

LABORATORY STUDY FOR INHIBITING THE GROWTH OF *MICROCYSTIS AERUGINOSA* USING *CHLORELLA* AND *SCENEDESMUS* IN THE NILE TILAPIA CULTURE

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Abstract

It is better to control the harmful algal bloom before the bloom develops. This study was a trial for the inhibition of *Microcystis aeruginosa* (Kutz) growth using *Chlorella elliposoidea* (Gerneck) and *Scenedesmus bijuga* (Turpin) mass culture via indoor experiment. The experiment was conducted using 12 glass aquaria, filled with canal water. These aquaria were seeded with green algae (*C. elliposoidea* & *S. bijuga*) at initial density of 20×10^3 cells ml^{-1} . 10 Nile Tilapia (*Oreochromis niloticus*) were stocked in each aquarium. The experimental water was fertilized by chicken manure as 3 mg l^{-1} . First 3 aquaria groups of each replicate were inoculated by *M. aeruginosa* collected from a pond that suffers from the problem. The inoculated doses were 10 , 30 and 50×10^3 cells ml^{-1} for the first three aquaria (D_1 , D_2 and D_3 , respectively). The 4th aquarium of each replicate served as a control without infection (D_0). The experiment was maintained for 10 days after infection.

The inoculated green algae (*Chlorella* and *Scenedesmus* sp.) and *Microcystis* (blue green alga) were counted after 5 and 10 days of infection. After 10 days of infection, there was no *Microcystis* in the 1st aquarium and a minimal number of *Microcystis* in the 2nd but a significant decrease in the number in the 3rd aquarium (which was inoculated with 50×10^3 cells ml^{-1}). On the other hand, the growth of green algae (*Chlorella* and *Scenedesmus*) increased gradually in numbers till the end of the experiment.

The c-phycoyanin was not detected in the control and D_1 after 5 days and in all treatments after 10 days of the experiment.

It could be concluded that, addition of *Chlorella* and *Scenedesmus* spp. at the beginning of the production season (or before the preferable time of cyanobacteria blooms can inhibit the growth and multiplication of *Microcystis* in the production pond.

Key words: Growth inhibiting, *Microcystis*, *Chlorella* and *Scenedesmus*, Nile tilapia, culture

INTRODUCTION

Fertilizing a pond with organic or inorganic fertilizer is often done to initiate an algal bloom, which is another term for an increased population of algae. The natural and artificial fertilizers provide principal chemical nutrients necessary for algal growth and reproduction. The principal nutrients are nitrogen (N), phosphorus (P), and potassium (K). The phytoplankton population takes up these chemical nutrients and at the proper temperature undergoes rapid population growth (Conte, 1990).

Algal blooms are essential to successful fish production because of the dissolved oxygen they produce through photosynthesis during the daylight hours and their uptake (assimilation) of nitrogenous waste products. In a sense, the fish help keep the bloom alive by fertilizing it, and the bloom helps keep the fish alive by producing oxygen and by breaking down and absorbing fish wastes, rendering them harmless to the fish population (Brunson, *et al.*, 2003).

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-offs 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).

A variety of schemes have been proposed for managing phytoplankton blooms, but most approaches have either been unreliable or impractical. Copper sulfate and certain chelated or complexed copper products are currently the only algaecides labeled for use in aquaculture ponds. Copper algaecides are not ideal for off-flavor management because they are not selectively toxic to odor-producing blue green algae. Copper also interacts strongly with other water quality variables, and one important consequence of those interactions is that copper products become more toxic to fish and algae as water hardness and alkalinity decrease (Tucker and van der Ploeg, 1999).

Potassium permanganate, KMnO_4 , is a chemical oxidizing agent that will react with any organic matter in a pond including algae, bacteria, and fish, particulate and dissolved organic and organic bottom sediments.

Copper or permanganate treatments can cause oxygen concentrations to drop, which may result in fish kills. Pond algae are a major source of oxygen production and by removing it this source of oxygen is also removed. In addition, oxygen will be consumed as the algae decompose.

