FIELD STUDIES ON PREVENTION AND BIOLOGICAL CONTROL OF THE CYANOBACTERIAL BLOOMS USING *CHLORELLA* AND *SCENEDESMUS* IN THE NILE TILAPIA FARMS

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Abstract

This study aimed to investigate the use of green algae *Chlorella elliposoidea* (Gerneck) + *Scenedesmus bijuga* (Turpin) Lageh as prevention and biological control treatment of cyanobacterial blooms as field application.

Twelve earthen ponds were randomly assigned to three groups with four replicates per each treatment. The first group served as a control. The second group (1st treatment) was seeded with *C. elliposoides* + *S. bijuga* at initial density; 20 x 103 cells ml-1 (16 tons acre-1 live algae) at the beginning of production season in June 2005 (as prevention treatment). The third group (2nd treatment) was seeded with the same previous density of live algae at the beginning of cyanobacterial bloom (as therapeutic treatment).

The alga found on most samples was Anabaena sp., recorded on 33 of the 36 samples and was absent from the counts only in January and February. The greatest number of species and individuals were seen between June and July. These species occurred in quantities that could be considered strong cyanobacterial blooms. The highly abundant species was *Microcystis aeroginosa*, which showed black blooms when the weather was cold (temperature ~7 ºC). Other species showed somewhat limited blooms (*Gloeocapsa ru*pestris and *Chroococcus sp*.) in January. All other species were scarce and their number was fluctuating throughout the study period.

The abundance of cyanobacteria was minimum in T1 in the first three months of grow-out periods. The green algae were the dominant in the T1 which inoculated by Chlorella + *Scenedesmus sp.* as prevention treatment for three months of grow-out period and reached a minimum level in the last months.

The highest number of cyanobacteria was in the control and T2 during all grow-out periods. On the other hand, the concentration of cyanobacteria decreased sharply after inoculation of Chlorella + Scenedesmus at the beginning appearance of cyanobacterial bloom in T2. The highest green algae lead to the highest biomass of rotifera. Cladocera were found to exhibit an opposite trend. In this study, the application of green algae at a dose of 20 x 103 cells ml-1 (16 tons acre-1 live algae) can prevent and control the cyanobacterial blooms for two months in Nile tilapia farms.

INTRODUCTION

In the last few decades, there is more and more evidence of harmful algal blooms (HABs) (Smayda and Reynolds, 2001). From the previous studies some fish ponds at Abbassa showed cyanobacteria especially *Microcystis* and *Anabaena* blooms which caused fish deaths and poisonous outbreaks (Ibrahim 1997). The use of pesticides or algaecides to control blooms of off-flavor-producing cyanobacteria can cause massive die-off of phytoplankton populations, resulting in more economic losses.

Cyanpbacteria (blue-green algae) are a diverse group of photosynthetic, prokaryotic organisms found in fresh water and marine environments. The origin of these organisms is dated back three or four billion years (Schopf and Packer, 1987). Their cell structure resembles that of Gram-negative bacteria, but as a rule they live photoautotrophically.

Cyanobacterial biomass is inefficiently utilized by zooplankton herbivore populations because the colonies or filaments may be too large to be effectively processed or biomass may be indigestible, toxic, or of poor food quality (Porter and Orcutt 1980).

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 flourish upon exposure to sunlight.

From the previous laboratory studies via glass aquaria, we concluded that the green algae *Chlorella elliposoidea* (Gerneck) + *Scenedesmus bijuga* (Turpin) can control the cynaobacterial growth (Dawah *et al*., 2006 a, b and Dawah 2007).

The main objectives of this field studies were: (1): To investigate the biological control behavior of *Chlorella elliposoidea* (Gerneck) + *Scenedesmus bijuga* (Turpin) Lageh against cyanobacterial blooms. (2): To prevent of the cyanobacterial blooms. (3): To find the reasons for cyanobacteria blooms by water quality and plankton assessment.

MATERIAL AND METHODS

Pond facilities

This work was carried out for one-year period (from May 2005 to May 2006). Twelve earthen ponds each of 4200-m^2 (1 feddan) areas with the same average water depth (~ 1.0 m) at the WorldFish Center (Abbassa, Sharkia Governorate, Egypt) were used in this study. Before the experiment, the ponds were drained, cleaned and exposed to the sun for one week. The ponds were filled by fresh Nile water from "Gadaon" channel branched from Ismailia canal; with average depth (~ 0.15 m) and disinfected by chlorine 10 mg L^{-1} and left for one weak. The water was filtered through saran screen to prevent the entrance of wild fish, their eggs and larvae to the experimental ponds. Water level was maintained at 1.0 m and any water loss due to evaporation or seepage was compensated periodically to maintain the depths of ~1.0 m.

All ponds were stocked with Nile tilapia fry (0.1±0.02 gm) obtained from the WorldFish Center stock ponds. Fish were stocked at a rate of 1.5 fish /m^2. At the first grow-out production for one week the ponds fertilized by 150 kg $feddan^{-1}$ $week^{-1}$ of chicken manure. After that the fertilizers decreased to 75 kg $feddan^{-1}$ $week^{-1}$ for two weeks. Finally the ponds fertilized by 50 kg chicken manure

feddan^{-1} week^{-1} for 10 weeks, then applied feeding at a rate of 3% of fish biomass for the rest period. No feeding was applied during the winter season.

