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Control of *Microcystis aeruginosa* using tannic acid

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ABSTRACT

The use of pesticides or algaecides to control blooms of cyanobacteria can cause massive die-off of phytoplankton populations, resulting in more severe problems. This study was conducted to determine the effectiveness of tannic acid for controlling the growth of the cyanobacteria (*Microcystis aeruginosa*) via indoor study, together with evaluating its safety to fish health and survival. The experiment was carried out in triplicates and extended for 10 days where 120 Nile Tilapia (*Oreochromis niloticus*) were equally stocked in 12 aquaria. First 3 aquaria of each replicate were filled with field surface water containing definite aliquots of cyanobacteria, and a known counted species of phytoplankton, chlorophyll "a", "b", "c" and c-phycocyanin. The tannic acid was thoroughly and homogeneously sprayed on the surface at three doses of 0.6, 1.2 and 2.4ppm for 1st, 2nd and 3rd aquaria; respectively. 4th aquaria served as a control. The count of *M. aeruginosa* was decreased by increasing the rate of tannic acid up to 1.2ppm, where the growth of *M. aeruginosa* was inhibited 96% at day 5 and day 10 after application. There was no observable growth at 2.4ppm. Also, the tannic acid inhibited the chlorophyll "a" content by increasing the dose. After 5 days of treatments, C-phycocyanin (CPC) was inhibited gradually to 61.32%, 99.61% and 100% by using 0.6, 1.2 and 2.4ppm, respectively. Moreover, the using of tannic acid at dose 1.2ppm inhibited CPC completely after 10 days of application. On the other hand, Chlorophyta and Bacillariophyta showed a pronounced growth elevation (-20% and -5.88%, respectively) in cultures treated with 0.6ppm during the first 5th days of exposure. The maximum growth of Chlorophyta and Bacillariophyta were recorded in cultures treated with 1.2ppm at the 5th days (-20% and -11.76%, respectively) in comparison with the control. Curiously enough, the tannic acid addition after 10 days inhibited the growth of Euglenophyta by (42.5%) at 0.6 and 1.2ppm and (57.5%) at 2.4ppm. The hematological parameters revealed a significant decrease in the erythrocytes and hemoglobin values in all groups in comparison to the negative control group. So, it is concluded that, treatment of *M. aeruginosa* (Cyanobacteria) with 1.2ppm tannic acid as a natural plant product resulted in a severe decline in their number that could be attributed to the inhibition of photosynstic pigments.

Introduction

One of the most difficult challenges facing the commercial fish producers is how to keep the constant balance that required maintaining a stable relationship among the water, fish and microscopic flora and fauna in their pond systems.

In commercial fish production ponds, however, natural carrying capacities are greatly exceeded, and a heavily laden artificial ecology is established among the various organisms and the environment in which they live. Important primary components of the ecosystem in a fish production pond are the microscopic algae, or phytoplankton. These microscopic, single cell plants are suspended in the water, and often collected as a "bloom". Like all green plants, phytoplankton produces oxygen during the daylight hours as a by-product of photosynthesis. This is a major source of oxygen in fish pond waters. Blooms are also responsible for consuming much of the oxygen produced. Fortunately, during daylight they usually produce more oxygen than they use, resulting in a surplus for fish and other organisms. At night or in cloudy weather, however, production of oxygen through photosynthesis ceases or is greatly reduced, but the consumption rate does not change, often results oxygen deficiency "budget". Under certain conditions, the level of oxygen can become critically low and fish may suffocate or at least become stressed to the point of being susceptible to some diseases (Brunson et al., 2003).

Significant losses occurred annually in aquaculture, livestock, and waterfowl due to problems of intoxication and "off-flavor" produced by microalgae blooms (Keven et al 2002).

The use of pesticides or algaecides to control blooms of cyanobacteria may cause massive die-off of phytoplankton populations, resulting in more severe problems (Zhao et al., 1997).