The time to control a toxic algal bloom is before the bloom develops. Preventing fertilizers, animal wastes and other sources of nutrients from reaching the water is the best prevention. Reducing nutrient and pollution runoff from the land has generally been accepted as vitally important in greatly reducing, though not eliminating, the frequency, toxicity and longevity, of harmful algal blooms. High phosphorus is often a precursor to an algal bloom. Nutrient-rich bodies of water such as estuaries, eutrophic lakes, agricultural ponds or catch basins may support a rapid growth of algae. Under

ideal conditions a clear body of water can become very turbid with an algal bloom within just a few days (Webster, *et al.*, 1996).

Virtually all freshwater blooms are caused by cyanobacteria and some of them are toxic. Freshwater algae rarely cause more than oxygen depletion problems due to high density blooms. The severity of the blooms varies from year to year depending on the climate; blooms tend to be worst in particularly dry summers or during droughts and least severe in wetter summers.

In the previous studies we could control the algal blooms using tannic acids (Dawah *et al.*, 2006a) or using *Chlorella* and *Scenedesmus* sp. (green algae) treatments (Dawah *et al.*, 2006b) but these treatments might cause oxygen depletion due to algae die off by tannic acid or increase the algae density by using *Chlorella* and *Scenedesmus* sp. So it might be better to prevent the algal blooms than treatments.

This study is a trial for the prevention of *Microcystis* infection by using *Chlorella* and *Scenedesmus* mass culture via indoor experiment.

MATERIALS AND METHODS

Isolates of *Chlorella elliposoidea* (Gerneck) and *Scenedesmus bijuga* (Turpin) were obtained from Nile water samples. The microalgae were subculture in a solid 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 at WorldFish Center 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 inoculate for production in indoor glass aquaria. The transfer of the algal cells to fish aquaria was achieved at a density of 5×10^6 cells ml⁻¹.

Indoor experiment was carried out in natural light using, 12 glass aquaria as four groups (each aquarium has 100 liters capacity) at WorldFish Center. 3 treatments and control groups were carried out in triplicates. 10 Nile tilapia (*Oreochromis niloticus*) with initial weight of 30 ± 3 g was stocked in each aquarium. The experimental water was fertilized by chicken manure as 3 mg L⁻¹ to allow the propagation of the green algae (*C. elliposoidea* and *S. bijuga*). Aeration was supplemented, provided by a regenerative blower with submerged stones at the bottom of each aquarium.

All aquaria were filled with canal water, having a known species composition count of phytoplankton, chlorophyll "a", "b", "c" and c-phyococyanin content. These aquaria were seeded with green algae (*C. elliposoidea* & *S. bijuga*) at initial density, 20×10^3 cells ml⁻¹ and left for 10 days to propagate the algae, where the average mean of green algae reached 1.2×10^6 cells ml⁻¹ in all aquaria. The first 3 aquaria in

each replicate were infected at day 11 by *Microcystis aeruginosa* collected from a pond that suffering from the problem as 10 , 30 and 50×10^3 cells ml^{-1} as dose rates D_1 , D_2 and D_3 , respectively. The 4th aquarium served as a control without infection by *Microcystis* (D_0). The experiment was maintained for another 10 days after infection. Sampling for chemical, physical and biological analysis in all treatments and control were carried out at the starting time, 5, 10 days intervals from infection by *Microcystis*.

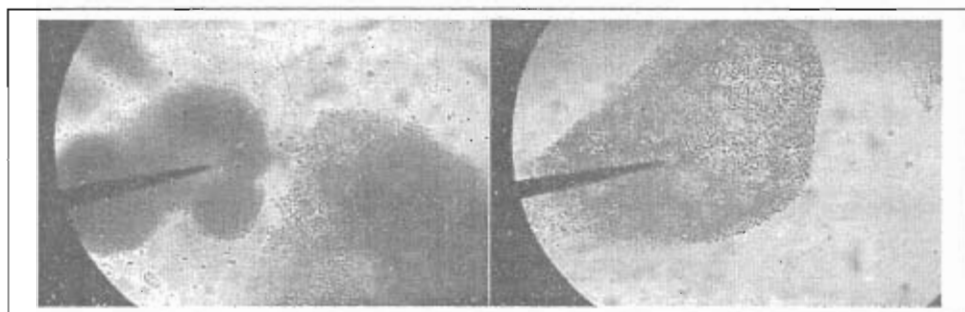


Fig. 1. *Microcystis* cells under microscope which were collected from ponds suffering from *Microcystis* blooms (X 400)

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

Volume to be added =

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

The laboratory investigations were done (by the end of 5th and 10th days of treatment) at WorldFish Center where chlorophyll, C-phycoyanin and phytoplankton were estimated. Also, the physicochemical characteristics of water were determined.

