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The Isolation , the purification and the identification of Hepatotoxin Microcystin-LR from two cyanobacterial species and studying biological activity on some aquatic organisms

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Emad Y. A. Al-Sultan
Dept. of Biology, College of Education, University of Basrah
Email: emad_yousif2000@yahoo.com

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Abstract:

The study was included the isolation , the purification and the cultivation two cyanobacterial species isolated from AL-Khandaq river , Basrah city/ southern of Iraq represented by species *Nostoc muscurum* (Agardh) and *Anabaena variabilis* (kuetz.) in addition to green algae (non- toxic alga) *Chlorella vulgaris* Beijerinck with measuring their growth constant (K) and generation time (G) . This study showed that the ability of two cyanobacterial species to produce toxins type hepatotoxins (Microcystin-LR) as the first time in Iraq especially for the two types above. Hepatotoxin was identified after the extraction and the purification by using HPLC technique (High performance liquid chromatography) to detect the purity and the concentration of this toxin , in addition Infra-red technique was used to investigate chemical active groups of hepatotoxoins. The Concentration of hepatotoxins reached to 28.57 µg/ml for alga *N. muscurum* and 4.61 µg/ml for species *A. variabilis* respectively . The two cyanobacterial species were used as food for three economic fish larvae represented by *Cyprinus carpio* L., *Hypophthalmicthyes* molitrix VaL. and *Ctenopharyngodon idella* Val. The results showed significant decreasing in mean of weight , length and survival percentage for all fish larvae when fed on toxic species compared with fed on non- toxic species *C. vulgaris* . Also the results showed toxic effect of two cyanobacterial species on some zooplankton species represented by *Artemia salina* , *Daphnia magna* and *Brachionus calyciflorous* after fed on toxic species . This feeding led to significantly decreasing $p \leq 0.05$ in the mean numbers of zooplankton compared with those fed on non- toxic microalga *C. vulgaris* .

Keywords: Hepatotoxins (Microcystin-LR) *Nostoc muscurum* , *Anabaena variabilis*, fish larvae , Zooplankton

Introduction:

One of the most interesting of five cyanobacterial orders is the Nostocales , defined by their ability to form specialized nitrogen-fixing cells known as heterocysts , many members of this order produce toxins , These metabolites include among others , the hepatotoxins Microcystins , Nodularins and Cylindrospermopsins , as well as Neurotoxins anatoxin-a , Anatoxin-a(s) and patalytic shell fish poisons (PSP) of which Saxitoxin (STX) is the parent compound [1] .

Microcystins (MCs) which are produced by several strains of planktonic genera *Microcystis* , *Plantothrix* (*Oscillatoria*) and *Anabaena* as well as by benthic or terrestrial *Nostoc* and *Hapalosiphon* are the most prevalent cyanobacterial toxins , cyanotoxins [2] .

Cyanobacteria (Blue - green algae) produce toxins that may present a hazard for drinking water safety and for other organisms , these toxins are structurally diverse and their effects range from liver damage , including liver cancer to neurotoxicity. Microcystin-LR (Leucine R, L-arginine) is the best studied cyanobacterial toxins and the World Health Organization has recently set a new provisional guideline value for Microcystin-LR of µg / L drinking water [3] .

Fish productivity in fresh water is correlated to flourishment of phytoplanktonic components , bacteria and algae, In addition [4 ,5] to Cyanobacterium blooms have been detected in fresh water ponds and lakes all over the world . Some of these cyanobacteria were found to be the source of some potent toxins [6] and Cyanobacteria were recorded to represent up to 17% of the phytoplankton investigated in fresh water ponds [7].

In spite of the great importance of cyanobacteria for aquatic life nutrition , specially for fish growth and productivity many of them were recorded to be highly toxic affecting the growth of aquatic organisms [8] .

The bioactivity of Microcystins is mainly based on an inhibition of eukaryotic protein phosphatases [9] However , it has been demonstrated that some of these substances (Hepato and Neurotoxins) also have a lethal , sub lethal and allelopathic impact on many aquatic invertebtates [10] Numerous physiological and metabolic processes in particular organisms are usually affected such as reducing grazing intensity reproduction and development of juvenile stages are inhibited [11] .

Toxic cyanobacteria are increasingly and environmental problem as consequence of their mass occurrences in aquatic ecosystems . Microcystins are cyclic heptapeptides produced by fresh water cyanobacteria including several strains from the genera *Anabaena* , *Microcystis* , *Plantothrix* and two strains of genus *Nostoc* [12] ,as well as in Iraq several species were detected as toxic cyanobacterial by their ability to produce Hepatotoxin (Microcystin-LR) represented by species *Microcystis aeruginosa* , *Microcystis flos-aque* , *Calothrix parietina* and *Hapalosiphon welwitshii* [13 , 14] .

The aim of this study is the illustration ability of two cyanobacterial species *Nostoc muscurum* and *Anabaena variabilis* to produce Hepatotoxin (Microcystin-LR) and their bioactivity on some economic fish larvae and several zooplankton tothe visualization of their toxicity .

Materials and Methods

1- Algal Samples collection and growth measurement

Water samples were collected from Al-Khandaq canal in (Basrah) Governorate / southern of Iraq by using phytoplankton net and then stored in plastic bottles (500 ml) until arriving to the laboratory .

Algal species were isolated by using streaking method on solid and then liquid ch-10 medium respectively [15] , Unialgal and axenic cultures were made according to [16].

Growth curve of two algal species was made by the measurement of the concentration of chlorophyll-a according to [17] , The growth constant (K) and the generation time (G) was measured .Algal species were classified according to [18 , 19, 20] .

2- Extraction and purification of hepatotoxins (Microcystin-LR)

The axienic culture for two toxic species were harvested after 5-6 days from the stationary phase by centrifuge at 3000 rpm , The harvested algal cells were lyophilized by using freezing drier type (Lab ConCo-18) .

Extraction toxins were made according [21] by taking 0.5 g from lyophilized alga and then mixed with organic mixture (Methanol: n-Butanol : water) in portion (4:1:15) respectively .

The purification of toxins was made by using gel filtration chromatography [22] , the column size (2×15) cm filling with silica gel mesh (100-200) . The column was washed with three eluents respectively (Deionized water , Methanol 20%, Methanol 80% The fraction eluated by 80% methanol) was concentrated and lyophilized .

3-The determination of quantity and purity of Hepatotoxin (MC-LR)

1- HPLC technique analysis

The toxin fraction was soluted in 1ml of absolute methanol speciallized used for HPLC analysis , and then put in sonicator for 5 minutes , 20 µl was injected by microsyring to the HPLC type (Shimadzu) have the following characters (Reverse phase (15×4.6 mm I.d)-C18 column 15 µm particles size) The mobile phase (65 : 35 v/v) of (Methanol : 5µm Buffer phosphate , flow rate (1ml / min) at wave length 239 nm [23] .The results were compared with an absorbance and retention time of standard hepatotoxin (Microcystin - LR) produced from Alexis Biochemical Company .