Now days, the application of synthetic compounds to the affected aquatic ecosystem is one of the management methods that used to control and prevent the growth of noxious phytoplankton. Unfortunately, many of these synthetic compounds have limitations for their usefulness in controlling phytoplankton blooms including restricted use by Government, broad-spectrum toxicity towards non- target organisms, and public

perception of adverse health risks associated with their use. However, the discovery, characterization, and use of natural compounds for phytoplankton control would provide environmentally safe alternatives to chemical compounds (Keven et al., 2002).

The interference with and/or influence on biological production in cyanobacteria is a feasible approach to avoid these problems in aquacultural management. Some researches have been directed towards the application of tannic acid into aquaculture as a regulator (inhibitor) of microorganism populations in fish pond systems (Zhao et al., 1997).

This study was conducted to determine the effectiveness of tannic acid for controlling the growth of the cyanobacteria (*Microcystis aeruginosa*) using several doses via indoor experiment. Together with evaluating its safety to fish health and survival.

Material and Methods

The experiment was carried out in natural light using 12 glass aquaria (100 liters capacity) at WorldFish Center. 120 Nile Tilapia (*Oreochromis niloticus*) with initial weight of 25±5 gm were used where ten were stocked in each aquarium. Experimented fish were fed daily at a rate 3% of 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 experiment was done in triplicates.

The tannic acid was purchased from Gomhoria Chemical Co. The tannic acid compound was freshly prepared at the specified concentrations by dissolving them in deionized water, pH adjusted to 7.4 with 1 N NaOH and then filter the solution (Chung et al., 1995a).

The aquaria of each replicate were filled with field surface water containing definite aliquots of cyanobacteria, (*Microcystis aeruginosa*) and a known counted species of phytoplankton, chlorophyll “a”, “b”, “c” and c-phycocyanin content that obtained from fish ponds that have the problems. The tannic acid was thoroughly and homogeneously sprayed on the surface at three doses (0.6, 1.2 and 2.4ppm for 1st, 2nd and 3rd aquaria groups; respectively). 4th aquaria group served as a control without any addition. The experiment was maintained for 10 days.

Sampling from all treatments and control were carried out at 0, 5, 10 days intervals while those for hematological and serum biochemical parameters were done by the end of experiment (10 days).

The laboratory investigations were done at WorldFish Center where chlorophyll, C-phycocyanin and phytoplankton were estimated. Also, the physicochemical characteristics of water were analyzed. In addition to, some hematological and serum biochemical analysis of experimental fish.

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 calculated using Beer's law and an extinction coefficient of 7.9 L g^{-1} cm^{-1} (Svedberg & Katsurai, 1929):

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

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 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 drown and placed into Sedgwick-Rafter cell, and then it was microscopically examined for counting after identification of phytoplanktonic organisms. The results were then 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).

Water temperature (^{o}C); and dissolved oxygen (DO, mgL^{-1}) were measured using an oxygen electrode. Water samples were collected to measure both the hydrogen ions (pH) by using the ACCUMET pH meter (model 25) and total ammonia (mgL^{-1}) by using HACH

Comparison (1982). Total alkalinity (as $CaCO_3$ mgL^{-1}), total hardness (mgL^{-1}) and nitrate (NO_3) were determined according to Boyd and Tucker (1992).

Blood samples were collected from the caudal vein of all experimented fish in different groups using sterile syringes; whole blood and serum were prepared. The whole blood used for the determination of hemoglobin, total erythrocytic and leukocytic count as well as differential leukocytic count according to Stoskoph (1993). The serum used for the estimation of alanine amino-tranferase (ALT) (Bergmeyer et al., 1986) and creatinin (Bartels, 1972).

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 statistical analyses were run on the computer, using the SAS program (SAS, 2003).

Results

The count of *M. aeruginosa* decreased with the increase of tannic acid rate up to 1.2ppm, where the growth of *M. aeruginosa* was inhibited by 96% at 5^{th} and 10^{th} day of application. There was no observable growth at 2.4ppm. The tannic acid inhibited the chlorophyll a content. A gradual increase in the inhibition percentage of C-phycocyanin (CPC) to 61.32%, 99.61% and 100% by using 0.6, 1.2 and 2.4ppm, respectively was noticed after 5 days of treatments. Moreover, the use of tannic acid at dose 1.2ppm inhibited CPC completely after 10 days of application (Table 1 and 2).