Experimental design

Samples were collected and analyzed monthly during the winter, biweekly during the spring and fall and weekly during the summer months.

The 12 ponds were randomly assigned to three groups with four replicates per each treatment. The first group served as a control. The second group (1st treatment) was seeded with *C. elliposoides* + *S. bijuga* (mixture 1:1) at initial density; 20 x 10^3 cells ml^{-1} (16 tons acre^{-1} live algae) at the beginning of production season in June 2005 (as prevention treatment). The third group (2nd treatment) was seeded with the same previous density of live algae at the starting of cyanobacterial bloom in May 2006 (as therapeutic treatment).

Water physicochemical analysis

Parameters measured at the time of collection included water temperature, (^{o}C); and dissolved oxygen (DO, mg L^{-1}) which was measured using an oxygen electrode meter. One-liter water samples were collected in 1-L polyethylene bottles, to measure hydrogen ions concentration (pH) at room temperature, using the ACCUMET pH meter (model 25), and total ammonia (mg L^{-1}) using HACH Comparison (1982). Total alkalinity, total hardness, available phosphorus and nitrate (NO_3) were also determined according to Boyd and Tucker (1992).

Outdoor and Indoor algae mass culture

Chlorella elliposoides and *Scenedesmus bijuga* 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. elliposoides* and *S. bijuga* were prepared at WorldFish Center laboratory in two liters capacity flasks for 5-6 days, then inoculated in carboy cultures at a density of 1 x 10^5 cells ml^{-1}. The carboy cultures were used as inoculums for two different phases of production in indoor and outdoor in glass aquaria. The carboy and aquaria were used to inoculate the fiberglass tanks (5 m^3)

and cement ponds (12 m^3). Commercial or agricultural grade components are used according to Oceanic Institute (OI) Algae Culture Medium for outdoor culture (Allen & Nelson 1910). The transfer of the algal cells to fish earthen ponds was achieved at a density of 5~6 x 10^6 cells ml^{-1}.

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

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

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. 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 phytoplankton organisms. The results were expressed as cell counts ml^{-1}. The phytoplankton cells were identified to four divisions as Chlorophyceae (green algae), Cyanobacteria (blue green algae), Bacillariophyceae (diatoms), and Euglenophyceae (euglenoids). For identification of the algal taxa, Fritsch (1979) and Komarek and Fott (1983) were followed.

Zooplankton estimation

Quantitative analysis for Zooplankton samples were taken biweekly. Ten liters of the pond water were filtered through zooplankton net of 55 µm mesh diameter. Samples were preserved immediately after collection in 4% neutral

formalin. Total zooplanktons were determined in each replicate following Ludwig (1993).

Statistical analysis

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

Over the course of the study (May 2005-May 2006), 36 water samples were collected and analyzed.

Water Quality

Water quality management is a key ingredient in a successful fish operation (Fig.1).

1-Hydrogen Ion Concentration (pH)

PH value plays an important role in many life processes. It may also reflect the redox potential productivity and pollution level of the aquatic environments. The pH of the ponds during the study period were found to lie on alkaline side generally ranged from pH 8.5 to 9.3, a range favorable to the growth of blue-green algae (Healey and Hendzel, 1975). The maximum average value of pH (9.31) was recorded at T_2. The high pH–value at these ponds is mainly related to the increase in the total count of phytoplankton.

2- Temperature

The temperature plays an important role in the distribution and productivity of phytoplankton with population variations in the levels of nutrient salts. It was found that the temperature changes during the period of study have a pronounced effect on the total count of phytoplankton and chlorophyll a in the summer months (July, August and September). This coincides with Kobbia (1982) who reported that the temperature variations had great influence on standing crop and

productivity. The water temperature of the ponds varied from season to season. The temperature remained fairly constant from the end of June until the end of September. The maximum average values of temp. (30 °C) were recorded in July, while the minimum one (11.8 °C) was recorded in December and January. Nile tilapia is sensitive to cool temperatures (< 25°C) that reduce their feeding activity (Boyd, 1990).

3 - Total Alkalinity

The total alkalinity values in the ponds during the study period revealed that the highest values were recorded during October, while the lowest value was recorded in January (Fig. 1). The average maximum value of $CaCO_3$ (195 mg /l) was determined at control ponds, while the minimum (128.8 mg $CaCO_3$ /l) was recorded at T_2. The low values of total alkalinity were recorded after flourishing of phytoplankton in T_2 during spring months. Some observations were recorded by Halim *et al.* (1976) who mentioned that a low total alkalinity values were recorded after the flourishing of phytoplankton.

4 - Total Hardness

The lowest value of total hardness was recorded at control in August (150 mg L^{-1} as $CaCO_3$), also the highest value was recorded at control in February (347.5 mg L^{1} as $CaCO_3$). Calcium and magnesium are the most abundant alkaline earths in normal freshwater, and their concentration as equivalent usually has been taken as a measure of total hardness (Boyd, 1990).

5 - Dissolved Oxygen

Dissolved oxygen concentration fluctuated mostly between 3 and 8.34 mg L^{-1}. The water quality in conventional mono-species culture of Nile tilapia exhibits extremely low DO and high ammonia (>2-3 mg/L) (Srisuwantach *et al*. 1981).