2- Infra - red spectrum

The Infra-red spectrum for purified toxins was measured by using fourier transform Infra-red spectrophotometer (FT IR – 84005) , 0.5 mg were mixed with potassium bromide (KBr) as disk in portion 3:1 and the wave number ranges between (600-3600 cm^{-1}) .

4-Source of fish larvae

Common carp (*Cyprinus carpio* L.) and Grass carp (*Ctenopharyngodon idella* Val.) larvae were obtained from invertebrates departments / marine science center /Basrah university .While Silver carp (*Hypophthalmicthyes molitrix* VaL.) Larvae were obtained from fish research center / Baghdad . Three types of fish larvae range between 7-10 days in age .

Carp larvae (mean weight 6mg , mean length 6 mm) silver carp (mean weight 6.2 mg , mean length 6.5 mm) and grass carp larvae (mean weight 6 mg , mean length 6.5 mm) each larval species were stocked separately in glass aquarium (35×60×30) cm containing 20 liter of dechlorinated tap water at the rate 2 larvae / L fed on yolk eggs during the adaptation period (24 hr) , at 25C^{o} ± 2 with continuous aeration .

5-Feeding experiment

a-Fish feeding

Three types of fish larvae were fed separately in glass aquarium (15×30×20) cm containing 6 liter of dechlorinated tap water under the same condition above .Three replicate were made for each treatment ,common carp and grass carp (10 larvae for each replicate) , While silver carp (6 larvae for each treatment) . The treatment group represented by two toxic algal species *Nostoc muscurum* , *Anabaena variabilis* and one species of green-algae (non -toxic) namely *Chlorella vulgaris* as a control group . Each aquarium was fed on 10% v/v from axienic

cultures for two types of toxic and non-toxic microalgae separately when the algal cultures reached to the stationary phase of its growth exactly (5-6) days after reaching this phase . The weight and the length of fish larvae were recorded at the beginning and end the of all treatments , while the survival rate of larvae was recorded at the long of experiments .

b-Zooplankton feeding

1. Daphnia magna

D. magna were collected from fresh water ponds in Basrah University / Garmat Ali by using zooplankton net (mesh size 200 µm) . salinity of water ponds reaches 1.3-1.5 ppt , pH = 7.77 and dissolved oxygen 3.6 mg / L . *D. magna* were acclimated (48 h) in aquarium (40 × 30 × 30) cm and fed on yeast cells with aeration before experiments . *D. magna* were fed on toxic microalgae *N. muscurum*, *A. variabilis* and non-toxic microalgae *C. vulgaris* in conical flasks 250 ml filling with 150 ml sterilized tape water , 10 individual were fed on each treatment (three replicate were made) , each treatment were inoculated with 2ml of algal cultures separately when reach to 5-6 days from stationary phase for each algal species . survival percentage were measured after 120 hr from feeding experiments by using Bogorove chamber .

2. Brachionous calyciflorus

B. calyciflorous were collected from pollutant canal with animal dung nearly Marine research center / Basrah university/ Iraq by using zooplankton net (mesh size 200µm). sample has salinity 21 ppt , pH = 8.53 . samples were filtered from filter paper type GF/C and then repeated washing with tape water , *B. calyciflorous* acclimate on class aquarium (15 × 30× 20) cm containing 6 L of filtration water samples , Rorifers were fed on yeast cells before experiments .

B. calyciflorus were fed on toxic microalgae *N. muscurum , A. variabilis* and non-toxic microalgae *C. vulgaris* on conical flasks 100 ml containing 50 ml sterilization filtering water sample , each one inoculated with 1ml of algal cultures after reaching 5-6 from stationary phase , 1ml were added from *B. calyciflorus* culture for each replicate containing (29-43) individuals, *Brachionus calyciflorus* were counted daily by using Sedgwich - Rafter counter .

3. Atremia salina

Atremia salina samples were collected from brine ponds in Basrah / karmat - Ali by zooplankton net (mesh size 200 µm) samples were filtration and put on class aquarium filling with (6 L) of tape water with salinity 21 % and acclimate for 48 hr before feeding experiments , *C. vulgaris* was used as food along the acclimate time and using as control group later . Toxic feeding experiments were done on conical flasks size 250 ml contain 150 ml of tape water (salinity 21 ppt) with aerator , each conical flasks inoculated with 5 ml of toxic and non-toxic microalgae . survival percentage and number of individual were counted daily by using Bogorove chamber .

6-Statistical analysis

The analysis of variance (Anova test one way anova were used) to detect the significant differences at ($p<0.05$) by using the program SPSS version-11) , three replicates were made for each treatment .

Results:

The present study included the isolation , the purification and the cultivation of two toxic cyanobacterial species *N. muscurum, A. variabilis* from water of Al- Kandaq River / Basrah / southern of Iraq , In addition to non-toxic microalgae *C. vulgris* . The two toxic algae *N. muscurum* and *A. variabilis* were belonging to the order Nostocales which appears as filamentous algae with heterocyst.

N. muscurum filaments consist of elongate cells 3-4 µm in diameter and 6-7 µm in length with heterocyst 3-5 in diameter and 6-7 µm in length . While *A. variabilis* filaments appeared as coiled filaments with elongate cells 4 - 5 in diameter and 7.5 - 10 µm in length with heterocyst 6 µm in diameter and 7.5 - 10 µm in length . *C. vulgaris* belonging to order Chroococcales , cells appeared as cycloid to avoid single cells 5 - 8.5 µm in diameter with cup-shape chloroplast , some times exactly in old cultures this alga appeared as palmelloid aggregation Plate- 1.

Results revealed that toxic algae *N. muscurum* and *A. variabilis* were grown well in Chu-10 medium , but green algae *C. vulgaris* were grown well more than two cyanobacterial species because it has short lag phase period (4 days) and low generation time (G=0.27) and highly growth constant (k=1.11) compared with toxic microalgae that needed long period for lag phase figures (1) . Table -1 has shown that two toxic species have low growth compared with green algae *C. vulgaris* there has been ageneration time and growth constant that reaches to (G=5.9 ,K=0.051) for and (G=5.101 , K= 0.059) for *N. muscurum* and *A. variabilis* respectively .

Table-1: Growth curve periods , generation time and growth constant for two toxic species *N. muscurum* , *A. variabilis* and non toxic species *C. vulgaris* .