Although, the tannic acid with a dose of 2.4ppm resulted neither cyanobacteria nor C-phycocyanin (CPC). However, the same dose showed no significant difference in cyanobacteria numbers and CPC by using 1.2ppm of tannic acid ($p<0.05$) (Tables 1 and 2).

On the other hand, the Chlorophyta and Bacillariophyta showed a pronounced growth elevation (-20% and -5.88%, respectively) in cultures treated with 0.6ppm during the first five days of exposure. The maximum growth of Chlorophyta and Bacillariophyta were recorded in cultures treated with 1.2ppm at the first five days (-20% and -11.76%, respectively) in comparison with control. However, the growth of Chlorophyta was decreased during the first five days in the culture treated with 2.4ppm and was

completely inhibited (0.00%) at the end of experiment, and associated with active growth of Bacillariophyta.

Curiously enough, the addition of tannic acid after 10 days inhibited the growth of Euglenophyta by 42.5% at 0.6 and 1.2ppm and 57.5% at 2.4ppm.

The chlorophyll "a" content was gradually inhibited to 81.62%, 90.56%, and 94.94% by increasing the dose of tannic acid on day 5 at the dose 0.6, 1.2 and 2.4ppm, respectively (Table 2). The chlorophyll "a" content showed significant difference between the control and other treatments.

A multiple correlation analysis including 9 biological variables was carried out for the experiment (Table 3). The correlation coefficient (r) of the significant relationships ($P<0.05$) are only listed. The phytoplankton abundance was very highly positively correlated with the count of cyanobacteria ($r = 1.0$). In addition, strong positive correlation was established between total standing crop and pigment fraction namely C-phycocyanin ($r = 0.98$).

The abundance of cyanobacteria showed strong positive correlation with chlorophyll "a" content ($r = 0.96$) and C-phycocyanin ($r = 0.98$) during the experiment.

Table (4) showed that, the initial pH was 8.38 then increased to 8.66 during the course of experiment. The starting ammonia level of the run experiment was 0.036, and then increased in 2.4ppm culture on day 10. The temperature, total alkalinity, total hardness, nitrate-nitrogen contents and the available phosphorus of the water showed insignificant differences in comparison to the control. The dissolved oxygen, pH, and ammonia-nitrogen contents of the water were significantly different ($P<0.05$) in all treatments and control. There was no mortality recorded among experimented fish along the period of experiment. The total harvest of Nile Tilapia showed non significant difference ($P<0.05$) in all treatments and control at the end of experiment.

The hemoglobin and RBCs showed significant decrease values in all treatments and the control positive group in comparison to the negative control group. The total leukolytic count in comparison to the negative control group was significantly high in the positive control and the groups used 0.6 and 1.2ppm but showed a non significant increase value in the group treated by 2.4ppm of tannic acid. The differential leukocytic

count, in comparison to the negative control group, revealed a significant increase in neutrophils and lymphocytes in the positive control group and the groups treated by 0.6 and 1.2ppm but showed a non significant increase in the group treated with 2.4ppm of tannic acid. No marked differences detected in eosinophils and basophiles. The numbers of monocytes were increased in all treatment in comparison to the negative control group. The increase was significant with the positive control group and non significant with other treatments. ALT showed significant (control positive) to non significant (other groups) decrease while creatinin (in all groups) was significantly lower than the negative control group.

Discussion

Blooms produce the greatest amount of oxygen at intermediate densities. As phytoplankton density increases, the amount of oxygen produced per algal cell drops off because of competition for light and nutrients. If it possible to regulate the density of the bloom in a pond, producers might be able to maximize the amount of oxygen produced during the daylight hours in relation to what would be needed at night. However, no practical and effective means of regulating plankton blooms in this manner has been developed. The use of chemicals to regulate algal densities in fish production ponds has generally proved unsuccessful, often resulting in the same problems as a partial or complete phytoplankton die-off. With large amounts of feed entering ponds, blooms return to their previous levels over several days or weeks unless nutrients are somehow removed (Brunson, et al., 2003).

