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Efficency of inoculating the green algae *Chlorella* and *Scenedesmus* to prevent cyanobacteria growing in Nile tilapia culture

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ABSTRACT

The purpose of this study was seeding or inoculating the *Chlorella elliposoidea* (Gerneck) + *Scenedesmus bijuga* (Turpin) as green algae at the beginning of the production season (or before the preferable time of cyanobacteria growing) to propagate and prevent the growth of harmful cyanobacteria in the Nile tilapia culture via glass aquaria.

Indoor experiment was carried out in natural light using 12 glass aquaria as four groups (3 replicates each). Ten Nile tilapia fingerlings were stocked (*Oreochromis niloticus*) in each aquarium.

The cyanobacteria were not appeared in the fish aquaria which were seeded or inoculated with *Chlorella* + *Scenedesmus* 20 x 10^3 cells ml^{-1} (T_3) at day 5 and 10. The cyanobacterial count was lower in the second treatment (T_2). Significant differences were observed between the cyanobacterial count on day 5 and 10 in the control aquaria and those inoculated with *Chlorella* + *Scenedesmus* sp. ($p < 0.05$). Higher counts of green algae were observed in aquaria inoculated with the highest dose of *Chlorella* + *Scenedesmus* sp. (T_3). Generally, the green algal count in control aquaria was the lowest, followed by those treated with 10 x 10^3 cells ml^{-1} *Chlorella* + *Scenedesmus* sp. (T_1). No significant differences were observed in the green algae and cyanobacteria counts on day 5 and 10 between T_2 and T_3 ($P < 0.05$).

The present experiment showed that the presence of *Chlorella* + *Scenedesmus* sp. is sufficient to control the growth of cyanobacteria for 10 days period in the Nile tilapia culture.

Key words: Efficiency, Cyanobacteria, *Chlorella* and *Scenedesmus, Tilapia*

INTRODUCTION

Commercial fish production ponds are usually operated as “static” systems, with little or no water going in or out. Food, as well as fertilizers are added to almost all ponds, and represent the major source of nutrients in fish ponds. Fortunately, uneaten feed and fish wastes are usually biologically degraded and reused by certain pond organisms. A large portion of these nutrients is chemically or biologically transformed and then released into the water and taken up by the phytoplankton bloom. In a healthy and balanced aquatic

ecosystem, algae are an important component of the natural plankton population (White *et al*., 2005).

Chlorella and *Scenedesmus* sp. are green algae with high chlorophyll content. Tendencia and Dela Pena (2003) reported that *Chlorella* sp. inhibits the growth of luminous bacteria after 48 h, although this was observed using 500 ml flasks. Corre *et al*., (2000) and Lio-Po *et al*., (2002) reported that *Chlorella* density in ponds using green algae ranged from 10^5 to 10^6 cells ml^{-1}. These micro-algae are found in pond water and could enhance upon exposure to sunlight. It is possible that these micro-algae could have antibacterial activity against some Gram negative bacteria (Lio-Po *et al*., 2002).

Cyanobacteria (blue-green algae) are a diverse group of photosynthetic, prokaryotic organisms found in fresh water and marine environments (Schoof and Packer, 1987). Their cell structure resembles that of Gram negative bacteria, but as a rule they live photoautotrophically.

In shrimp ponds, rearing water containing *Chlorella* is considered ideal for disease prevention and it could inhibit growth of pathogenic bacteria isolated from diseased shrimp (*Vibrio harveyi, V. parahaemolyticus* and *V. penaeidida*) (Direkbusarakom *et al*., 1997).

The purpose of the present study was to prevent the harmful algal growth of cyanobacteria by inoculating or seeding the *Chlorella elliposoidea* (Gerneck) + *Scenedesmus bijuga* (Turpin) as green algae at the beginning of the production season (or before the preferable time of cyanobacteria growing) in the Nile tilapia culture via glass aquaria.

MATERIALS AND METHODS

The study was conducted at WorldFish Center, Abbassa Sharkia. *Chlorella elliposoidea* (Gerneck) and *Scenedesmus bijuga* (Turpin) were isolated from Nile water samples according to Pascher (1915). The microalgae were subcultured in Bold's basal medium (BBM) (Bischoff & Bold, 1963). The cultures were allowed to grow in the algae culture room at 25 °C and 14/10 light-dark cycle (5000 lux). Stock cultures of *C. elliposoidea* & *S. bijuga* were prepared in two liters capacity flasks in the laboratory for 5-6 days, then inoculated in carboy cultures at a density of 1×10^5 cells mL^{-1}. The carboy cultures were used as inocula for two different phases of production in indoor and outdoor in glass aquaria. The transfer of the algal cells to fish aquaria was achieved at a density of 5×10^6 cells mL^{-1}, chlorophyll content was 2.9 mg L^{-1} and dry weight gain was 660 mg L^{-1}.