Algal isolates	Lag phase Period (days)	Exponential phase (days)	Stationary phase (days)	Harvested time (days)	Growth constant (K)	Generation time(G)
C. vulgaris	4	11	15	20-21	1.11	0.271
N. muscurum	7	11	14	19-20	0.051	5.90
A. variabilis	8	10	16	20-22	0.059	5.101

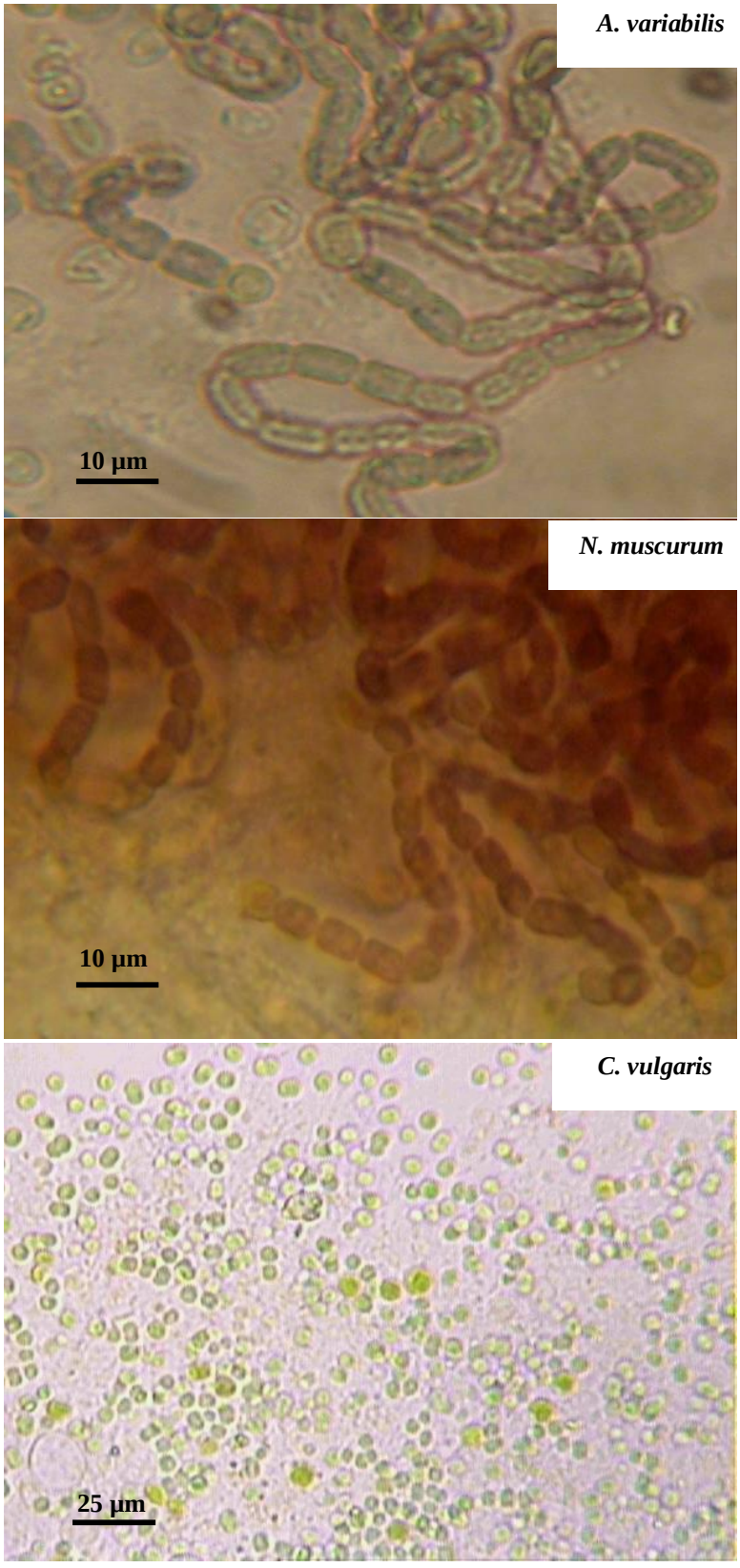


Plate-1 : Vegetative structures of algal species isolates

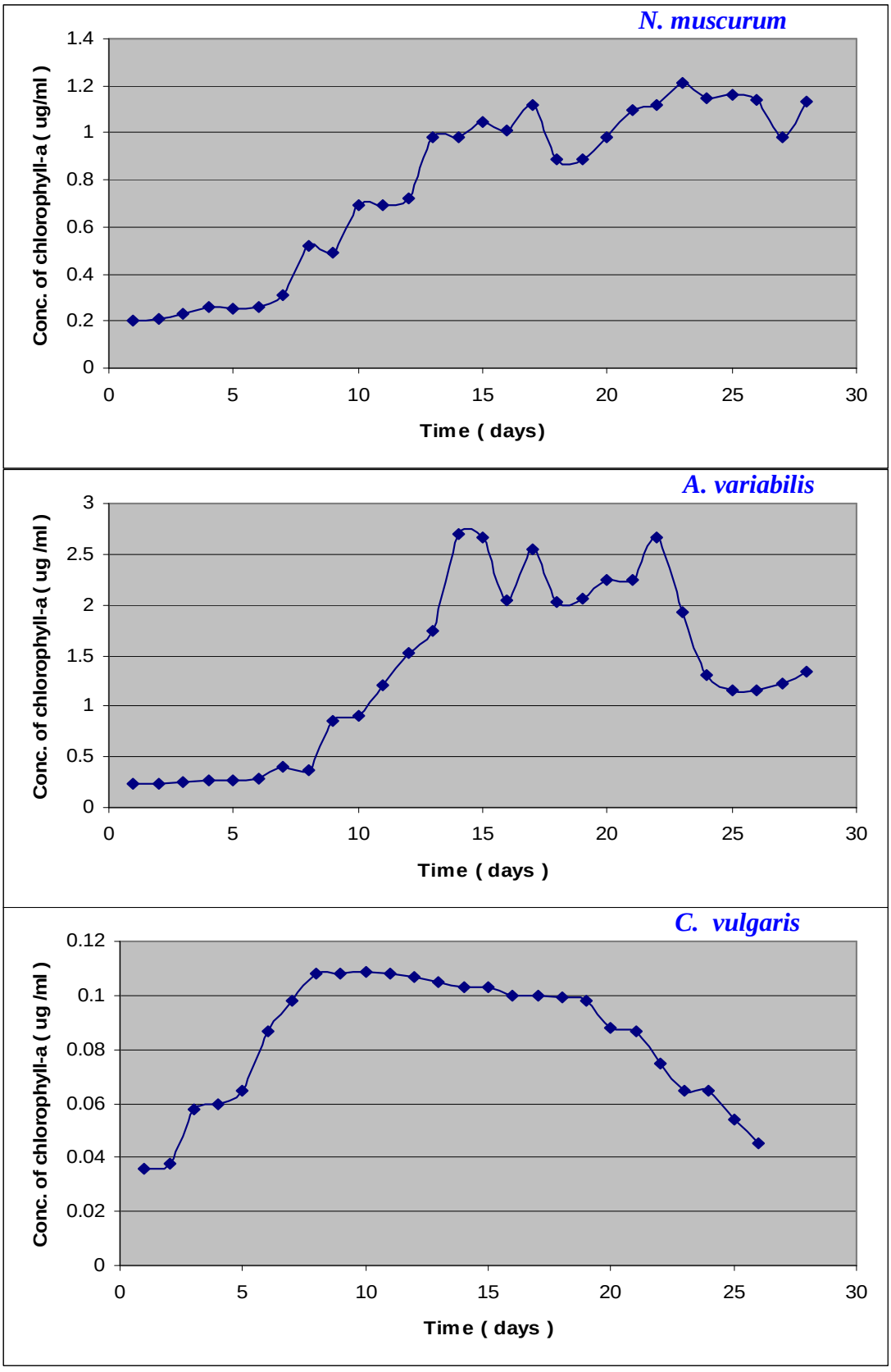


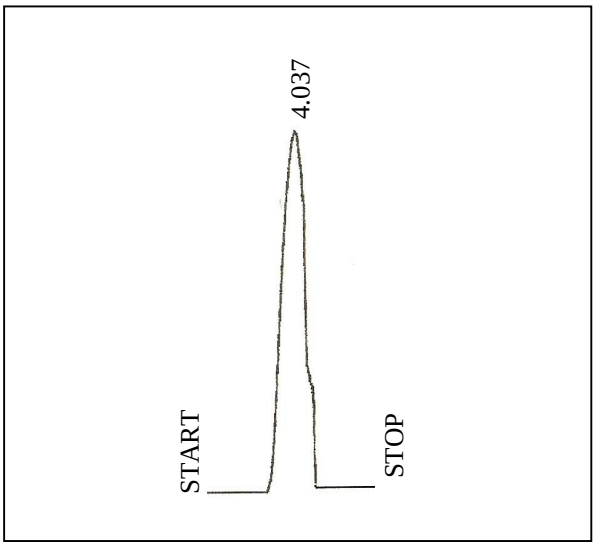
Figure -1 : Growth curves of algal isolates

Detection toxicity of cyanobacterial algae

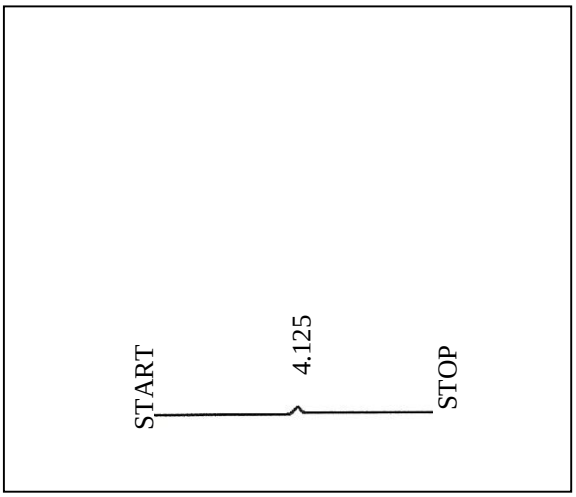
The purified toxins from two cyanobacterial species were detected by using HPLC analysis , they showed the ability to produce toxin called hepatotoxins exactly named Microcystin-LR because there has been retention time (4.124 and 3.968) min. so this close related with retention time of standard toxins (Microcystin-LR) reached (4.037) min. Hepatotoxin (Microcystin-LR) concentration