Other work has shown that tannic acid and related compounds were inhibitory to the growth of cyanobacteria both in laboratory growth medium, in augmented pond water and caused pigment release (Chung et al., 1995b).

Tannic acid, astringent vegetable product found in a wide variety of plants. Tannin varies somewhat in composition, having the approximate empirical formula ($C_{76}H_{52}O_{46}$). Tannic acid is a colorless to pale yellow solid; it is believed to be a glucoside in which each of the five hydroxyl groups of the glucose molecule is esterified with a molecule of digallic acid (Leu et al 2002). Tannic acid, a polymeric compound with multiple hydroxyl groups, had a greater capacity for binding protein and glycogen (Zhau et al., 1998). These

bioactive, naturally occurring plant products may offer new opportunities for managerial intervention in the blooming of cyanobacteria.

On the basis of previous work on the growth inhibition and pigment release induced by tannic acid (Chung et al., 1995b). This study showed the interaction between cyanobacteria and tannic acid in order to understand the effect of tannic acid against cyanobacteria especially *M. aeruginosa*. The experimental evidence here has shown, that tannic acid reduced *M. aeruginosa* biomass production. Furthermore, it is quite clear from the above mentioned that, pigment biosynthesis, including chlorophyll a (the dominant photosynthetic pigment) and C-phycocyanin (the major accessory pigment), were almost totally suppressed by the tested compound. This might be due to disruption of the thylakoid membrane (Rai et al., 1989), and the subsequent degradation of photosynthetic pigments.

Tannin compounds induced a release of photosynthesis- related pigments in cyanobacteria (Chung et al., 1995b). This indicates that, the tested compound caused membrane damage and increased cell permeability (Cabral, 1991; Rai, et al., 1990). Zhao, et al., (1998) recorded that treatment of *Nostoc* sp. strain MAC with subinhibitory concentrations of tannic acids and related compounds resulted in a severe decline in biological production, this may be attributed to the inhibition of biosynthesis and to biomaterial leakage.

However, Chlorophyta and Bacillariophyta showed a pronounced growth elevation in cultures treated with 0.6 and 1.2ppm during the first five days of exposure. The role of tannic acid on the growth elevation of Chlorophyta and Bacillariophyta is not clear and need further study.

The changes in dissolved oxygen, pH and ammonia levels were associated with the growth of phytoplankton community (McLarney, 1998).

The hematological parameters revealed a significant decrease in the erythrocytes and hemoglobin values in all groups in comparison to the negative control group. However, the group treated by the higher dose of tannic acid (2.4ppm) showed higher values than other groups including the control positive groups. This observation could indicate that, the decrease in erythrocytes and hemoglobin contents referred as a side

effect to the *Microcystis* and/or their toxins on the experimented fish and prove the safety of tannic acid doses.

The total leukocytic count was increased in all treatments and the positive control group. This increase resulted from the increase in neutrophils and lymphocytes. The increase in these cells and the total count was significant in the control positive group and the groups treated with 0.6 and 1.2ppm but non significant in group treated with 2.4ppm. This observation could confirm that, the higher the dose of the tannic acid, the lower the inflammatory reaction and the immunological response which was parallel to the lower effect of *Microcystis* and/or their toxins on the experimented fish.

The serum analysis revealed no marked changes that might indicate the safety of tannic acid and that the concentration of *Microcystis* and/or their toxins was not enough to affect the liver and kidney functions.

Because the tannic acid concentrations that are toxic to *Microcystis* are not harmful to fish or most other organisms, The aim is that, tannic acid compounds may be safer alternative to copper sulfate or to other biocides that are currently added to water supplies to limit *Microcystis* blooms. Leu et al., (2002) mentioned that, tannic acid extracts from macrophyte *Myriophyllum spicatum* (Haloragaceae) is a highly competitive freshwater macrophyte that produces and releases algaecidal and cyanobactericidal polyphenols.

An accurate assessment of the environmental role of tannic acids and of its potential for the control of *Microcystis* and Euglenophyta species blooms needs more detailed and comprehensive field studies.