6 - Nitrate

The maximum amount of nitrates (2.32 mg L^{-1} as an average) was observed at T_2, while the minimum (0.04 mg L^{-1} as an average) was recorded at control. At the same time their highest concentrations were observed during winter, while the lowest ones were observed during summer. The absence or deficiency of nitrate,

decrease the production of phytoplankton, (Fayed and Shehata 1980 and kobbia 1982).

7-Ammonia

Total nitrogen concentrations showed a trend of slight increase towards the later part of the grow-out. Ammonia is toxic to fish. The maximum average values of ammonia (1.3 mg L^{-1}) were recorded in May at T_2 after inoculated by green algae, while the minimum one (0.06 mg L^{-1}) was recorded at T_2 in December. The level of ammonia toxicity depends on the species of fish, water temperature, and pH (Boyd 1990). The European Inland Fisheries Advisory Commission (EIFAC, 1973) stated that the toxic concentration of unionized ammonia to freshwater fish for short-term exposure is 0.7-2.7 mg L^{-1}.

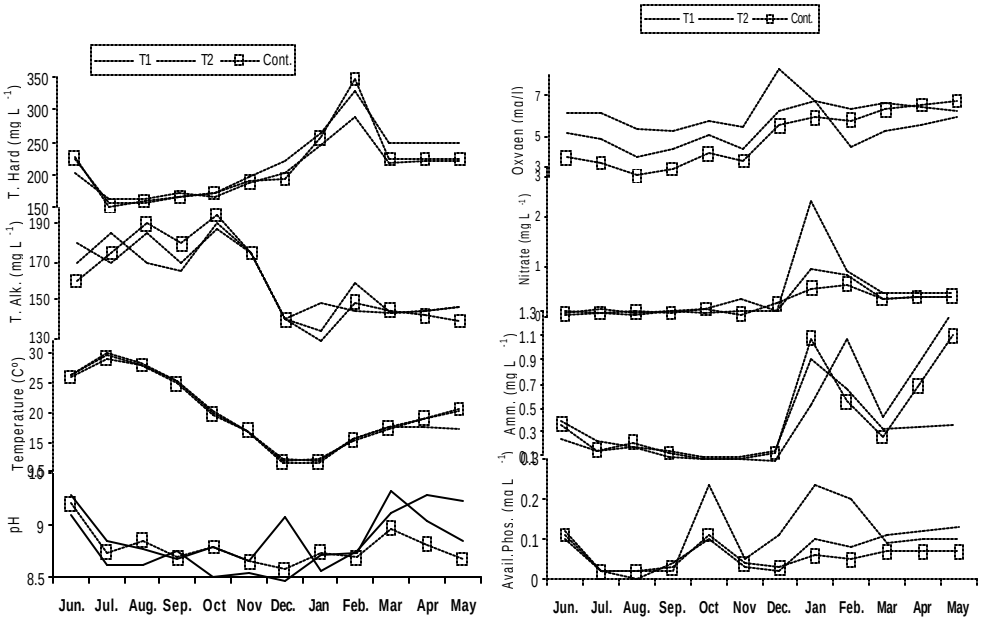

Figure 1. Some physical and chemical parameters during the study period.

8 – Phosphate

Phosphate is known to be the main limiting nutrient for the growth of phytoplankton and chlorophyll a. An increase in soluble phosphate-phosphorus from a reported zero mg L^{-1} at all ponds on September to reading of 0.1 to 0.23 mg L^{-1} for all ponds on October may have been a result of fish feeding

The maximum amounts of phosphate were demonstrated at control, giving remarkable growth of phytoplankton counts and chlorophyll a. highly significant positive correlation exists between phosphate concentration and phytoplankton numbers (Radwan, 1994).

II- Plankton

i- Phytoplankton Standing Crop

At the first grow-out production the ponds fertilized by 150 kg /feddan of chicken manure. These quantity led to more surface scum of cyanobaterial blooms especially *Anabaena* sp. over populations of fish, and oxygen depletion problems. After that the fertilizers decreased to 75 kg acre-1 but the problems were still existed. Finally the ponds were fertilized by 50 kg chicken manure $acre^{-1}$ for 10 weeks, then feeding by 3% w/w for other periods and feeding was checked in the winter season.

The total number of identified and recorded phytoplankton species at all the investigated ponds during the period of study were 69 species (Appendix A) belonging to 4 classes namely; Bacillariophyceae (25), Cyanophyceae (16), Chlorophyceae (22) and Euglenophyceae (6). The maximum count of phytoplankton was recorded during June at T_1 ponds (6167.7 x 10^5 unit L^{-1}) after inoculating green algae, followed by control ponds (4062.8 x 10^5 unit L^{-1}), while the lowest number occurred at T_1 (1878.7 x 10^5 unit L^{-1}) during February (Table 1). It was generally observed that the maximum number of counted species was belonging to class Cyanobacteria which represent the most productive group at all ponds during the period of study.

Phytoplankton community structure in both treatments and control varied significantly over the grow-out period, although the most abundant species varied with time and between treatments and control. The total phytoplankton biomass in the inlet and drainage canals was 44.8 and 247.7 x 10^5 unit L^{-1}, respectively. The inlet and drainage canals were significantly different ($P < 0.05$).