reaches to 27.57 µ/ml and 4.61 µ/ml from two cyanobacterial species *N. muscurum* and *A. variabilis* respectively figure - 2 and Table -2 . Also purified toxins (MC-LR) from two toxic species *N. muscurum* and *A. variabilis* showed similar active chemical groups their consist the structure of standard hepatotoxins (MC-LR) figure-3 . and table -3 .

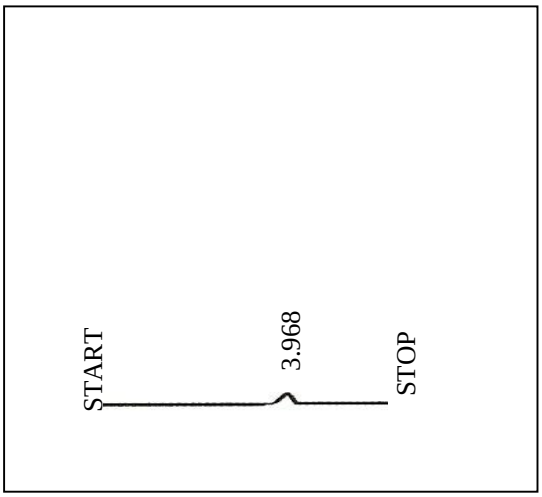
Table-2: Retention time , area under curve and concentration of hepatotoxins (MC-LR) For standard toxins and purified toxins from two cyanocacterial species .

Algal toxins	Area under curve	Retention time(min)	Conc. of hepatotoxins (Microcystin-LR) µg/ml
Standard toxin (Microcystin-LR)	35190	4.037	20
N. muscurum	50270	4.125	27.57
A. variabilis	8128	3.968	4.61



Standard toxins (Microcystin-LR)





N. muscurum ***A. variabilis***

Figure -2 : HPLC analysis of standard toxins (Microcystin-LR) and Purified toxins from two cyanobacterial species .