Although, the tannic acid with a dose of 2.4ppm was better in inhibition of cyanobacteria and C-phycocyanin (CPC). Also, the fish group treated by the higher dose of tannic acid (2.4ppm) showed higher values in hematological parameters than other fish groups including the positive control groups. However, the same dose (2.4ppm) showed no significant difference in cyanobacteria numbers, CPC and hematological parameters by using 1.2ppm of tannic acid. So, economically, it is advised to treat *M. aeruginosa* (cyanobacteria) with 1.2ppm tannic acid and field application is recommended.

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Table (1): Effect different concentrations of tannic acid on some phytoplankton divisions (x 10^3 cells mL^{-1}) along with the period of experiment

Divisions	Initial	Days	control	0.6ppm		1.2ppm		2.4ppm	
			count	count	% Inh.	count	% Inh.	count	% Inh.
Cyanobacteria	6560±35.2	5	1170±26.5^{Aa}	310±11.5^{Ba}	73.50	46.7±5.78^{Ca}	96.01	0.0±0.0^{Ca}	100.00
		10	846.7±68.9^{Ab}	243.3±24.04^{Ba}	71.26	32.7±4.1^{Ca}	96.14	0.0±0.0^{Cb}	100.00
Chlorophyta	0.12±0.004	5	0.15±0.006^{Ba}	0.18±0.003^{Aa}	-20.00	0.18±0.006^{Aa}	-20.00	0.17±0.01^{ABa}	-13.33
		10	0.18±0.01^{Aa}	0.19±0.017^{Aa}	-5.56	0.19±0.012^{Aa}	-5.56	0.18±0.006^{Aa}	0.00
Bacillariophyta	0.15±0.002	5	0.17±0.003^{Ba}	0.18±0.006^{ABa}	-5.88	0.19±0.006^{Aa}	-11.76	0.2±0.009^{Aa}	-17.65
		10	0.183±0.009^{Ba}	0.23±0.009^{Ab}	-25.68	0.2±0.012A^{Ba}	-9.29	0.21±0.012^{ABa}	-14.75
Euglenophyta	0.05±0.002	5	0.03±0.006^{Ba}	0.033±0.003^{ABa}	-10.00	0.04±0.006^{ABa}	-33.33	0.05±0.003^{Aa}	-66.67
		10	0.04±0.006^{Aa}	0.023±0.003^{Ba}	42.50	0.023±0.003^{Ba}	42.50	0.017±0.003^{Bb}	57.50
Total standing crops	6560.32±35.3	5	1170.35±26.46^{Aa}	310.39±11.5^{Ba}	73.48	47.08±5.79^{Ca}	95.98	0.413±0.018^{Ca}	99.96
		10	847.07±68.9^{Ab}	243.7±24.04^{Ba}	71.23	33.08±4.08^{Ca}	96.09	0.4±0.007^{Ca}	99.95

% 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

Table (2): Effects different concentrations of tannic acid on some pigments contents (µg mL-1) along the period of experiment

Pigment	Initial	Days	control	0.6ppm		1.2ppm		2.4ppm	
			Amount	Amount	% Inh.	Amount	% Inh.	Amount	% Inh.
Chlorophyll "a"	9.14±0.1	5	5.93±0.57^{Aa}	1.09±0.1^{Ba}	81.62	0.56±0.05^{Ba}	90.56	0.3±0.05^{Ba}	94.94
		10	3.01±0.21^{Ab}	0.61±0.06^{Bb}	79.73	0.33±0.04^{Bb}	89.04	0.173±0.03^{Cb}	94.35
Chlorophyll "b"	1.12±0.07	5	0.84±0.03^{Ba}	1.1±0.06^{Aa}	-30.95	1.11±0.06^{Aa}	-32.14	1.07±0.09^{Aa}	-27.38
		10	0.67±0.04^{Ab}	0.7±0.06^{Ab}	-4.48	0.6±0.06^{Ab}	10.45	0.7±0.06^{Ab}	-4.48
Chlorophyll "c"	1.1±0.05	5	1.02±0.04^{ABa}	1.2±0.06^{Aa}	-17.65	0.99±0.06^{Ba}	2.94	1.0±0.06^{Ba}	1.96
		10	0.7±0.06^{Ab}	0.8±0.06^{Ab}	-14.29	0.77±0.09^{Ab}	-10.00	0.77±0.03^{Ab}	-10.00
C-phycocyanin	8.35±0.5	5	5.17±0.47^{Aa}	2.0±0.12^{Ba}	61.32	0.02±0.003^{Ca}	99.61	0.0±0.0^{Ca}	100.00
		10	3.63±0.26^{Ab}	1.3±0.15^{Bb}	64.19	0.0±0.0^{Cb}	100.00	0.0±0.0^{Cb}	100.00