Indoor experiment was carried out in natural light using 12 glass aquaria as four groups (each aquarium has 100 liters capacity. Three treatments and control groups were carried out in triplicates. Ten Nile tilapia (*Oreochromis niloticus*) with initial weight each of 30±3 g $fish^{-1}$ were stocked in each aquarium. Experimented fish were fed daily at a rate of 3% their body weight on commercial formulated feed containing 25% protein. Aeration was

supplemented, provided by a regenerative blower and stones submerged at the bottom of each aquarium.

The first 3 aquaria groups were filled with canal water, having a known species composition count of phytoplankton, chlorophyll "a", "b", "c" and c-phycocyanin content. These aquaria were seeded with green algae *C. elliposoidea* + *S. bijuga* (mixture 1:1) at 3 initial different densities; 10 x 10^3 cells ml^{-1} (T_1), 15 x 10^3 cells ml^{-1} (T_2) and 20 x 10^3 cells ml^{-1} (T_3) for 1st, 2nd and 3rd aquaria groups; respectively. The 4th aquaria group served as a control without addition of green algae (T_0). Water samples for chemical, physical and biological analyses in all treatments and control were carried out at starting time, 5, 10 days intervals.

The following formula was used to compute for the required volume of stock green algae to be added into the aquaria (Tendencia *et al*., 2005).

$$\text{Volume to be added} = \frac{(\text{desired density} - \text{existing density}) \times \text{volume of water in aquarium}}{\text{Density of stock culture}}$$

Chlorophyll a, b, and c contents were determined in water photometrically by using spectrophotometer. Water samples (100 ml) were filtered through a membrane filter (0.45 µm pore size) then extracted with 90% acetone. Calculation of the chlorophyll a, b, and c was carried out using the equation adopted by APHA (1985). Spectrophotometrically, the C-phycocyanin (CPC) concentration was carried out according to O'Carra and Oh'eocha (1976) and calculated using Beer's law and an extinction coefficient of 7.9 L g^{-1} cm^{-1} (Svedberg and Katsurai, 1929):

$$CPC\ gL^{-1} = A_{625}/7.9\ L/g/cm \times 1\ cm.$$

Quantitative estimation of phytoplankton was carried out by the technique adopted by APHA (1985) using the sedimentation method. Phytoplankton samples were preserved in Lugol's solution at a ratio of 3 to 7 ml Lugol's solution to one liter sample and concentrated by sedimentation of one liter water sample in volumetric measuring jars for about 2 to 7 days. The surface water was siphoned and the sediment was adjusted to 100 ml. From the fixed sample, 1 ml was drown and placed into Sedgwick-Rafter cell, and then it was microscopically examined for counting after identification of phytoplanktonic organisms. The results were expressed as cell counts ml^{-1}. The phytoplankton cells were identified to four divisions as (Chlorophyta), (Cyanobacteria), (Bacillariophyta), and (Euglenophyta). For identification of the algal taxa, Fritsch (1979) and Komarek and Fott (1983) were followed.

Water temperature (^oC); and dissolved oxygen (DO, mgL^{-1}) were measured using an oxygen electrode meter. One liter water samples were collected to measure the hydrogen ions (pH) using the ACCUMET pH meter (model 25), and total ammonia (mg L^{-1}) using HACH Comparison (1982). Total alkalinity, total hardness and nitrate (NO_3) were determined according to Boyd & Tucker (1992).

One-way ANOVA was used to evaluate the significant difference among treatments and duration. A probability at level of 0.05 or less was considered significant. All statistical analyses were run on the computer, using the SAS program (SAS, 2003).

RESULTS AND DISCUSSION

Results showed that cyanobacterial count was generally highest in control aquaria (Table 1). The cyanobacterial count in aquaria with *Chlorella* + *Scenedesmus* was not appeared and completely inhibited in the fish aquaria which were inoculated with 20 x 10^3 cells ml^{-1} (T_3) either at day 5 or day 10. The cyanobacterial count was lower in the second treatment (inoculated with 15 x 10^3 cells ml^{-1} of green algae). Significant differences were observed in the cyanobacterial count on both day 5 and 10 in the control aquaria and those seeded with *Chlorella* + *Scenedesmus* sp. ($p < 0.05$).