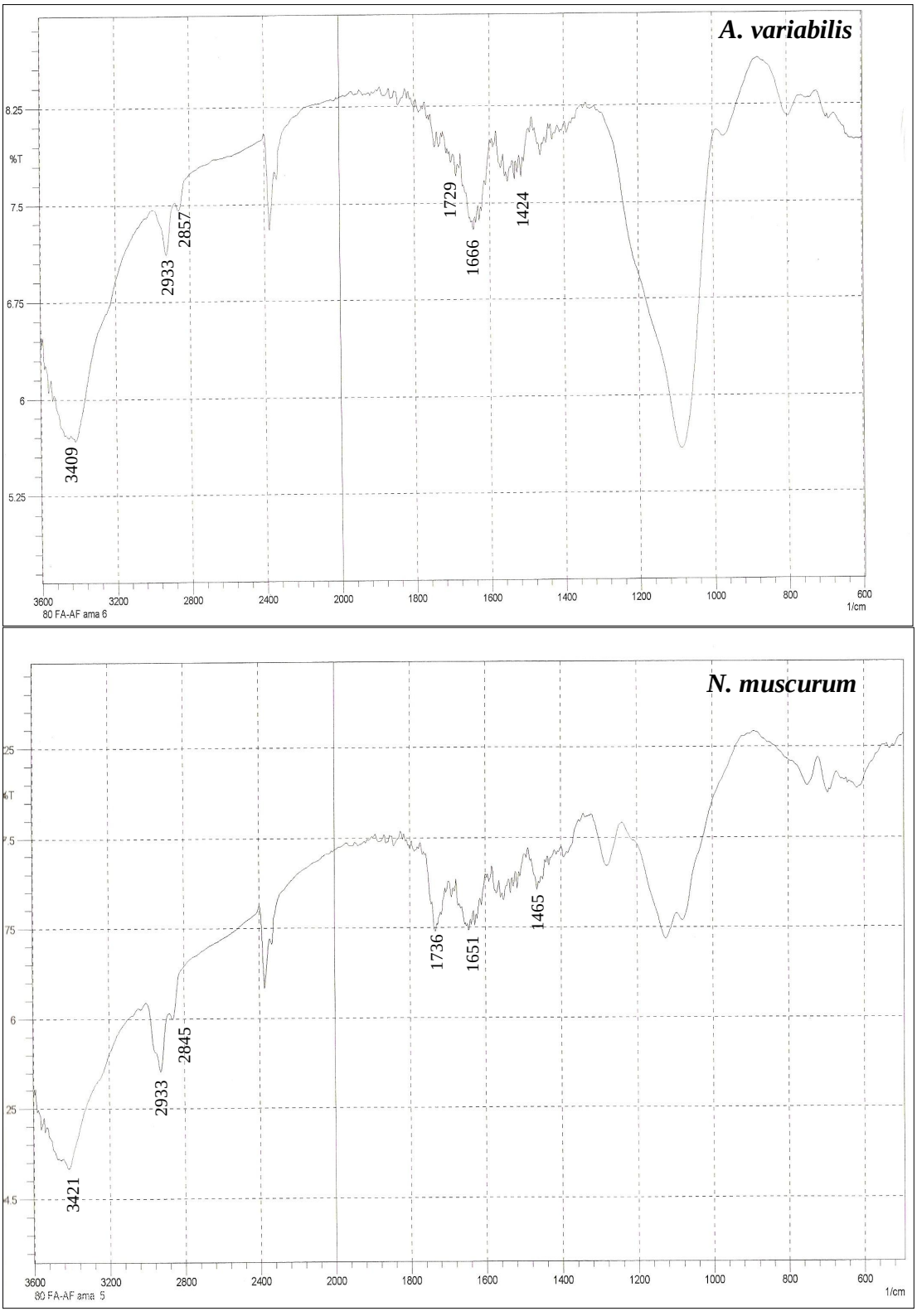


Figure-3 : Infra- red spectra of purified hepatotoxins from two cyanobacterial species

Table -3 : IR spectrum of Microcystin-LR purified from two toxic algae *N. muscurum* and *A.variabilis* .

Functional group	***N. muscurum***	***A. variabilis***
OH , NH Str.	3421 Str. , Br.	3409 Str. , Br.
Alkyl group (CH, CH2 , CH3)	2933 V. , Str. , Br.	2933 V. , Str. , Br.
Carbonyl in Carboxyl group Str.	1736 Str.	1726 M. Br.
Carboxyl in amide Str.	1465 - 1651	1661-1424

Str. , Br. = strong braud , V. , Str. , Br. = very strong braud , M.,Br = medium braud

Toxicity of algae on fish larvae

The results showed that feeding on non -toxic microalgae *C. vulgaris* led to highly survival percentage for three types of fish larvae reached to (70 , 83.33 , 83.33) % for larvae *C. carpio* , *H. molitrix* and *C. idella* respectively , in contrast the results showed decreasing on survival percentage especially for *C. carpio* reached to (0% , 20%) after fed on toxic microalgae *N. muscurum* and *A. variabilis* respectively followed by *H. molitrix* and *C. idella* larvae figure - 4 . *C. idella* larvae showed more resistance against two toxic algae compared with other larvae .

Also feeding on toxic microalgae showed that significant decreasing ($p \leq 0.05$) in the weight and the length of three fish larvae especially weight of *H. molitrix* larvae (6.28 mg) when fed on toxic microalgae *N. muscurum* and length of *C. carpio* larvae (6.46 mm) , *C. idella* larvae (8.50 mm) when fed on toxic microalgae *A. variabilis* compared with these fed on non-toxic microalgae *C. vulgaris* Tables (4 , 5) respectively .

