB in water and pond sediment reported in this study are very low when compared with those reported by Gutierrez *et al*. [18]. This might be due to the effect of frequent rainfall during the study period (rainy season), particularly from July to August, and to the abundance of cyanobacteria. Preparation of the ponds before culture might have reduced the population of some organisms in the sediments. If culture time was increased from 6 to 8 months, tilapia in ponds may have acquired off-flavor, as Pimolrat *et al*. [35] observed in their study.

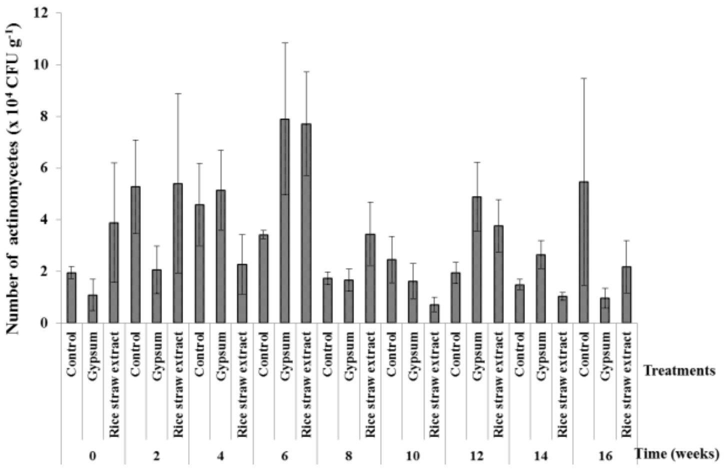


Figure 5 Number of actinomycetes in sediments (CFU g^{-1}). (Control; 200 ppm gypsum, and 1 ppm rice straw tannin extract).

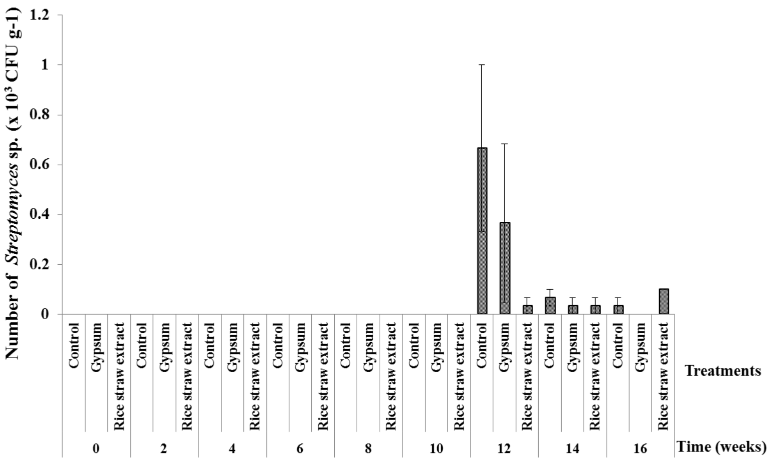


Figure 6 Number of *Streptomyces* spp. in sediments (CFU g^{-1}). (Control; 200 ppm gypsum, and 1 ppm rice straw tannin extract).

Diversity and composition of phytoplankton in fish pond

The phytoplankton found in fish ponds were categorized into 58 genera and 6 divisions, namely Chlorophyta (46.6 %), Cyanophyta (24.1 %), Bacillariophyta (13.8 %), Euglenophyta (6.9 %), Pyrrhophyta (5.2 %), and Cryptophyta (3.4 %). Chlorophyta was the most abundant in terms of composition; however, the highest biomass in pond water was Euglenophyta (0.39±0.12 µl L^{-1}), followed by Chlorophyta, Bacillariophyta, Cyanophyta, Pyrrhophyta, and Cryptophyta, which showed average biomasses of 0.22±0.08, 0.13±0.08, 0.12±0.08, 0.09±0.04, and 0.06±0.02 µl L^{-1}, respectively (**Figure 7**). Species and biomass of cyanobacteria are known to be important factors influencing MIB and geosmin concentrations in water [41].