The alga found on most samples was *Anabaena* sp., recorded on 33 of the 36 samples and absent from the counts only in January and February. The greatest number of species and individuals were seen between June and July. These species occurred in quantities that could be considered strong cyanobacterial blooms. The species highly abundant was *Microcystis aeroginosa*, which on January reached a concentration of 1984 x 10^5 unit L^{-1} of pond water at control. That species showed black blooms when the weather was cold (temperature ~7 ºC). Other species (*Gloeocapsa rupestris* and *Chroococcus* sp.) showed somewhat limited blooms in January. All other species were scarce and showed fluctuation in number throughout the study period (Table 2).

The common blue green algae (Cyanobacteria) in the studied ponds are considered less desirable for Nile tilapia growth than green algae (Chlorophyceae) (Turker *et al*., 2003).

Table (3) shows that the abundance of cyanobacteria was minimum in T_1 in the first three months of grow-out periods which reached 130.3 x 10^5 unit L^{-1} in August 2005. The green algae were dominant in the T_1 which inoculated by *Chlorella* + *Scenedesmus* sp. as prevention treatment for three months of grow-out period and reached a minimum in the April 2006.

The highest number of cyanobacteria was in the control and T_2 during all grow-out periods. On the other hand, the concentration of cyanobacteria decreased sharply after inoculation of *Chlorella* + *Scenedesmus* on April 2006 at the beginning appearance of cyanobacterial bloom in T_2. In this study, the green algae can prevent and control the cyanobacterial blooms for two months in Nile tilapia farms at Abbassa, Sharkia , Egypt. This may be these ponds have runoff from Ismaillia canal. The water level of the pond exceeded over the watersheds.

So, sometimes the water drained from the ponds to the drainage canal to maintain the water level in the ponds and the density of green algae decreased. The green algae not only controlled algal blooms but also provides a food source for zooplankton and fish.

Based on results of previous work during the control of cyanobacterial blooms using tannic acid treatments where oxygen depletion was seen that could be due to algae die off (Dawah *et al*., 2006a), *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.

Chlorella and *Scenedesmus* sp. are not drug depressant but may be the perfect food. These algae contain 50-60% protein, much vitamin C and more vitamin B-12, minerals and essential amino acids (Halama 1990).

ii- Pigments

From the results obtained for chlorophyll a contents of the phytoplankton at the ponds, it was more or less similar to the pattern of phytoplankton counts (Table 4). The maximum average contents of chlorophyll a were determined during summer season (July) at control (733 g L^{-1}) while the average minimum values of chlorophyll a were measured during winter (February) at T_1 (130.1 g L^{-1}). In general, inlet canals recorded the lowest content of chlorophyll a. The maximum average contents of chlorophyll b were determined in May at T_2 (241.1 g L^{-1}) while the average minimum values of chlorophyll b were measured in September at T_2 (15.1 g L^{-1}). The chlorophyll c content was increased by increasing chlorophyll b content. On the other hand, c-phycocyanin pigment was decreased by increasing chlorophyll b content.

A multiple correlation analysis including 14 biological variables was carried out for the experiment (Table 5). The correlation coefficient (r) of the significant relationships ($P<0.05$) are only listed. The abundance of chlorophyceae showed negative correlation with the abundance of blue green algae ($r = -0.7$). In

addition, the same negative correlation was established between cyanobacteria and rotifera, copepods and nauplii (r = -0.6). On the other hand, the chlorophyceae count was high positively correlated with rotifera and total zooplankton r = 0.87 and r = 0.85 respectively. Also, the growth of bacillariophyceae showed positive correlation with the rotifera, total zooplankton and chlorophyceae counts r = 0.79, r = 0.82 and r = 0.88 respectively. While, bacillariophyceae numbers showed negative correlation with the abundance of chlorophyceae (r = -0.6). The Chlorophyll c content showed positive correlation with Chlorophyll b content (r = 0.76).

III- Zooplankton

Biomass of zooplankton was estimated monthly during the study period (Table 6). The biomass of zooplankton was increased by inoculating the green algae in T_1 (1866 org. L^{-1}) in June and in T_2 (2270 org. L^{-1}) in April. The total zooplankton biomass in the inlet varied over a smaller range than in the drainage canal. The mean average reached to 120.3 org. L^{-1} in the inlet canal and 249 org. L^{-1} in the drainage canal. Inlet and drainage canals were significantly different ($P < 0.05$).

Results showed that the biomass of rotifera was lower in the control during all grow-out period and T_2 until April (inoculated green algae). The concentration of rotifera was increased sharply during April and May 2006 in T_2 reached to 1930 and 1060 org. L^{-1} that represented 78% and 63% from total zooplankton biomass respectively. Also, the biomass of rotifera was higher in T_1 during the first month of grow-out periods reached 1360 org. L^{-1} that represented 65% from total zooplankton biomass, where these ponds have highest number of green algae. So, the highest green algae lead to the highest biomass of rotifera. Cladocera were found to exhibit an opposite trend. The cladocera was lower in T_1 reached 60 org. L^{-1} in November and higher in the control ponds reached 491 org. L^{-1} in March. No obvious trends were observed for copepoda biomass and nauplii. Nauplii represented the lower biomass in all ponds (Table 7).

CONCLUSION

The green algae can control and protect the fish ponds from cyanobacterial blooms that provide the aquaculturists with a promising management tool for controlling of nuisance cyanobacteria especially *Microcystis* and *Anabaena* sp. However, additional research will be needed to know the effect of continuous addition of *Chlorella* + *Scenedesmus* sp. in the Nile tilapia farms.