Chlorophyll a, b and c contents were determined in 100 ml water photometrically by using spectrophotometer APHA (1985).

Spectrophotometrically, the C-phycoyanin (CPC) concentration was calculated using Beer's law and an extinction coefficient of $7.9 \text{ L g}^{-1} \text{ cm}^{-1}$ (Svedberg and Katsurai, 1929):

$$\text{CPCgL}^{-1} = A_{625} / 7.9 \text{ L per g cm} \times 1 \text{ cm.}$$

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 a volumetric measuring 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 drawn and placed into Sedgwick-Rafter cell, and then it was microscopically examined for counting after

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

Water temperature ($^{\circ}\text{C}$), and dissolved oxygen (DO , mgL^{-1}) were measured using an oxygen electrode. Water samples were collected to measure the hydrogen ions (pH) by using the ACCUMET pH meter (model 25), total ammonia (mg L^{-1}) by using HACH Comparison (1982), whereas total alkalinity (as CaCO_3 mg L^{-1}), total hardness (mg L^{-1}) and nitrate (NO_3) were determined according to Boyd and Tucker (1992).

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

RESULTS AND DISCUSSION

Results demonstrated in Table (1) revealed the inhibitory effect of *Chlorella* and *Scenedesmus* on the growth of *M. aeruginosa*. The number of *Microcystis* (Cyanobacterium) cells decreased rapidly after 5 days of incubation and continued to decline till disappearing after 10 days of incubation in all aquaria inoculated with *Microcystis* cells in the three doses used (D_1 , D_2 and D_3) as a high dose. In contrary, the growth of green algae (*Chlorella* and *Scenedesmus*) increased gradually in numbers from after incubation with *Microcystis* cells to the end of the experiment. The *Microcystis* was not detected in the group infected by 10×10^3 cells ml^{-1} of *Microcystis* (D_1) at 5 days of incubation. No *Microcystis* was detected in the control group (D_0) using *Chlorella* and *Scenedesmus*.

Diatoms (Bacillariophyta) were increased with increasing *Microcystis* dose from day 5 to day 10. On the other hand, Euglenophyta was inhibited by increasing the dose of *Microcystis* cells.

The cell structure of Cyanobacteria (blue-green algae) resembles that of Gram negative bacteria, (Schoof and Packer, 1987) since it might be possible to use *Chlorella* and *Scenedesmus* in Nile Tilapia ponds not only for control of *Microcystis* but also to control bacterial disease and maintain ecological balance in the pond (Tendencia *et al.*, (2005).

It is better to prevention *Microcystis* cells by (green algae) *Chlorella* and *Scenedesmus* which were used for feeding fish. These are single-cell algae and are not drug depressant but may be the perfect food. Certain freshwater *Chlorella* and *Scenedesmus* are cultured as health foods for humans and animals. These algae

contain 50-60% protein, much vitamin C and more vitamin B-12, minerals and essential amino acids (Halama, 1990). They have also been used in medicinal products and as an antibiotic (Suwapepan, 1984, Hill and Nakagawa 1981).

The results of this study revealed clearly that *Chlorella* and *Scenedesmus* could inhibit the growth of *Microcystis*. Pratt *et al.*, (1944) suggested that *Chlorella* produced an antibacterial substance called chlorellin, also Jones (1988) suggested that *Chlorella* produces more than one antibiotic substance and that one of these may be chlorophyllid, a precursor of chlorophyll. In aquaculture, they are used as food for aquatic animal larvae as well as a biological control agent. Therefore, the efficacy of growth inhibition may be due to released antibacterial substances. This is consistent with the results of Tendencia and dela Pena (2003) who reported that luminous bacteria were not detected in flasks with *Chlorella* sp. after 2 days. Also, Tendencia *et al.* (2005) showed that the luminous bacterial counts in tanks with *Chlorella* sp. were lowest only from day 1 to day 5.