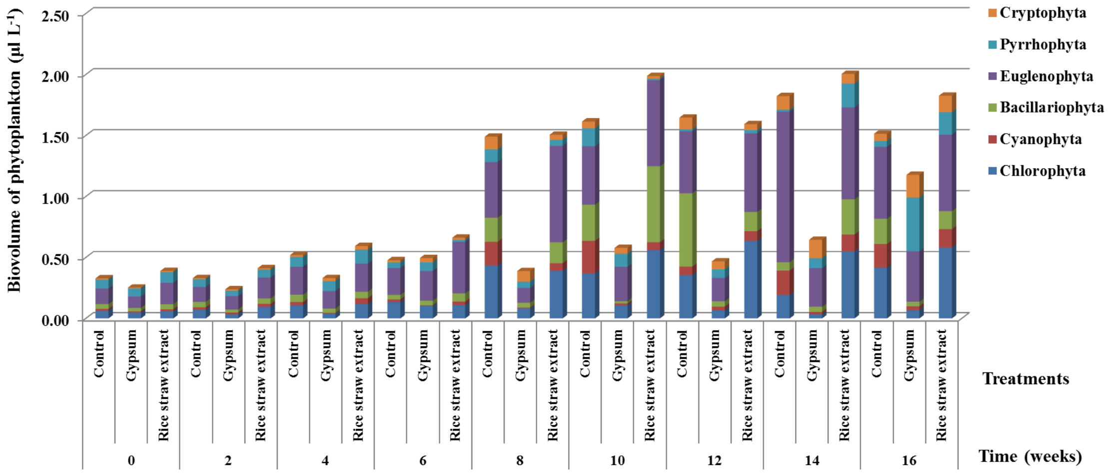


Figure 7 Diversity and composition of phytoplankton in fish ponds. (Control; 200 ppm gypsum, and 1 ppm rice straw tannin extract).

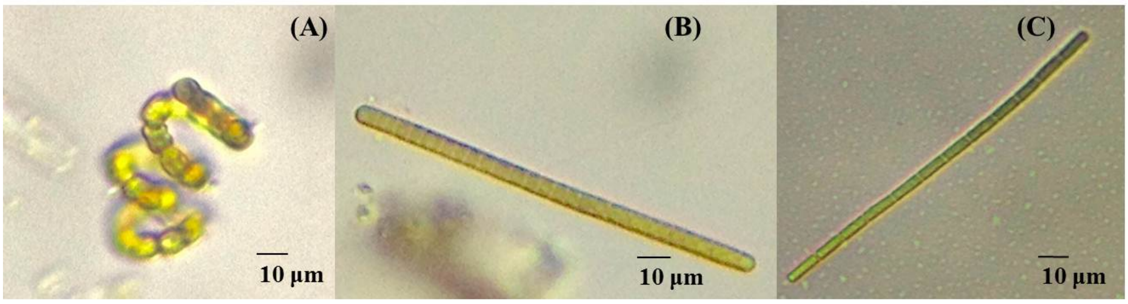


Figure 8 Photomicrographs of earthy-musty odor-producing cyanobacteria in fish ponds. (A) *Anabaena* sp., (B) *Phormidium* sp., and (C) *Pseudanabaena* sp.

Table 4 Biovolume of cyanobacteria (×10^{-3} µl L^{-1}) in fish ponds during the experimental period (mean±SE).

Div. Cyanophyta	Treatment		
	Control	**200 ppm gypsum**	**1 ppm rice straw tannin extract**
Anabaena circinalis	1.97±1.20	1.71±1.17	-
Aphanocapsa sp.	9.96±4.47	2.54±0.75	5.57±1.27
Aphanothece sp.	-	-	0.05±0.05
Chroococcus limneticus	3.52±1.24	0.28±0.14	1.04±0.37
Coelomoron sp.	1.31±0.43	0.43±0.12	1.01±0.13
Cylindrospermopsis philippinnensis	50.20±23.67	-	4.48±1.35
Cylindrospermopsis raciborskii	-	-	2.44±1.23
Leptolyngbya limnetica	4.77±3.31	0.63±0.37	2.39±0.63
Merismopedia punctata	0.84±0.24	0.74±0.36	0.92±0.26
Merismopedia tenuissima	0.78±0.50	0.33±0.12	0.32±0.10
Microcystis sp.	5.35±3.13	0.69±0.69	5.48±2.62
Microcystis aeruginosa	21.42±13.09	-	28.27±13.23
Phormidium limnetica	4.92±1.94	0.50±0.39	0.49±0.21
Phormidium sp.	6.84±1.98	6.42±2.60	2.45±1.60
Planktolyngbya limnetica	2.92±0.81	1.07±0.50	3.71±1.16
Pseudanabaena limnetica	0.22±0.22	0.83±0.36	-
Pseudanabaena sp.	0.46±0.17	0.22±0.10	0.09±0.07
Romeria gracilis	0.20±0.07	-	0.25±0.12
Synechococcus sp.	0.03±0.02	-	-

In this study, even though earthy-musty odor-producing cyanobacteria, including *Phormidium* sp., *Anabaena* sp., and *Pseudanabaena* sp. (**Figure 8**), were found at lower bio-volume than other species, these species are well known producers of odorous compounds [1,5,42,43], and might have somehow contributed to the geosmin concentration in pond water, especially in the particulate-bound fractions.