RECOMMENDATION

The dense cyanobacterial growths could be usually found in the same ponds at almost all times of the year, therefore, we must disinfect the pond by chlorine (10 ppm is enough, for cyst reduction and decline the problem). Moreover, seeding the ponds by 16 ton live algae per acre (*Chlorella* + *Scenedesmus* sp.) before fish culture or at the starting the cyanobacterial blooms is found to be the best management.

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Table 1. Total monthly counts of phytoplankton in Nile tilapia ponds, inlet and drainage canals

Total count (org x 10^5 L^{-1})					
Month after stocking	T_1	T_2	cont.	Inlet Canal	Drainage Canal
June*	6167.7 [a]	3238.8 [b]	4062.8 [b]	40.7 [d]	373.6 [c]
July	4988.6 [a]	3654.0 [b]	3828.5 [b]	42.03[d]	284.9 [c]
August	2252.9[a]	1900.8[b]	2009.2[b]	53.6[d]	154.7[c]
September	3202.9[a]	2709.3[a]	2914.1[a]	59.2[d]	197.1[c]
October	3184.7[a]	3480.9[a]	3593.5[a]	48.5[d]	198.1[c]
November	3312.9[a]	3615.4[a]	3783.9[a]	51.5[d]	183.1[c]
December	2086.4[a]	2201.7[a]	2184.6[a]	36.5[d]	160.76 [c]
January	2560.2[a]	2859.5[a]	2964.1[a]	32.1[d]	176.0[c]
February	1878.7[a]	2055.2[a]	2364.1[a]	35.2[d]	127.5[c]
March	1922.1[a]	2267[a]	2455.0[a]	39.34[d]	144.8[c]
April**	2437.2[b]	5022.4[a]	3569.5[b]	47.3[d]	510.5[c]
May	2561[b]	4635.4[a]	3358.3[b]	52.2[d]	461.22 [c]
Max	6167.7	5022.4	4062.8	59.2	510.5
Min	1878.7	1900.8	2009.2	32.1	127.5
Mean	3046.3	3136.7	3090.6	44.8	247.7

*Month seeding with *Chlorella* + *Scenedesmus* at the beginning of the fish culture season

**Month inoculating with *Chlorella* + *Scenedesmus* at the starting of cyanobacteria bloom

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

Table 2 The genera of the most abundant phytoplankton found monthly in Nile tilapia ponds, inlet and drainage canals. Values are number (x 10 5 cells L-1) of the dominant species and (in parentheses) the percentage of the total cells L-1 represented by the most abundant genera

Total amount (x 10^5 cell L^{-1})				
Month	T_1	T_2	cont.	Drainage Canal
June*	*Chlorella* 2274 (36.9%) *Scenedesmus* 1674 (27.1%)	*Anabaena* 2964 (91.5%)	*Anabaena* 3364 (94.2%)	*Anabaena* 90.1 (24.1%)
July	*Chlorella* 1574 (31.6%) *Scenedesmus* 1074 (21.5%)	*Anabaena* 3354 (91.8%)	*Anabaena* 3564 (93.1%)	*Anabaena* 103 (36.2%)
August	*Chlorella* 674 (29.9%) *Scenedesmus* 554 (24.6%)	*Anabaena* 1074 (56.5%)	*Anabaena* 1455 (93.1%)	*Anabaena* 42.3 (27.3%)
September	*Anabaena* 774 (23.2%)	*Anabaena* 2044 (75.4%)	*Anabaena* 2342 (72.4%)	*Anabaena* 67.4 (34.2%)
October	*Anabaena* 1553 (48.8%)	*Anabaena* 3112 (89.4%)	*Anabaena* 3254 (80.4%)	*Anabaena* 112.1 (56.6%)
November	*Anabaena* 1984 (59.9%)	*Anabaena* 3211 (88.8%)	*Anabaena* 3411 (90.6%)	*Anabaena* 99.2 (54.2%)
December	*Anabaena* 958 (45.9%)	*Anabaena* 1541 (70%)	*Anabaena* 1612 (90.1%)	*Anabaena* 87.8 (54.4%)
January	*Microcystis* 1456 (56.9%) *Gleocapsa* 527 (20.6%)	*Microcystis* 1735 (60.7%) *Gleocapsa* 878 (30.7%)	*Microcystis* 1984 (66.9%) *Gleocapsa* 900 (30.4%)	*Microcystis* 76.3 (45.1%) *Gleocapsa* 47.6 (27%)
February	*Microcystis* 923 (49.1%) *Gleocapsa* 354 (18.8%)	*Microcystis* 988 (42.7%) *Gleocapsa* 534 (26%)	*Microcystis* 1465 (62.0%) *Gleocapsa* 782 (31.8%)	*Microcystis* 65 (51%) *Gleocapsa* 22.5 (17.6%)
March	*Anabaena* 1056 (54.9%)	*Anabaena* 1665 (73.4%)	*Anabaena* 1998 (81.4%)	*Anabaena* 101.3 (70%)
April**	*Anabaena* 1432 (58.8%)	*Chlorella* 2430 (48.4%) *Scenedesmus* 1240 (24.7%)	*Anabaena* 2055 (50.6%)	*Anabaena* 123.1 (42.1%)
May	*Anabaena* 1874 (73.2%)	*Chlorella* 1345 (29%) *Scenedesmus* 1113 (24%)	*Anabaena* 2544 (75.8%)	*Anabaena* 37.6 (29.8%)