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).

The c-phycoyanin was not detected in the control and D₁ treatments after 5 days of incubation and in all treatments after 10 days of incubation. There was significant difference between D₂ and D₃ after 5 days of incubation but there was insignificant different at 10 days of experiment ($p < 0.05$) Fig. (2). Although, all treatments and control seeded with the same numbers of *Chlorella* and *Scenedesmus*, there was significant difference between the control and the other treatments in the number of green algae and in the chlorophyll b content. The number of green algae and chlorophyll b content increased gradually where the control $< D_1 < D_2 < D_3$ after 5 days of incubation to the end of experiment. This means that the *Chlorella* and *Scenedesmus* could produce antibacterial substances that inhibit the growth of *Microcystis*.

Survival and growth of many fish larvae such as sand goby and hybrid catfish was found to increase when *Chlorella* was added to nursing ponds (Viputanumart *et al.*, 1986. Watcharagonyotin *et al.*, 1992). 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).

On the other hand, among the problems associated with the use of algal cells as fish feed is that the low digestibility of the algal cells which makes the algal biomass unsuitable for rearing fishes. The rigid cell walls of the green algae make them even

more difficult for the fishes to digest (Soeder, 1976). Segner *et al.* (1987) even reported that the larvae of milk fish (*Chanos chanos*) were found to suffer 100% mortality when reared on *Chlorella* sp. Moreover, mixed diets containing several species of microalgae have been reported to give better results for some organisms (Hu 1990). So, in this study, it was used two different microalgae species (*Chlorella* and *Scenedesmus*) for prevention of *Microcystis* sp. and feeding Nile tilapia in aquaculture.

Water quality is an important factor which might affect the growth of fish. The results of water quality are summarized in (Table 2) during the experiment. All chemical parameters of culture waters remained all time with minor changes, but there were significant differences in oxygen and nitrate content between D₃ and other treatments ($p < 0.05$). The dissolved oxygen was significantly decreased with increasing the *Microcystis* dose. In contrary, the nitrate content increased by decreasing the *Microcystis* cells in D₃ treatment. The pH values in all treatments were always toward the alkaline side and insignificantly differences. The available phosphorus was decreased from starting day to the end of experiment in all treatments. Generally, the decrease in available phosphorus (AP) concentration in aquaria culture water may be related to green algae growth. Thus, AP content might be consumed by phytoplankton community. There were positive correlation among AP, chlorophyll "a" and algal density. No mortality appeared during the experiment. All chemical parameters in this study, temperature, oxygen, pH, ammonia, nitrate, total hardness, total alkalinity, and available phosphorus were suitable for fish growth and survival (Boyd 1990).

The total weight gain of Nile Tilapia was insignificantly different between control and all other treatments at the end of experiment.

From the above mentioned study, it could be concluded that, addition of *Chlorella* and *Scenedesmus* species at the beginning of the production season (or before the preferable time of cyanobacteria bloom may inhibit the growth and multiplication of *Microcystis* in the production pond. In order to maintain a stable culture of *Chlorella* and *Scenedesmus* sp in Nile tilapia cultures, further studies are required.

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Table 1. Phytoplankton divisions (cell $\times 10^3$ ml⁻¹) of aquaria culture water inoculated with *Chlorella* and *Scenedesmus* (20×10^3 cells ml⁻¹) on day 5 and 10 after infection by *Microcystis aeruginosa*