On the basis of previous work on the control of cyanobacterial blooms using tannic acid treatments might cause oxygen depletion due to algae die off (Dawah *et al*., 2006a) or *Chlorella* and *Scenedesmus* sp. (green algae) treatments, might cause increase in the algal density (Dawah *et al*., 2006b). The prevention of cyanobacterial growth by inoculating or seeding *Chlorella* + *Scenedesmus* sp. (green algae) to propagate and prevent any growth for Cyanobacteria in the Nile tilapia culture was the aim of this study.

Higher green algae counts were observed in aquaria inoculated with high dose of *Chlorella* + *Scenedesmus* sp. (20 x 10^3 cells ml^{-1} (T_3)). Generally, the green algae count in control aquaria had the lowest count of *Chlorella* and *Scenedesmus* sp. No significant differences were observed in the green algae and Cyanobacteria counts on day 5 and 10 in T_2 and T_3 ($P < 0.05$) Table. (1).

The aim of the work was based on differences between *Chlorella* + *Scenedesmus* and Cyanobacteria (*Microcystis, Anabaena* and *Aphenocapsa*) which WorldFish Center, Abbassa, Sharkia suffered from them. *Chlorella* and *Scenedesmus* have fast and high growth rates (μ at 25ºC = 0.7 ˜0.83 d^{-1}), tolerant high temperature up to 35ºC, strongest antimicrobial activity against gram-negative bacteria, (Shi-Li. *et al*., 2001), fast and very high nutrient uptake capabilities and no aggregation properties but colonization (no blooms)**.** Cyanobacteria are the only prokaryotic organisms carrying out an oxygen-evolving photosynthesis (Schopf and Packer, 1987).

To minimize the harmful algal blooms, control of eutrophication or selection of aquaculture sites was suggested as preventive measures (Anderson, 1997), but these strategies were not widely accepted (Sengco *et al*., 2001). Several chemical methods are employed (Jhingran, 1995), but they are too expensive, ineffective and may have some residual effects in the aquatic organisms (Anderson, 1997). The time to control a harmful algal bloom is before the bloom develops (Webster *et al*., 1996).

Results of the present experiment showed that cyanobacteria growth was decreased by increasing the dose of *Chlorella* + *Scenedesmus*, and were not detected in fish aquaria with high green algal dose. Aquaria in this study were not covered during sunny days to allow sunlight penetration and encourage growth of micro-algae. Tilapia is herbivorous and filters phytoplankton and it could feed on the seeded *Chlorella* and *Scenedesmus* sp. (Turker *et al*., 2003). Certain freshwater *Chlorella* and *Scenedesmus* are cultured as health foods for humans and animals because of the proteins, vitamins, minerals, amino acids and other substances they contain (Halama 1990). *Chlorella* was added at a density of 50–350 × 10^4 cells per ml starting at day 1 to day 21 to maintain water quality for larval rearing of milkfish (Liao, 1979).

Bacillariophyta was observed in all treatments from day 5 to day 10 increased with increasing of green algal concentration in all treatments and control aquaria, while Euglenophyta was inhibited by increasing inoculates of green algae cells (Table 1).

Table (1): Phytoplankton dynamics (x 10^3 cells ml^{-1}) by using different concentration of *Chlorella* + *Scenedesmus* sp. in Nile tilapia culture through out the experimental period 10 days)