*Month seeding with *Chlorella* + *Scenedesmus* at the beginning of the fish culture season

**Month inoculating with *Chlorella* + *Scenedesmus* at the start of cyanobacteria blooms

Table 3. Monthly average cyanobacteria, chlorophyceae, bacillariophyceae and euglenophyceae abundance found in Nile tilapia grow-out ponds, inlet and drainage canals

cyanobacteria (x 10^5 cell L^{-1})						chlorophyceae (x 10^5 cell L^{-1})				
Month after stocking	T_1	T_2	cont.	Inlet Canal	Drainage Canal	T_1	T_2	cont.	Inlet Canal	Drainage Canal
June*	285.3	3151.2	3480.1	20.7	112.3	5766.1	21.2	18.1	13.7	220.1
July	313.3	3573.3	3743.1	19.1	123.7	4462	20.3	16.2	15.7	130.3
August	130.3	1816.4	1920.2	26.1	50.5	2015	25.1	20.3	18.7	90.5
September	1516.1	2630.3	2819.3	30.3	105.2	1602.2	30.7	25.6	20.3	78.2
October	2015.2	3416.3	3522.2	27.2	130.3	1100.2	28.3	22	14.4	55.2
November	2205.4	3552.1	3721.3	30.1	126.6	1050.3	30	25.2	15.2	45.2
December	1125.6	2152.3	2126	18.2	111.3	910.5	22.3	23.1	12.2	40.2
January	2015.1	2818.1	2916.1	16.6	139.3	502.6	18.1	19.9	10.1	30.3
February	1525.2	2015.2	2315	17.7	90.9	313.4	19.9	18.8	12.3	31
March	1662.2	2225.4	2415	19.3	112.5	215.4	16.2	15.3	13.3	19.9
April**	2216.3	818.3	4015.3	20.7	144	190.3	4051	16.3	18.7	331.1
May	2317.4	905.2	3312.2	22.1	160.6	220.3	3610.1	17.7	20.7	273.1
Max	2317.4	3573.3	4015.3	30.3	160.6	5766.1	4051	25.6	20.7	331.1
Min	130.3	818.3	1920.2	16.6	50.5	190.3	16.2	15.3	10.1	19.9
Mean	1444	2422.8	3025.5	22.3	117.3	1529	657.8	19.9	15.4	112.1
bacillariophyceae (x 10^5 cell L^{-1})						euglenophyceae (x 10^5 cell L^{-1})				
Month after stocking	T_1	T_2	cont.	Inlet Canal	Drainage Canal	T_1	T_2	cont.	Inlet Canal	Drainage Canal
June*	114.1	57.3	61.2	5.1	40.3	2.2	9.1	10.1	1.2	0.9
July	210.2	50.2	57.1	5.9	30.1	3.1	10.2	12.1	1.33	0.8
August	98.6	44.1	52.3	7.3	13.1	9	15.2	16.4	1.5	0.6
September	77.3	37.1	56	8.6	12.9	7.3	11.2	13.2	0. 9	0.8
October	63.1	29.2	41.1	6.3	12.1	6.2	7.1	8.2	0.6	0.5
November	53.1	30.1	33.1	5.8	10.2	4.1	3.2	4.3	0.4	1.1
December	47.3	26.1	32.1	6.1	9.2	3	1	3.4	0	0.06
January	42.5	23.3	28.1	5.4	6.4	0	0	0	0	0
February	40.1	20.1	30.1	5.2	5.6	0	0	0.2	0	0
March	44.3	25.1	24.1	6.7	12.1	0.2	0.3	0.6	0.04	0.3
April**	30.2	153.1	26.1	7.8	35.2	1.4	0	5.1	0.1	0.2
May	23.2	120.1	22.1	9.2	27.3	2.1	0	6.3	0.2	0.22
Max	210.2	153.1	57.1	9.2	35.2	9	15.2	16.4	0. 9	1.1
Min	114.1	57.3	61.2	5.1	40.3	2.2	9.1	10.1	1.2	0.9
Mean	70.3	51.3	38.6	6.6	17.9	3.2	4.8	6.7	0.5	0.5

Table 4. Monthly average of chlorophyll a, b, c and c-phycocyanin (CPC) content in Nile tilapia grow-out ponds, inlet and drainage canals