Divisions	Initial*	Days	control (D ₀)	<i>Microcystis</i> dose ($\times 10^3$ cell ml ⁻¹)		
				10 (D ₁)	30 (D ₂)	50 (D ₃)
Cyanobacteria		5	0±0 ^a	0±0 ^b	0.05±0.01 ^{Ba}	1.2±0.1 ^{Aa}
	0±0	10	0±0	0±0	0±0 ^b	0±0 ^b
Chlorophyta		5	1350±60 ^{Bb}	1530±50 ^{ABb}	1580±30 ^{Ab}	1620±40 ^{Aa}
	1205±50	10	1545±65 ^{Ba}	1670±30 ^{ABa}	1790±70 ^{Aa}	1795±85 ^{Aa}
Bacillariophyta		5	11.8±0.3 ^{Ca}	12.95±0.15 ^{Bb}	13.55±0.25 ^{Ab}	14±0.2 ^{ABb}
	7.75±0.55	10	13.3±0.5 ^{Ba}	16±0.2 ^{Ab}	15±0.2 ^{ABa}	16.1±0.7 ^{Aa}
Euglenophyta		5	0.09±0.004 ^{Aa}	0.007±0.0005 ^{Bab}	0.006±0.001 ^{Bab}	0.007±0.001 ^{Bab}
	0.04±0.01	10	0.025±0.003 ^{Ab}	0.001±0 ^{Bb}	0.002±0.001 ^{Bc}	0.003±0.0005 ^{Bb}
Total standing crops		5	1361.9±59.7 ^{Bb}	1543±50.2 ^{Ab}	1593.61±30.14 ^{Ab}	1635.2±39.85 ^{Aa}
	1212.79±5.56	10	1558.33±5 ^{Ba}	1686.0±30.7 ^{ABa}	1805±10.2 ^{Aa}	1811.1±84.8 ^{Aa}

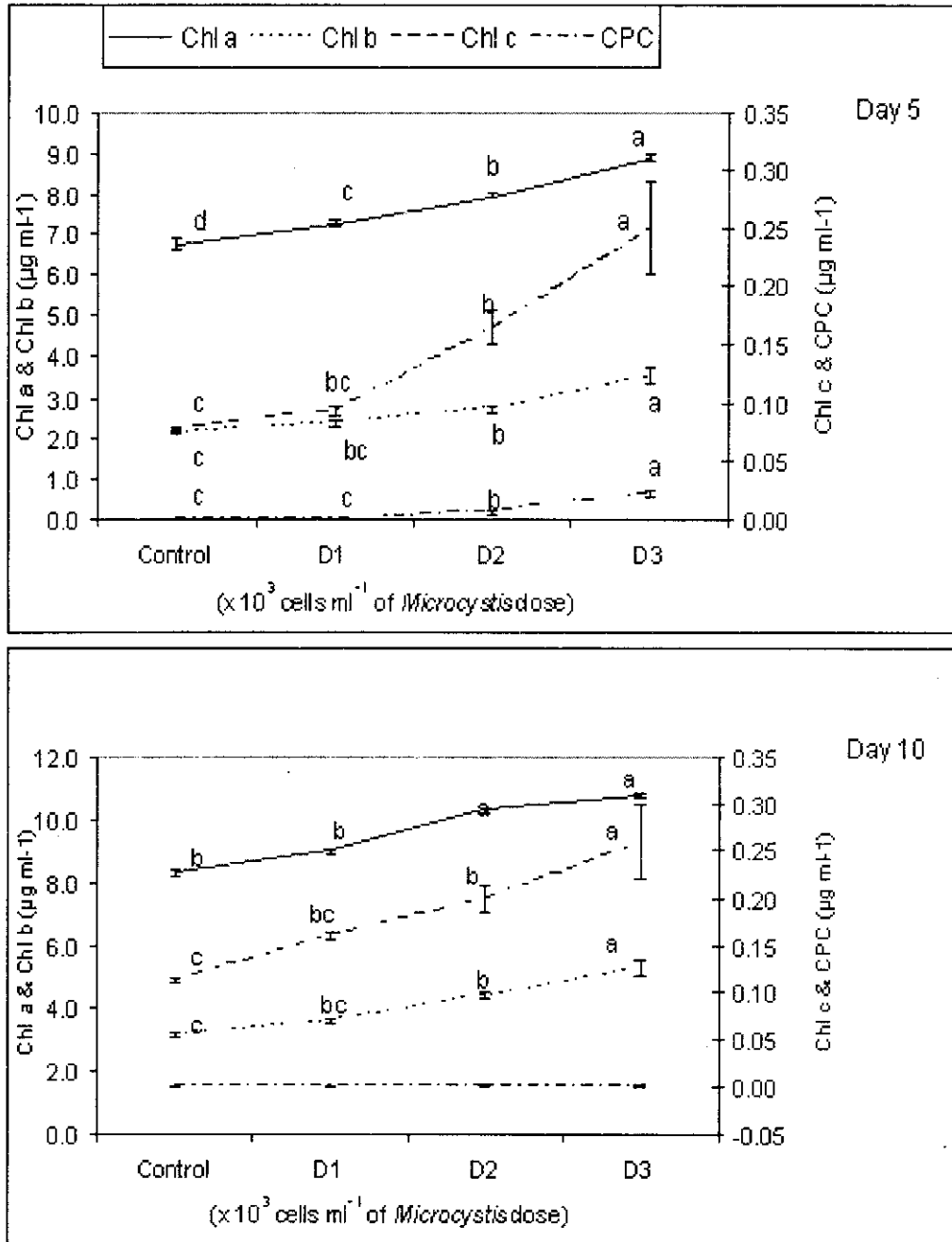
Normal count within the canal water as Cyanobacteria = 0.022±0.005, Chlorophyta = 0.2±0.05, Bacillariophyta = 0.06±0.003, Euglenophyta = 0.007±0.001 and total standing crops = 0.289±0.05 (cell $\times 10^3$ ml⁻¹).