	Initial		control	10 x 10^3 cell ml^{-1} (T_1)		15 x 10^3 cell ml^{-1} (T_2)		20 x 10^3 cell ml^{-1} (T_3)	
Divisions	count	Days	count	count	% Inh.	count	% Inh.	count	% Inh.
		5	95±3.5^{Ab}	8.57±0.5^{Ba}	91.0	2.1±0.5^{Ca}	97.8	0.0±0^{Ca}	100.0
Cyanobacteria	0.02±0.003	10	115±1.73^{Aa}	2.67±0.3^{Bb}	97.7	0.733±0.09^{Bb}	99.4	0.0±0^{Bb}	100.0
		5	11.67±0.35^{Ca}	512.05±54.5^{Ba}	-4287	962.67±37.8^{Ab}	-8149	1343.33±35.3^{Ab}	-11411.0
Chlorophyta	0.1±0.02	10	8.63±0.44^{Db}	802.67±47.9^{Ca}	-9200	1143.33±24.0^{Ba}	-13148	1546.67±37.6^{Aa}	-17822.0
		5	3.27±0.2^{Db}	7.47±0.5^{Ca}	-128	9.53±0.35^{Ba}	-191.4	11.57±0.3^{Ab}	-253.8
Bacillariophyta	0.05±0.003	10	4.27±0.2^{Da}	8.47±0.4^{Ca}	-98.4	10.6±0.5^{Ba}	-148.2	13.23±0.3^{Aa}	-209.8
		5	0.667±0.03^{Ab}	0.262±0.03^{Ba}	60.7	0.2±0.02^{Ba}	70.0	0.08±0.005^{Ca}	88.0
Euglenophyta	0.008±0.001	10	1.24±0.06^{Aa}	0.14±0.03^{Bb}	88.7	0.081±0.005^{Bb}	93.5	0.027±0.003^{Bb}	97.8
		5	110.6±3.1^{Bb}	528.35±253^{Ba}	-377	974.5±38^{Ab}	-781.1	1354.98±35.2^{Ab}	-1125.1
Total standing crops	0.178±0.05	10	129.14±1.65^{Da}	813.95±48.0^{Ca}	-530	1154.75±23.8^{Ba}	-794.2	1559.96±37.3^{Aa}	-1108.0

% Inh. = percentage inhibition

A, B, C, D. Values-having different script at the same row are significantly (P<0.05) different

a, b, c, d. Values-having different script at the same column are significantly (P<0.05) different

The C-phycocyanin pigment in (T_3) as a high dose was zero and completely inhibited at day 5 until day 10. The c-phycocyanin pigment content showed significant difference between control and all treatments inoculated with *Chlorella* + *Scenedesmus* sp. ($p < 0.05$).

The results of the pigment content are shown in Fig. (1). The chlorophyll "a" content in aquaria treated with high dose of *Chlorella* + *Scenedesmus* sp. (T_3) was significantly higher than in the other two treatments and control ($p < 0.05$). The chlorophyll "b" content was increased by increasing the dose of *Chlorella* + *Scenedesmus* sp. as $T_3 > T_2 > T_1$ .The chlorophyll "b" contents showed significant differences between control and all treatment ($p < 0.05$).