% 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

Table (3): Correlation coefficients of biological parameters for inhibitory effect of tannic acid on *Microcystis aeruginosa* at all periods
Listed is only the coefficient of the significant correlations (p< 0.05)

		Cyano.	Chlo.	Bac.	Eug.	Tot.	Chl. a	Chl. b	Chl. C	CPC
Cyanobacteria	Cyano.	1.00								
Chlorophyta	Chlo.	-0.39	1.00							
Bacillariophyta	Bac.	-0.51	0.40	1.00						
Euglenophyta	Eug.	0.08	-0.23	-0.23	1.00					
Total standing crops	Tot.	1.00	-0.39	-0.51	0.08	1.00				
Chlorophyll "a"	Chl. a	0.96	-0.50	-0.56	0.12	0.96	1.00			

Chlorophyll "b"	Chl. b	-0.15	-0.31	-0.17	0.49	-0.15	-0.09	1.00		
Chlorophyll "c"	Chl. C	0.04	-0.22	-0.37	0.45	0.04	0.13	0.66	1.00	
C-phycocyanin	CPC	0.98	-0.42	-0.51	0.04	0.98	0.91	-0.10	0.10	1.00

Table (4): Some chemical parameters and total fish harvest along the period of experiment

Item	Initial	Days	control	0.6ppm	1.2ppm	2.4ppm
Dissolved oxygen (mg L^{-1})	6.52±0.8	5	$7.3±0.83^{AB}$	$5.0±1.4^{B}$	$8.3±2.03^{AB}$	$9.87±0.17^{A}$
		10	$6.4±0.85^{A}$	$7.2±1.3^{A}$	$6.03±1.6^{A}$	$7.73±0.91^{A}$
Temperature (ºC)	19.3±0.16	5	$19.6±0.13^{A}$	$19.2±0.19^{A}$	$19.4±0.21^{A}$	$19.07±0.23^{A}$
		10	$20.6±0.27^{A}$	$21.1±0.06^{A}$	$20.6±0.35^{A}$	$21.3±0.15^{A}$
pH	8.38±0.05	5	$8.04±0.07^{AB}$	$7.9±0.06^{B}$	$8.21±0.23^{AB}$	$8.5±0.08^{A}$
		10	$8.6±0.04^{A}$	$8.27±0.14^{A}$	$8.6±0.25^{A}$	$8.66±0.05^{A}$
Ammonia (mg L^{-1})	0.036±0.02	5	$0.04±0.02^{B}$	$0.053±0.015^{B}$	$0.14±0.07^{AB}$	$0.27±0.05^{A}$
		10	$0.15±0.1^{B}$	$0.14±0.02^{B}$	$0.24±0.11^{AB}$	$0.42±0.04^{A}$
T. alkalinity (mg L^{-1})	265±20.2	5	$251.7±17.4^{A}$	$248.3±1.7^{A}$	$250±2.89^{A}$	$245±10.4^{A}$
		10	$283.3±6^{A}$	$285±14.4^{A}$	$290±2.89^{A}$	$295±13.2^{A}$
T. hardness (mg L^{-1})	208±5.1	5	$214.7±4.7^{A}$	$225.3±3.7^{A}$	$218.7±9.3^{A}$	$213.3±5.7^{A}$
		10	$235.3±6.6^{A}$	$235.3±6.1^{A}$	$240.7±14.6^{A}$	$251.3±3.53^{A}$
NO3 (mg L^{-1})	1.442±0.06	5	$1.35±0.04^{A}$	$1.61±0.1^{A}$	$1.4±0.12^{A}$	$1.46±0.22^{A}$
		10	$1.9±0.26^{A}$	$1.7±0.19^{A}$	$2.16±0.09^{A}$	$2.68±1.02^{A}$
Available phosphorus	0.113±0.03	5	$0.022±0.003^{A}$	$0.007±0.002^{B}$	$0.007±0.003^{B}$	$0.016±0.007^{AB}$
		10	$0.0±0.0^{B}$	$0.014±0.01^{AB}$	$0.0±0.0^{B}$	$0.075±0.04^{A}$
Initial body weight (g/10 fish)		10	$209.7±14.8^{B}$	$242.7±9.2^{AB}$	$247.7±11.57^{A}$	$219.3±1.9^{AB}$
Final body weight (g/10 fish)		10	$228±15.6^{B}$	$261.3±9.87^{AB}$	$266±10.7^{A}$	$237.3±1.45^{AB}$
Total harvest (g/aquarium)		10	$18.3±1.85^{A}$	$18.7±0.7^{A}$	$18.3±0.88^{A}$	$18±0.58^{A}$