* At day 11 infection of 10, 30 and 50 $\times 10^3$ ml⁻¹ from *Microcystis aeruginosa* as dose rate
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.

Table 2. Some chemical parameters of aquaria culture water inoculated with *Chlorella* and *Scenedesmus* (20×10^3 cells ml⁻¹) on day 5 and 10 after infection by *Microcystis aeruginosa*

Parameters	Initial	Days	control (D ₀)	<i>Microcystis</i> dose ($\times 10^3$ cell ml ⁻¹)		
				10 (D ₁)	30 (D ₂)	50 (D ₃)
Dissolved oxygen (mg L ⁻¹)	7.2±0.2	5	8.15±0.15 ^A	8.15±0.05 ^A	8.1±0.2 ^A	7.55±0.05 ^C
		10	8.35±0.05 ^A	8.15±0.05 ^A	8.23±0.08 ^A	7.75±0.15 ^B
Temperature (°C)	21.3±0.2	5	21.1±0.5 ^A	21.25±0.7 ^A	21.7±0.1 ^A	21.9±0.1 ^A
		10	21.75±0.25 ^A	21.2±0.6 ^A	21.2±0.4 ^A	22.25±0.15 ^A
pH	8.0±0.05	5	8.95±0.05 ^A	8.73±0.03 ^A	8.85±0.25 ^A	8.8±0.2 ^A
		10	8.95±0.25 ^A	8.45±0.15 ^A	9.05±0.45 ^A	9.1±0.1 ^A
Ammonia (mg L ⁻¹)	0.07±0.01	5	0.18±0.02 ^A	0.18±0.04 ^A	0.2±0.04 ^A	0.215±0.005 ^A
		10	0.305±0.005 ^A	0.31±0.01 ^A	0.25±0.01 ^B	0.26±0.0 ^B
T. alkalinity (mg L ⁻¹)	220±2	5	235±15 ^A	230±10 ^A	232±2 ^A	232.5±7.5 ^A
		10	217±1 ^C	256±4 ^A	252±2 ^A	237±3 ^B
T. hardness (mg L ⁻¹)	230±6	5	231±1 ^A	223±1 ^{AB}	207±1 ^B	228±12 ^{AB}
		10	207±1 ^A	215±5 ^A	217±1 ^A	211±1 ^A
NO ₃ (mg L ⁻¹)	0.100±0.02	5	0.05±0.01 ^B	0.09±0.01 ^B	0.09±0.01 ^B	0.25±0.05 ^A
		10	0.1±0.02 ^A	0.11±0.01 ^A	0.06±0.02 ^A	0.15±0.05 ^A
Available phosphorus	1.40±0.03	5	0.239±0.18 ^A	0.01±0.002 ^A	0.015±0.003 ^A	0.028±0.016 ^A
		10	0.017±0.001 ^A	0.006±0.002 ^A	0.01±0.005 ^A	0.01±0.005 ^A
Initial body weight (g/10 fish)		10	292±5 ^A	299.5±6.5 ^A	328.5±31.5 ^A	327±17 ^A
Final body weight (g/10 fish)		10	313±7 ^A	325±5 ^A	351±31 ^A	348.5±17 ^A
Weight gain (g/aquarium)		10	21±2 ^A	25.5±1.5 ^A	22.5±0.5 ^A	21.5±0.5 ^A