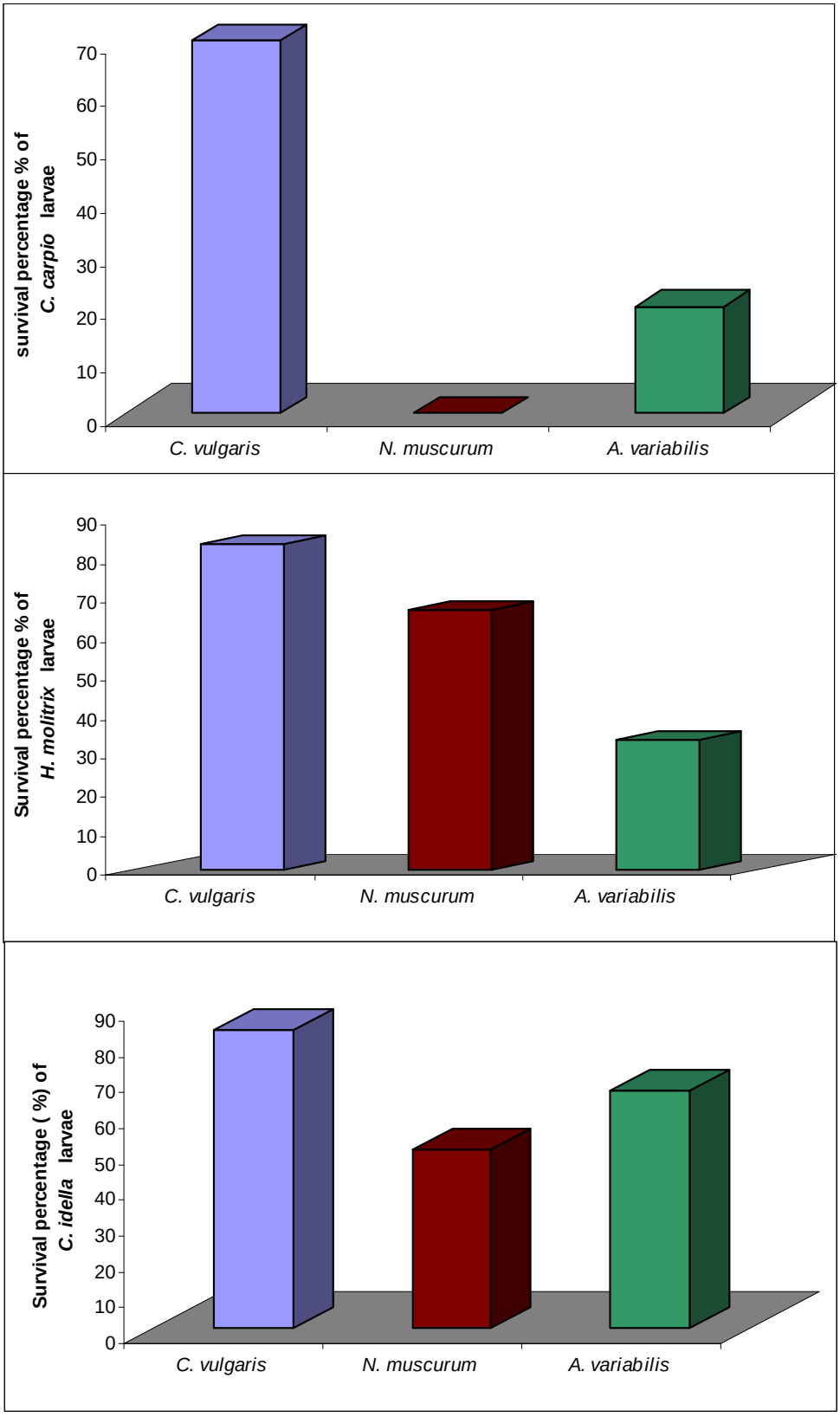


Figure - 4 : Survival percentage of fish larvae *C. carpio* , *H. molitrix* and *C. idella* after fed on toxic and non-toxic microalgae .

Table -4: Mean weight of *C. carpio* , *H. molitrix* and *C. idella* larvae fed on toxic and non- toxic microalgae after 12 days of feeding.

Algal species	*C. carpio*	*H. molitrix*	*C. idella*
C. vulgaris	$7.31 \pm 0.10^{a*}$	7.72 ± 0.02^{a}	10.55 ± 0.58^{a}
N. muscurum	6.83 ± 0.05^{b}	6.28 ± 0.02^{c}	6.92 ± 0.01^{b}
A. variabilis	6.30 ± 0.10^{b}	6.74 ± 0.04^{b}	6.65 ± 0.01^{b}
RLSD	0.14	0.05	0.55

(*) : similar letters mean non significant differences and converse .

Table -5 : Mean Length of *C. carpio* , *H. molitrix* and *C. idella* larvae fed on toxic and non- toxic microalgae after 12 days of feeding.

Algal species	*C. carpio*	*H. molitrix*	*C. idella*
C. vulgaris	$8.35 \pm 0.05^{a*}$	9.08 ± 0.07^{a}	10.76 ± 0.25^{a}
N. muscurum	7.20 ± 0.1^{b}	6.86 ± 0.05^{b}	8.76 ± 0.05^{b}
A. variabilis	6.46 ± 0.05^{c}	6.92 ± 0.02^{b}	8.50 ± 010^{c}
RLSD	0.12	0.09	0.08

(*) : Similar letters mean non significant differences and converse .

Toxicity on zooplankton

The results showed obvious decreasing ($p \leq 0.05$) in the mean numbers of zooplankton individuals after feeding on two toxic species *A. vaiabilis* and *N. muscurum* . Figure (5) showed non significant differences between mean number of two zooplankton *D. magna* and *B. calyciflorous* after feeding on two toxic species . while increasing in their mean number of individual in control group represented by non-toxic microalgae *C. vulgaris* reach mean number to 71.77 and 60.66 individuals for two zooplankton respectively. *A. Salina* was also affected by the toxicity of two cyanobacterial species exactly *N. muscurum* which led to decreasing significanty on their mean number of individuals that reached to 5 individuals followed by *A. variabilis* reaching to 12 individuals , in contrast non significant decreasing on mean number of individuals was show after feeding on green algae *C. vulgaris* , In addition to that increasing in the mean number of *D. magna* and *B. calyciflorous* was shown 48-72 hrs to 120 hrs after feeding on non-toxic *C. vulgaris* compared with significant decreasing without significant differences especially in the first time of feeding periods (0 - 45 hrs) figure (6) . *A. salina* also showed significant decreasing number of individuals on periods (24 – 120) hrs after feeding on two toxic species , while non- significant differences showed on their mean numbers a long the feeding periods after feeding on non-toxic microalgae *C. vulgaris* .