Chlorophyll a content (µg L^{1})						Chlorophyll b content (µg L^{-1})				
Month after stocking	T_1	T_2	cont.	Inlet Canal	Drainage Canal	T_1	T_2	cont.	Inlet Canal	Drainage Canal
June*	241.8	608.0	703.8	52.7	113.35	203.1	31.3	27.1	15.2	79.9
July	291.1	670.1	733.0	50.1	118.17	179.3	26.1	24.3	20.1	51.6
August	146.9	271.1	325	57.1	67	141.3	33.1	29.1	22.1	23.1
September	203.0	443.2	513	62	78	90.7	15.1	20.3	20.4	19.3
October	230.1	463.3	562	60.1	87	74.5	17.2	18.2	17.1	17.1
November	263.1	387.1	423.1	61.7	85.5	65.0	15.3	16.5	17.9	17.2
December	167.4	210.0	352.3	46.1	73.1	42.1	20.6	19.1	16.0	16.0
January	271.0	531.1	525	47	77.3	37.9	15.1	16.3	15.7	14.2
February	130.1	202	397	49.3	63.7	37.2	31.2	18.9	17.1	13.5
March	140.1	401	422.7	53.2	69.1	34.8	33.7	20.4	19.2	15.2
April**	230.0	510	532.6	61.2	86.2	35.1	228.1	27.1	20.1	67.6
May	256.0	434	630.0	60.0	97.3	36.7	241.1	29.7	22.2	60.2
Max	291.1	670.1	733	62	118.17	203.1	241.1	29.7	22.2	79.9
Min	130.1	202	325	46.1	63.7	34.8	15.1	16.3	15.2	13.5
Mean	214.2	427.6	510.0	55.0	84.6	81.5	59.0	22.3	18.6	32.9
c-phycocyanin CPC content (µg L^{-1})						Chlorophyll c content (µg L^{-1})				
Month after stocking	T_1	T_2	cont.	Inlet Canal	Drainag Canal	T_1	T_2	cont.	Inlet Canal	Drainage Canal
June*	69.7	966.3	981.2	15.1	98.2	150.1	26.1	24.1	15.9	37.7
July	77.3	971.0	991.3	15	110.0	140.0	23.3	23.1	16.3	28.8
August	53.8	168	184.3	17.2	40.3	110.2	25.3	25.5	17.1	19.1
September	87.3	271.2	271	19.3	58.1	97.3	20.1	20.1	16.3	17.1
October	96.0	240	298.3	18.3	57.2	80.2	18.1	19.3	15.1	16.3
November	108.2	204	312.2	19.0	63.1	67.2	17.7	18.1	15.2	16.0
December	56.5	101.0	116.2	20.3	30.3	57.3	18.1	19.3	14.9	15.1
January	93.2	197.2	234.3	15.5	60.3	51.6	15.9	16.1	13.3	15.3
February	87.0	135.2	147.2	14.1	50.7	48.2	19.9	17.7	14.0	14.2
March	115.2	193.2	221.1	21.1	70.2	44.1	20.1	18.9	14.7	14.0
April**	145.2	68.0	243.3	19.2	77.9	45.2	56.3	19.1	15.0	31.2
May	168.1	56.2	263.1	19.3	89.1	48.1	60.2	20.5	18.2	39.9
Max	168.1	971	991.3	21.1	110	150.1	60.2	25.5	18.2	39.9
Min	53.8	56.2	116.2	14.1	30.3	44.1	15.9	16.1	13.3	14
Mean	96.5	297.6	355.3	17.8	67.1	78.3	26.8	20.2	15.5	22.1

Table 5. Correlation coefficients of biological parameters for biological control and prevention of the cyanobacterial blooms using *Chlorella* and *Scenedesmus* in the Nile tilapia farms, the coefficient of the significant correlations ($p < 0.05$) are listed only

		Roti	Clad	Copi	Naup	Tzoo	Tphy	Cyano	Chlo	Bacil	Eug	Chla	Chlb	Cpc	Chlc
Rotifera	Roti	1													
Cladocera	Clad	0.45	1												
Copepoda	Copi	0.37	0.38	1											
Nauplii	Naup	0.33	0.33	0.8	1										
Total zooplankton	Tzoo	0.95	0.63	0.5	0.46	1									
Total standing crops	Tphy	0.66	0.27	0.01	-0.1	0.59	1								
Cyanobacteria	Cyano	-0.6	-0.3	-0.6	-0.6	-0.7	-0.1	1							
Chlorophyceae	Chlo	0.87	0.37	0.42	0.36	0.85	0.71	-0.7	1						
Bacillariophyceae	Bacil	0.79	0.55	0.25	0.24	0.82	0.64	-0.6	0.88	1					
Euglenophyta	Eug	-0.3	-0.2	-0.2	-0.1	-0.3	-0.0	0.27	-0.2	0.01	1				
Chlorophyll "a"	Chla	-0.0	0.17	-0.4	-0.3	-0.1	0.38	0.69	-0.2	-0.1	0.33	1			
Chlorophyll "b"	Chlb	0.87	0.36	0.37	0.38	0.84	0.64	-0.7	0.95	0.87	-0.2	-0.2	1		
C-phycocyanin	Cpc	-0.3	0.2	-0.2	-0.2	-0.2	0.15	0.61	-0.3	-0.1	0.48	0.75	-0.3	1	
Chlorophyll "c"	Chlc	0.53	0.28	0.41	0.31	0.57	0.46	-0.8	0.85	0.76	-0.1	-0.5	0.76	-0.0	1

Table 6. Total zooplankton found monthly in Nile tilapia ponds, inlet and drainage canals.

Total count (org L^{-1})					
Month after stocking	T_1	T_2	cont.	Inlet Canal	Drainage Canal
June*	1866 [a]	770 [b]	696 [b]	115 [d]	315 [c]
July	1546 [a]	610 [b]	730 [b]	105 [c]	404 [b]
August	870 [a]	620 [a]	810 [a]	99 [c]	310 [b]
September	330 [a]	370 [a]	410 [a]	101 [c]	220 [b]
October	312 [a]	350 [a]	301 [a]	89 [c]	170 [b]
November	216 [a]	220 [a]	310 [a]	112 [c]	133 [b]
December	593 [a]	501 [a]	515 [a]	90 [c]	118 [b]
January	870 [a]	733 [a]	815 [a]	87 [c]	121 [b]
February	767 [a]	713 [a]	669 [a]	77 [c]	130 [b]
March	810 [a]	880 [a]	993 [a]	122 [c]	276 [b]
April**	630 [b]	2270 [a]	530 [b]	211 [d]	376 [c]
May	540 [b]	1680 [a]	556 [b]	235 [c]	415 [b]
Max	1866	2270	993	235	415
Min	216	220	301	115	118
Mean	847	813.1	567.4	120.3	249

*Month seeding with *Chlorella* + *Scenedesmus* at the beginning of the Nile tilapia production season

**Month inoculating with *Chlorella* + *Scenedesmus* at the start of cyanobacteria blooms

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

Table 7. The abundance of zooplankton (org L^{-1}) found monthly in Nile tilapia ponds, inlet and drainage canals.