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



Chl a= Chlorophyll "a", Chl b= Chlorophyll "b", Chl c= Chlorophyll "c" and CPC= C-phycoyanin.

Fig 2. Pigments contents of aquaria culture water inoculated with *Chlorella* and *Scenedesmus* (20×10^3 cells ml^{-1}) on day 5 and 10 after infection by *Microcystis aeruginosa*

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دراسة معملية لوقف نمو الميكروسيستس اروجينوزا باستعمال نوعين من الخضراء الدقيقة (الكلوريللا و السينيديسمس) في مزارع البلطي النيلي

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١. المعمل المركزى لبحوث الثروة السمكية - مركز البحوث الزراعية- جيزة - مصر
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للسيطرة على إنفجار أعداد الطحالب الضارة (الخضراء المزرقة). أجريت هذه الدراسة كانت لوقف نمو الميكروسيستس اروجينوزا باستعمال الإنتاج الكمي للكلوريللا و السينيديسمس عن طريق التجربة المعملية. أجرت التجربة باستعمال ١٢ حوض زجاجي؛ ملئت كلها بماء القناة (الترعة). وهذه الأحواض السمكية بُدِرت بالطحالب الخضراء (الكلوريللا و السينيديسمس) في الكثافة الأولى؛ 20×10^3 خلية مليلتر⁻¹. وُجِّهَ في كل حوض زجاجي عشرة سمكات من البلطي النيلي. وخصب ماء التجربة بسماذ الدواجن (٣ ملجم لتر⁻¹). و قد لُحِثَت مجموعات الأحواض الثلاثة الأولى من كل معاملة من قبل الميكروسيستس. التي جمعت من بركة كانت تعاني من المشكلة. وكانت الجرعات من الميكروسيستس ١٠، ٣٠، ٥٠، 10×10^3 خلية مليلتر⁻¹ لمجموعات الأحواض الثلاثة الأولى ١٥، ٣٥، و استخدمت مجموعة الأحواض الرابعة كمجموعة ضابطة بدون عدوى (دمر). وأستمرت التجربة ١٠ أيام بعد العدوى.

و قد تم حساب عدد خلايا الطحالب الخضراء (الكلوريللا و السينيديسمس) و خلايا الميكروسيستس (طحالب خضراء مزرقة) الملقحة بعد ٥ و ١٠ أيام من العدوى. إختفت الميكروسيستس في أحواض السمك الأولى بعد ١٠ أيام من العدوى، والعدد الأقل كان في المجموعة الثانية وكان هناك نقص ملحوظ هام في عدد الميكروسيستس في أحواض السمك الثالثة (التي تلقت مع 50×10^3 خلية مليلتر⁻¹ ميكروسيستس). و كان نمو الطحالب الخضراء (الكلوريللا و السينيديسمس) زاد بشكل تدريجي في الأحواض المصابة بالميكروسيستس حتى نهاية التجربة.

إختفت صبغة الفيكوسيانين في المجموعة الضابطة و ١٥ بعد ٥ أيام من الإصابة وفي كل المعاملات بعد ١٠ أيام من الإصابة حيث لم يعطى الكشف عنها أي نتائج.

و يُستنتج من ذلك، أن إضافة الكلوريللا و السينيديسمس في بداية فصل الإنتاج (أو قبل الوقت المفضل لظهور السيانوبكتيريا قد يمنع أو يقلل تضاعف نمو الميكروسيستس في بركة الأسترزاع السمكي.