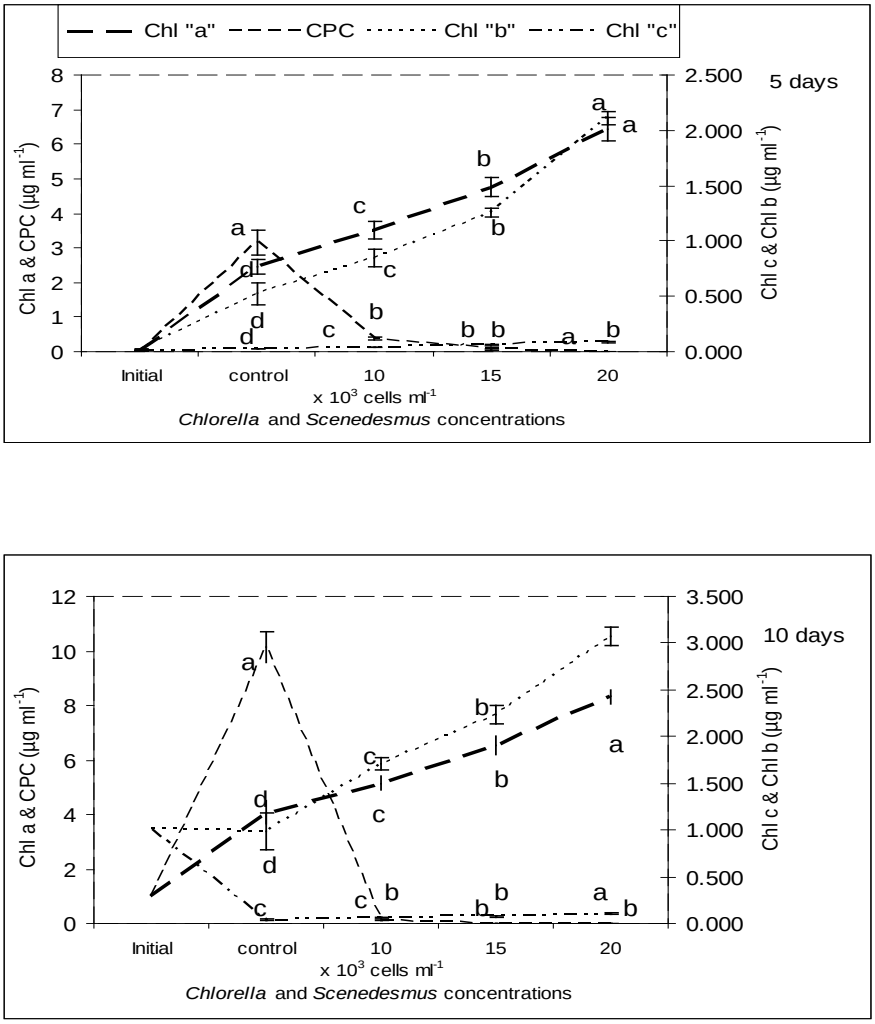

Fig (1): Pigments contents (µg ml^{-1}) by using different concentration of *Chlorella* + *Scenedesmus* sp. in Nile tilapia culture along the period of experiment (10 days)

A multiple correlation analysis including 9 biological variables was carried out for the experiment (Table 2). The correlation coefficient (r) of the significant relationships (P<0.05) are only listed. The abundance of green algae showed negative correlation with the abundance of blue green algae (r = -0.83) and Euglenophyta abundance (r = -0.82). A negative correlation was noticed between chlorophyll "b" content and C-phycocyanin pigment (r = -0.46). The green algal count had high positively correlated with chlorophyll "a" (r = 0.87), chlorophyll "c" (r = 0.87) and Bacillariophyta abundance (r = 0.944).

Table (2): All over means of Correlation coefficients of biological parameters for prevention of *cyanobacteria* using different concentration of *Chlorella* and *Scenedesmus* at all periods Listed is only the coefficient of the significant correlations (p< 0.05)

		ChlA	*ChlB*	*ChlC*	*CPC*	*Cyano*	*Chloro*	*Bacill*	*Eug*	*Total*
Chlorophyll "a"	*ChlA*	1								
Chlorophyll "b"	*ChlB*	0.9536	1							
Chlorophyll "c"	*ChlC*	0.9019	0.9521	1						
C-phycocyanin	*CPC*	-0.406	-0.457	-0.507	1					
Cyanobacteria	*Cyano*	-0.606	-0.613	-0.645	0.898	1				
Chlorophyta	*Chloro*	0.8742	0.8705	0.874	-0.705	-0.83	1			
Bacillariophyta	*Bacill*	0.8785	0.876	0.892	-0.685	-0.85	0.944	1		
Euglenophyta	*Eug*	-0.594	-0.616	-0.642	0.957	0.948	-0.82	-0.82	1	
Total standing crops	*Total*	0.8841	0.8796	0.88	-0.677	-0.81	0.999	0.938	-0.79	1

All over means of chosen chemical parameters of water and total gain of Nile tilapia fish at the end of the experiment are shown in Table (3). Results showed no significant difference ($P < 0.05$) in temperature and nitrate-nitrogen contents of the water in comparison to the control at day 5. The total alkalinity and ammonia-nitrogen content of the water were significantly higher ($P < 0.05$) in T_3 than other treatments and control. The total gain of Nile tilapia in T_3 was the highest and significantly different ($P < 0.05$). This is means that fish fed on the green algae *Chlorella* + *Scenedesmus* sp. as natural food whereas the lowest value was in control group at the end of experiment.

A number of problems are associated with dense algal blooms. In waters that have a low or moderate buffering capacity (alkalinity), dense blooms create wide fluctuations in pH during the day. Occasionally, phytoplankton populations cause pH to reach afternoon values of 10 or above, which depress fish growth and health. Algal die-off can result in high ammonia concentrations that can affect fish appetites and growth rates for extended periods. This can result in reduction of the growing season for fish producers each time an algal bloom dies back in a pond (Brunson *et al*., (2003).