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

Table (5): Effects different concentrations of tannic acid on some hematological and biochemical parameters of experimented fish at day 10 of experiment

Parameters	$Cont_{-ve}$	$Cont_{+ve}$	0.6ppm	1.2ppm	2.4ppm
Hb (g dL^{-1})	$4.942±0.22^{A}$	$3.54±0.25^{B}$	$3.737±0.22^{B}$	$3.693±0.32^{B}$	$4.07±0.4^{B}$
RBCs (10^6 uL^{-1})	$1.4±0.04^{A}$	$1.1±0.05^{B}$	1.165±0.05B	$1.142±0.06^{B}$	$1.23±0.07^{B}$
TLC (10^3 uL^{-1})	$39.8±0.76^{B}$	$48.5±1.8^{A}$	$46.9±1.49^{A}$	$47.9±1.9^{A}$	$44.0±1.5^{AB}$
Neutrophils (10^3 uL^{-1})	$5.06±0.44^{B}$	$8.76±0.78^{A}$	$8.266±0.68^{A}$	$8.287±0.77^{A}$	$7.01±0.9^{AB}$
Lymphocyte (10^3 uL^{-1})	$33.3±0.4^{B}$	$37.81±1.02^{A}$	$36.98±0.84^{A}$	$37.83±1.17^{A}$	$35.56±0.58^{AB}$
Esinophi (10^3 uL^{-1})	$0.35±0.04^{AB}$	$0.24±0.11^{B}$	$0.187±0.08^{B}$	$0.517±0.08^{A}$	$0.32±0.1^{AB}$
Basoph (10^3 uL^{-1})	$0.12±0.06^{A}$	$0±0^{A}$	$0.0540.05^{A}$	$0.039±0.04^{A}$	$0.05±0.05^{A}$
Monocyte (10^3 uL^{-1})	$0.94±0.17^{B}$	$1.68±0.2^{A}$	$1.460.23^{AB}$	$1.223±0.2^{AB}$	$1.06±0.2^{AB}$
ALT (U L^{-1})	$56.4±7.2^{A}$	$27.6±8.3^{B}$	$40.47.6^{AB}$	$22.67±7.1^{B}$	$32.2±7.0^{B}$
Creatinin (m dL^{-1})	$0.64±0.03^{A}$	$0.4±0.04^{B}$	$0.427±0.05^{B}$	$0.428±0.05^{B}$	$0.42±0.04^{B}$

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

مكافحة نموالميكروسيست ايروجينوزا بإستعمال

حامضَ التنّيك

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إن إستعمال مبيداتِ الحشرات أو مبيدات الطحالب للسَيْطَرَة على ظهور السيانوبكتريا يُمْكِنُ أنْ يُسبّبا موتَ هائلَ للهائمات النباتية مما ، يُؤدّي إلى مشاكل أكثر حدّةِ.