The bio-volume of phytoplankton was positively correlated with chlorophyll *a* ($r = 0.88$; $p < 0.05$) in pond water (**Figure 9**), and both parameters continued to increase from 8 weeks of the culture period onwards. In this study, it can be observed that the addition of gypsum at 200 ppm resulted in lower bio-volume and chlorophyll-*a* values, compared to other treatments. This could be due to the positively-charged calcium ions in gypsum, which interact with the negatively-charged algal surfaces and bind them, resulting in flocculation . Similarly, Sun *et al.* [44] reported that all cells of *Microcystis aeruginosa* were removed by the surface charge neutralization with polyaluminum chloride (PACl) in the coagulation process of conventional drinking water treatment through colloid charge neutralization, followed by aggregation into flocs that are amenable to solid/liquid separation with subsequent processes, such as sedimentation and filtration. Gypsum treatment also reduces phytoplankton abundance by lowering the dissolved orthophosphate concentration, the nutrient most commonly limiting phytoplankton growth in freshwater ecosystems, through calcium phosphate precipitation in water [8].

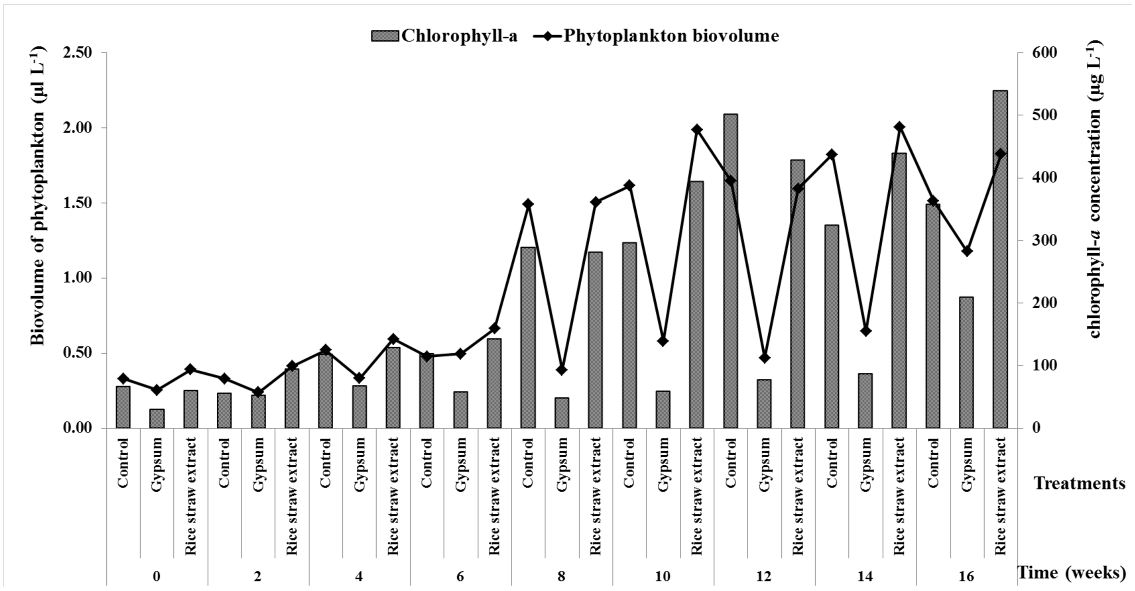


Figure 9 Biovolume of phytoplankton and chlorophyll-*a* in fish ponds. (Control; 200 ppm gypsum, and 1 ppm rice straw tannin extract).

Conclusions

The use of 1 ppm rice straw tannin extract appeared to be the most effective in mitigating off-flavor occurrences in tilapia ponds, due to the non-detection of both geosmin and MIB in the treatment. Sediment samples from the rice straw extract-treated ponds had lower geosmin levels compared to the control and gypsum-treated ponds. The use of gypsum at 200 ppm in minimizing off-flavor in ponds was also more effective than the control. In addition, both rice straw extract and gypsum also increased the fish biomass and %FCE. Thus, these methods, which are simple, practical, and cost effective, can be recommended as alternative methods to control phytoplankton and reduce off-flavor, turbidity and TSS in tilapia ponds, as well as to improve tilapia productivity.

Acknowledgements

The work was carried out with the aid of a grant from the Thailand Research Fund (TRF), and with the support of Maejo University and their laboratory instruments and research facilities.

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