	Rotifera (org L^{-1})			Cladocera (org L^{-1})			Copepoda			Nauplii		
Month after stock	T_1	T_2	cont.	T_1	T_2	cont.	T_1	T_2	cont.	T_1	T_2	cont.
June*	1360	130	122	123	471	481	260	104	63	123	65	30
July	1120	177	219	263	291	390	106	82	89	57	60	32
August	350	160	316	310	207	331	112	151	91	98	102	72
Septemb	140	130	141	101	153	160	60	44	62	29	43	47
October	104	101	83	131	170	151	43	39	36	34	40	31
Novembe	100	93	113	60	100	150	30	12	28	26	15	19
Decembe	183	214	234	229	210	212	120	46	45	61	31	24
January	210	337	471	380	291	320	156	52	13	124	53	11
February	193	174	204	313	309	241	171	136	152	90	94	72
March	321	209	261	333	410	491	106	126	176	50	135	65
April**	201	1930	172	231	160	312	160	105	24	38	75	22
May	192	1060	191	237	416	306	66	118	38	45	86	21
Max	1360	1930	471	333	410	491	260	151	176	124	135	72
Min	100	93	83	60	100	150	30	12	13	26	15	11
Mean	372.8	392.9	210.6	225.9	265.7	295.4	295.4	115.8	84.6	68.1	64.6	66.6

*Month seeding with *Chlorella* + *Scenedesmus* at the beginning of the Nile tilapia production season

**Month inoculating with *Chlorella* + *Scenedesmus* at the start of cyanobacteria blooms

Appendix A: Check list of phytoplankton recorded during the study periods

1- Bacillariophyceae :
Amphora ovalis kütz.
Bacillaria paradoxa Gmel.
Cocconeis placentule Ehr.
Cyclotella comta (Her.)Kütz.
Cyclotella ocellata Pant.
Cyclotella opaerculata (Ag.)
Cymbella affinis Kütz.
Diatoma hiemale
Diploneis didyma Ehr.
Melosira granulata (Ehr.) Ralfs.
*Melosira varians*Ag.
Navicula cryptocephala Kütz.
Navicula cuspidata Kütz.
Navicula gracilis Ehr.
Navicula humerosa Breb.
Navicula anglica Ralfs
Navicula viridula Kütz.
Nitzschia closterium (Ehr.) W.Sm.
Nitzschia longissima (Breb) Ralfs.
Nitzschia acicularis Smith
Surirella stratula (Turp.)
Synedra acus (Kütz.)
*Synedra longissima*W.Sm.
Synedra tabulata Kütz.
Synedra ulna Nitzsch.
2- Cyanophyceae :
Anabaena spiroides Lemmer.
Anabaena flos- aquae
Anabaena circinalis
Anabaenopsis circularis (F.S.West.)Wol&Miller .
Chroococcus dispersus (Keissl.) Lemmer.
Chroococcus limneticus Lemm.
Chroococcus minor (Kütz.) Naegelli
Gloeocapsa rupestris kuetzing . Kütz .
*Merismopedia tenusisema*Lemmer.
Merismopedia punctata Meyen .
Microcystis aerioginosa .kutz. ; emend .Elenkin .
Microcystis incerta Lemmer.
Mrismopedia tenuissima Lemmer .
*Oscillatoria limnetica*Lemmer.
*Oscillatoria tenus*var. *natans* Gomont
Spirulina sp.
3- Chlorophyceae :
Actinastrum hantzschii Lagerh.
Ankistrodesmus falcatus (Chorda) Ralf
Botryococcus braunii Kütz .
Chlorella vulgaris Beij.
Chlorella saccharophila (Kruged)
Chlorella elliposoidea (Gerneck)
Chlorococcum humicola (Nag.)
Closterium kuetzingii Bréb
Crucigenia reciangularis Nag
Dictyospharium polchellam wood
Gonium sociale (Duj.) warming
Oocystis locustris Chodat
Pediastrum boryanum (Turp.) Menegh
Pediastrum duplex Meyen.
Pediastrum simplex var. radianus (af. Chodat)
Scenedesmus acuminatus (Largerh) Chodat
Scenedesmus bijugatus (Turp Kütz)
Scenedesmus quadricauda (Turp) Bréb
Scenedesmus bijuga (Turpin) Lageh
Spirogyra loxissima G.S. West.
Straurastrum paradoxam Meyen
Tetraedron trigonium (af. Reinsch)
4- Euglenophyceae :
Euglena acus Ehr.
Euglena gracilis Kelbs .
Euglena spirogyra Her.
Phacus longicauda (Ehr.) Dujadin.
Phacus pleuronectes (Muell) Dujardin .
Phacus orbicularis Heubner

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