Table (3): Water quality parameters and total fish gain by using different concentrations of *Chlorella* + *Scenedesmus* in Nile tilapia culture along the experimental period (10 days)

Parameters	Initial	Days	control	10x 10^3 cell ml^{-1} (T_1)	15 x 10^3 cell ml^{-1} (T_2)	20 x 10^3 cell ml^{-1} (T_3)
Dissolved oxygen ($mg\ L^{-1}$)	6.5±0.1	5	$7.83±0.14^{A}$	$8.06±0.1^{A}$	$7.8±0.12^{A}$	$7.97±0.2^{A}$
		10	$8.0±0.18^{B}$	$8.23±0.06^{A}$	$8.35±0.03^{A}$	$8.5±0.09^{A}$
Temperature (ºC)	20.5±0.12	5	$21.8±0.17^{A}$	$22.2±0.2^{A}$	$22.5±0.35^{A}$	$22.1±0.26^{A}$
		10	$21.4±0.15^{AB}$	$22±0.09^{A}$	$21.2±0.3^{B}$	$21.6±0.23^{AB}$
pH	8.2±0.03	5	$8.7±0.04^{A}$	$8.6±0.09^{AB}$	$9.02±0.25^{A}$	$8.4±0.09^{B}$
		10	$8.7±0.07^{B}$	$8.6±0.06^{B}$	$9.0±0.06^{A}$	$8.7±0.15^{AB}$
Ammonia (mgL^{-1})	0.04±0.006	5	$0.08±0.009^{D}$	$0.113±0.007^{C}$	$0.21±0.01^{B}$	$0.15±0.007^{A}$
		10	$0.13±0.018^{D}$	$0.16±0.02^{BC}$	$0.21±0.02^{AB}$	$0.26±0.02^{A}$
T. alkalinity ($mg\ L^{-1}$)	254±2.6	5	$216.3±0.88^{C}$	$246±3.1^{B}$	$254.7±3.3^{B}$	$273.7±4.7^{A}$
		10	$238.7±8.1^{A}$	$227.3±10.3^{A}$	$230±7.5^{A}$	$258.3±13.6^{A}$
T. hardness ($mg\ L^{-1}$)	220±3.7	5	$231.3±4.7^{A}$	$237.7±2.3^{A}$	$207.3±2.4^{B}$	$200.3±0.33^{B}$
		10	$208.7±4.7^{B}$	$234.7±2.9^{A}$	$218±7.6A^{B}$	$208.7±4.4^{B}$
NO_3 ($mg\ L^{-1}$)	0.06±0.03	5	$0.003±0.002^{B}$	$0.002±0.0009^{B}$	$0.02±0.006^{A}$	$0.007±0.004^{AB}$
		10	$0.0007±0.0006^{B}$	$0±0^{B}$	$0.0007±0.0006^{B}$	$0.03±0.02^{A}$
Available phosphorus	0.54±0.04	5	$0.09±0.005^{A}$	$0.09±0.02^{A}$	$0.13±0.06^{A}$	$0.113±0.009^{A}$
		10	$0.13±0.02^{B}$	$0.118±0.032^{B}$	$0.254±0.06^{A}$	$0.13±0.008^{B}$
Initial body weight (g/10 fish)		10	$307.7±9.02^{A}$	$260.7±23.8^{B}$	$287±9^{AB}$	$299±7.6^{AB}$
Final body weight (g/10 fish)		10	$321.7±9.8^{A}$	$275.7±24.06^{A}$	$302.7±7.7^{A}$	$315.7±8.09^{A}$
Total gain (g/aquarium)		10	$14±1.15^{B}$	$15±0.58^{A}$	$15.7±1.3^{A}$	$16.7±0.88^{A}$

A, B, C, D. Values-having different script at the same row are significantly ($P<0.05$) different

The present experiment showed that the presence of *Chlorella* + *Scenedesmus* sp. is sufficient enough to control the growth of cyanobacteria for 10 days period in the Nile tilapia culture.

The presence of *Chlorella* spp. alone was efficient for controlling the growth of Cyanobacteria but maintaining the algal density of a single kind of microalgae in a simulated environment was labor intensive. Among the problems associated with the use of algal cells as fish feed is that the low digestibility of the algal cells makes the algal biomass unsuitable for rearing fishes. Moreover, mixed diets containing several species of microalgae have been reported to give better results for some organisms (Hu, 1990).

This study has also shown that it is advisable to encourage or allow the growth of more than one green alga, especially *Scenedesmus* to control Cyanobacteria in Nile tilapia culture.

This study serves as a guide to know the green algal density for controlling Cyanobacteria in Nile tilapia ponds. It also provides a basic information for future research field studies along the same line.

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