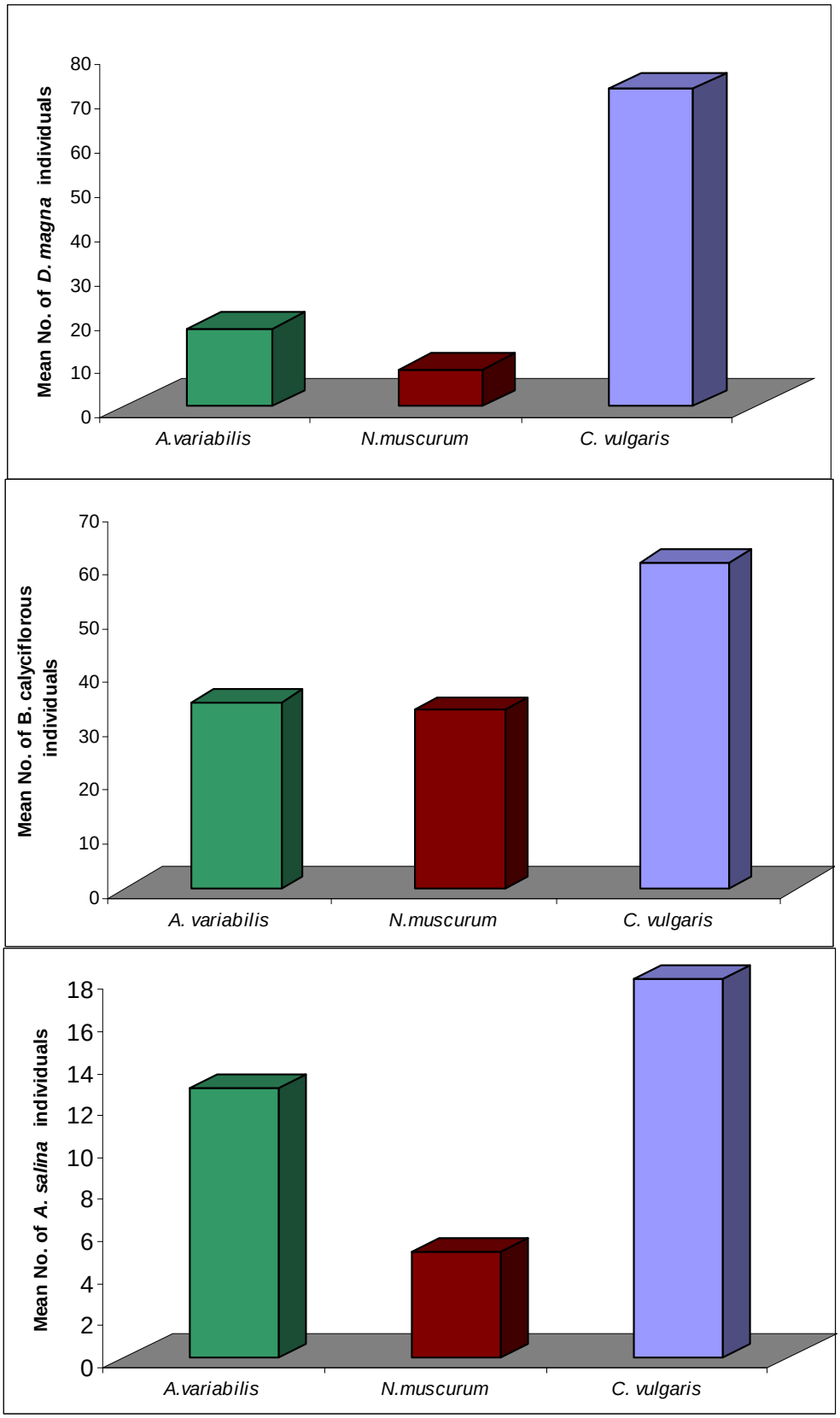


Figure-5 : Total Mean numbers of *D. magna* , *B. calyciflorous* and *A. salina* after fed on Toxic and non-toxic microalgae .

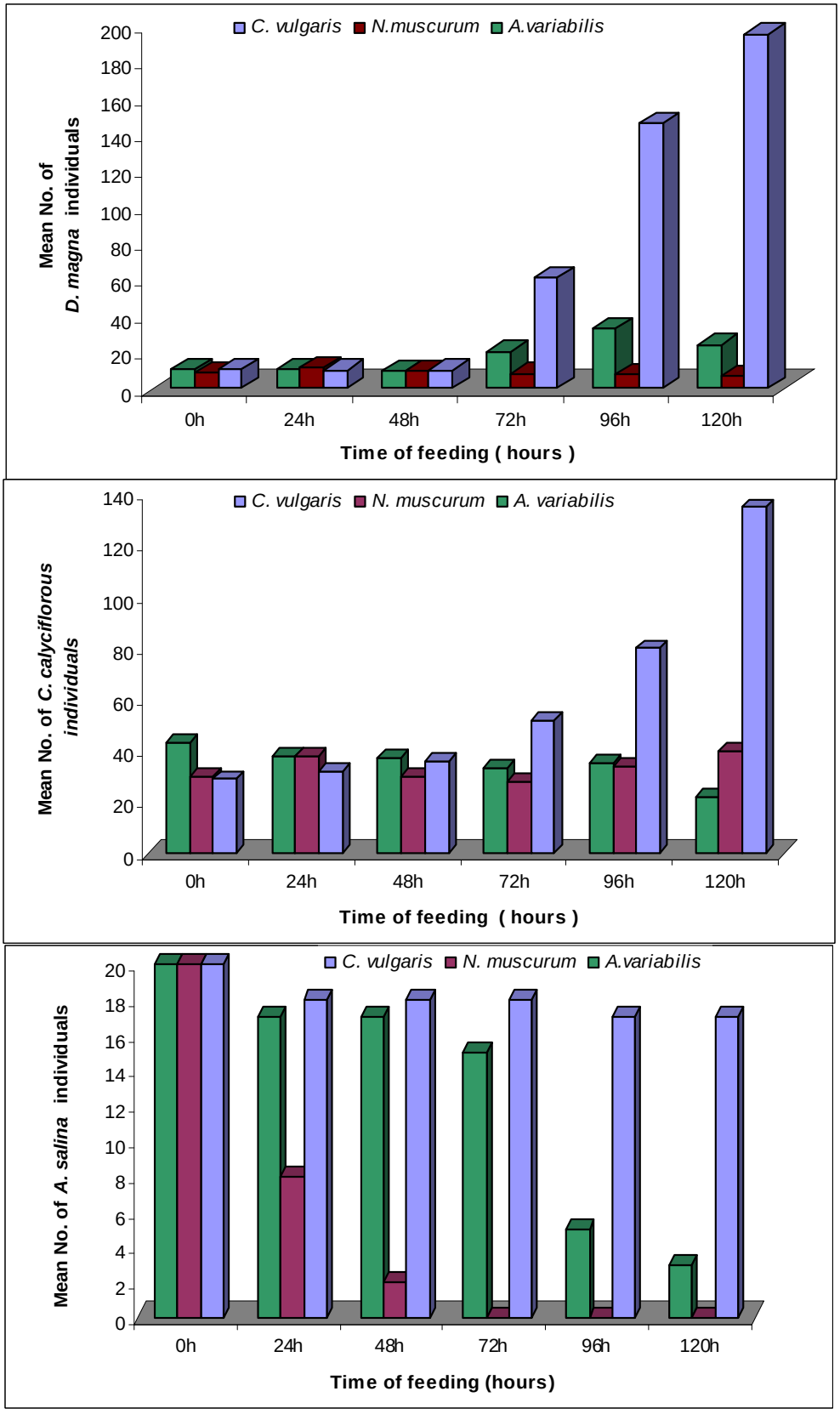


Figure-6: Mean numbers of *D. magna* , *B. calyciflorous* and *A. salina* after fed on toxic and non-toxic microalgae daily along the feeding period (120 h) .

Discussion

In Iraq several studies were published about toxic algae especially hepatotoxins (microcystins) [24] and studying their toxicity on laboratory mice [25] fish larvae [14] and on Molli fish [26] . In the present study two new toxic species of algae *A.variabilis* and *N.muscurum* belonging to division: Cyanobacteria was isolated and purified as axenic cultures in addition to detection the type of toxins which their produce hepatotoxin exactly (Microcystin-LR) and study their effects on some economic fish larvae and zooplankton , so this study may be considered the first time in Iraq for the two cyanobacterial species . The two toxic cyanobacterial species showed high and good growth in the Chu-10 medium , but less than growth compared with non-toxic species *C. vulgaris* that showed highly growth constant and low generation time in contrast , this result was in agreement with some studies showed that green algae was grow well in this medium compared with cyanobacterial algae [27 , 24] .

The two cyanobacterial species *A. variabilis* and *N. muscurum* show their ability to produce hepatotoxins (Microicystin-LR) with highly concentration (4.61 , 27.57) µ/ml for two species respectively , but this toxicity is considered less than the toxicity for another cyanobacterial species isolated southern of Iraq such as *Microcystis aeruginosa* , *Microcystis flos-aque* , *Hapalosiphon welwitschii and Calothrix parietina* [13].