أجرتْ هذه الدراسةِ لتَحديد فعالية حامضَ التنّيك للسَيْطَرَة على نمو الطحالبِ الخضراء المزرقهِ (الميكروسيست ايروجينوزا) عن طريق الدراسةِ المعمليةِ، مع تَقييم مدى أمانِه للإبقاء على صحةِ الأسماك.

نُفّذتْ التجربة في تكرار ثلاثى لمدة 10 أيامِ حيث جُهّزَ 120 سمكة من البلطى النيلى موزعة بالتساوى على 12 حوض زجاجى. مُلِى كُلّ أحواض السمك بماءِ الحقل السطحي يَحتوي عدد معروف مِنْ السيانوبكتريا ، عَرفَ نوعَه وعدد كُلّ من الهائمات النباتية ، و كلوروفيل "ا" ، "ب" ، "ج" و صبغة الفيكوسيانبن (CPC) تم استخدام ثلاثة جرعات من حامض التنّيك 0.6, 1.2 ، 2.4 ملجم لتر$^{-1}$ ماء وقد تم رُشّهاَ بتجانس على أسطح أحواض الأسماك الأولى و الثانيةِ و الثالثةِ ؛ على التوالي. عَملتْ أحواضُ السمك الرابعة كمجموعة ضابطة.

نُقِصَ عدد الميكروسيست بزيَادَة نسبةِ حامضِ التنّيك حتى 1.2 ملجم لتر$^{-1}$ ماء ، حيث ثبت نمو الميكروسيست بحوالى 96 % في يومِ الخامس واليومِ العاشربعد التطبيق. أيضاً لم يلاحظ نمو الميكروسيست باستخدام حامضِ التنّيك بجرعة 2.4 ملجم لتر$^{-1}$ ماء ، مَنعَ حامضَ التنّيك محتوى الكلوروفيلَ" أ " بزيَادَة الجرعةِ بعد 5 أيامِ مِنْ المعالجةِ، وقل صبغة الفيكوسيانبن بشكل تدريجي إلى 61.32 %, 99.61% و100 % بإستعمال حامضِ التنّيك بجرعات 0.6, 1.2 ،2.4 ملجم لتر$^{-1}$ ماء ، على التوالي. علاوة على ذلك، إستعمال حامضِ التنّيك في جرعةِ 1.2ملجم لتر$^{-1}$ ماء مَنعَ منع ظهور الفيكوسيانبن بالكامل بعد 10 أيامِ مِنْ التطبيق.

من الناحية الأخرى، أوضحت الدراسة إرتفاعَ نمو الطحالب الخضراء والدياتومات شوّفا a أعلنَ (-20% و-5.88%، على التوالي) في الأحواضِ المعالجةِ ب0.6 ملجم لتر$^{-1}$ ماء أثناء اليومِ الخامس. وسُجّل النمو الأقصى للطحالبِ الخضراءِ والدياتومات في الأحواضِ المعالجةِ ب1.2 ملجم لتر$^{-1}$ ماء في اليومِ الخامس (-20% و-11.76%، على التوالي) بالمقارنة بالمجموعة الضابطةِ.

وأتضح من التجربة ان إضافة حامضَ التنّيك بعد 10 أيامِ مَنعتْ نمواليوجيلينات بأستخدام كل مِن 0.6 و 1.2 ملجم لتر$^{-1}$ ماء بنسبة (42.5 %) و كانت النسبة بأستخدام تركيز2.4 ملجم (57.5%).

وقد أوضحت اختبارات الدم الى حدوث نقص فى كرات الدم الحمراءِ في كُلّ المجموعاتِ المعالجة بالمقارنة مع المجموعة القياسية السلبية.

وقد خلصت الدراسة الى ان معالجة الميكروسيست (السيانوبكتريا) ب1.2 ملجم حامضِ تنّيك لتر$^{-1}$ ماء كمنتج نباتي طبيعي أدّى إلى نقص شديد فى أعدادها الذي يُمْكِنُ أنْ يُنْسَبَ إلى منع صبغاتِ النباتية.