Feeding on two toxic species led to decreasing in survival percentage in all three fish larvae especially *C. carpio* after fed on toxic species *N. muscurum* reach to 0 % at the end of experiment compared with those fed on non-toxic microalgae *C. vulgaris* . In addition feeding on non-toxic microalgae was led to significantly increasing in the weight and length for all fish larvae , but feeding on two toxic microalgae led to significantly decreasing in the weight and length , so this result was in agreement with the study of Teneva *et al*., [8] found that *Phormidium* extracts caused weight loss as well as neuro/hepatotoxic symptoms in mice . In addition to that Microcystin-LR inhibited the biosynthesis of glucosidases and revealed a destructive effect on membranes of lysosomes and endoplasmic reticulum[28]. Also Al-Asarajy and Al-sultan [14] found significant decreasing in the mean of length and weight in addition the survival percentage for two fish larvae *C. carpio* and *H. molitrix* after fed on toxic cyanobacterial represented by *Microcystis aeruginosa ,Microcystis flos-aque , Hapalosiphon welwitschii and Calothrix parietina* .

All zooplankton species *D. magna , B. calyceflourous and D. salina* increasing in their survival individual when fed on non-toxic microalgae as compared with two toxic cyanobacterial species so this result agree with the study of [29] . Several studies have reported a wide variety of changes in the feeding behavior of daphnids after exposure to cyanobacterial cells or colonies and their toxins [10 , 7 , 30 , 31] .

Recent studies suggested that *Daphnia* feeding can be controlled by the mechanism of chemosensory stimulation or " bad taste factor " [10] . so that was leading to possibility of selective feeding by *Daphnia* in the presence of filamentous cyanobacteria [32] .In contrast it is suspected that large cyanobacterial colonies and filamentous could be simply the cause of feeding inhibition in *Daphnia* [30 , 31] .

Kurmayer and Jutter [29] hypothesized that more lipophilic and less toxic compounds may cause the food avoidance of plantothrix by copepods and their results demonstrated that chemical defenses are more important than morphological features and microcystin probably causes food avoidance in *Daphnia* , In addition to that the chemical stimuli for copepods may be related to some kinds of toxin which is unknown or alipophilic microcystins or both .

DeMott and Dhawale [33] demonstrated that cells of wild type *Microcystis aeruginosa* strain PCC7806 are able to poison *D. galeata* causing the death of the animals in a short time . but *Daphnia* sp. remaind alive when myc^- mutant (non-toxic strain) was offered as food . Also Mackintosh *et al*., [9] , showed that wild type *Microcystis aeruginosa* synthesize s relatively large amounts of two microcystin variants , which are known to be potential inhibitors of *Daphnia* protein phosphatase 1 and 2A . The inhibition of these enzymes by

microcystins plays an important role in poisoning of warm - blooded animals , which supports the idea that microcystins are the toxins which cause the death of *Daphnids* . One possible function of microcystins is that play a role in the defense of *M. aeruginosa* cells against zooplankton grazing . This hypothesis is supported by the observation that the exposure to microcystis cells reduces the life spam of *Daphnids* [34 , 35 , 36] .

Kurmayer and Jüttner [29] Also showed that zooplankton species studied *Eudiptomus* , *Cyclops* , *Daphnia and Thamnocephallu*s exhibited defferent sensitivity when fed on toxic *Plantokthrix rubescence* as compared with starvation treatment . The survival of *Thamnocephallu*s and *Eudiptomus* was significantly reduced by *Plantokthrix* , but they showed that the survival of *Cyclops and Daphnia* was higher in the presence of *Plantokthrix* as compared with control without food . Also in the prescence of non-toxic alga *Anabaena* all species showed higher survival than in the controls without food .

Conclusion : This study revealed that two cyanobacterial species *Nostoc muscurum* and *Anabaena variabilis* isolated from sewage water in Basrah southern Iraq have the ability to produce bioactive toxins namely Microcystin-LR with highly bioactivity and toxicity against fish larvae and zooplankton in aquatic environments .

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عزل السم الكبدي Microcystin-LR وتنقية وتشخيصه من نوعين من الطحالب الخضر - المزرقة وبيان تأثيراته الحيوية على بعض الأحياء المائية

عماد يوسف عواد السلطان

قسم علوم الحياة ،كلية التربية، ، جامعة البصرة

Email: emad_yousif2000@yahoo.com

الملخص :

تضمنت الدراسة عزل وتنقية وإكثار نوعين من الطحالب الخضر - المزرقة المعزولة من نهر الخندق / محافظة البصرة جنوب العراق تمثلت بالطحلب *Nostoc muscurum* (Agardh) و *Anabaena variabilis* (kuetz.) أضافة الى الطحلب الأخضر *Chlorella vulgaris* Beijerinck ، أظهرت الدراسة قدرة هذين النوعين من الطحالب الخضر – المزرقة على انتاج السم الكبدي Microcystin-LR وذلك لأول مرة في العراق. تم تشخيص السم الكبدي MC-LR بعد استخلاصه وتنقيته باستخدام تقنية الغاز السائل عالي الأداء High performance liquid chromatography (HPLC) لمعرفة تركيز السم ونقاوته إضافة الى استخدام تقنية Infra-red لبيان أهم المجاميع الفعالة في تركيبة السم الكبدي ، أذ بلغ تركيز السم الكبدي 28.57 مايكروغرام / مل للطحلب *N. muscurum* و 4.61 مايكروغرام / مل للطحلب *A. variabilis* . استخدم النوعان السامان من الطحالب غذاءً ليرقات بعض الأسماك المهمة أقتصادياً تمثلت بيرقات الكارب الأعتيادي *Cyprinus carpio* L. ، الكارب الفضي *Hypophthalmicthyes molotrix* VaL. والكارب العشبي *Ctenophayngodon* VaL. وقد بينت الدراسة نقصان معنوي $P\leq0.05$ في معدل أوزان الانواع الثلاثة من اليرقات وأطوالها ونسبة بقاءها *idella* . Survival percentage عند التغذية على النوعين السامين مقارنة عند التغذية على الطحلب الأخضر *C. vulgaris* ، كما بينت الدراسة التأثيرات الحيوية للطحلبين السامين على بعض الهائمات الحيوانية التي تمثلت بالحيوان القشري *Artemia salina* ومتفرع اللوامس *Daphnia magna* والحيوان الدولابي *Brachionus calyciflorous* والذي أدى إلى نقصان معنوي واضح جداً تحت مستوى أحتمال $P\leq 0.05$ في معدل أعداد الهائمات الحيوانية مقارنة بالتغذية بالطحلب غير السام *C. vulgaris* .

كلمة المفتاح : السموم الكبدية (Microcystin-LR) ،*Nostoc muscurum* ، *Anabaena variabilis* ، يرقات الاسماك ، الهائمات الحيوانية .