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Detection of the ability of cyanobacterial Chroococcidiopsis cubana isolated from the Shatt al-arab River in southern Iraq to produce hepatotoxins MCs

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A R T I C L E I N F O

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A B S T R A C T

The study involved the isolation, purification and culturing of the cyanobacterial *Chroococcidiopsis cubana* from the Shatt al-Arab River in the Basrah Governorate, southern Iraq. The growth curve of *Ch. cubana* was schemed after its cultivation in the liquid culture medium BG-11. The growth analysis indicated a growth constant (k) = 1.3 and a generation time (G) = 0.5 days. *Ch. cubana* ability to produce hepatoxic microcystins (MCs) was detected after isolating and purifying the toxins. The toxins were determined quantitatively and qualitatively using the technique of High-Performance Liquid Chromatography (HPLC), which revealed that *Ch. cubana* exhibited a high production capacity of these toxins, with a concentration rate = 4.053 ug/l.

1. Introduction

Obtaining pure and safe drinking water is a paramount concern for maintaining human health, as the availability of water resources and the potential for their contamination are of fundamental importance. However, the apprehension regarding toxins produced by cyanobacteria in water is a relatively recent trend that began in the late twentieth century. Cyanobacterial blooms have been a cause for concern since the 1950s, and there is substantial evidence of the adverse effects of cyanobacterial toxins on human health due to water contamination, particularly hepatotoxic microcystins (MCs)[1, 2].

Despite the actuality of diverse communities of cyanobacteria in aquatic environments, it is essential to identify the toxic species among them, particularly those capable of producing hepatotoxic microcystins (MCs). Several analytical methods have been utilized to monitor and detect hepatotoxicity in water samples, such as the Protein Phosphatase Inhibition Assay (PPIA), Enzyme-Linked Immuno-Sorbent Assay (ELISA), and High-Performance Liquid Chromatography (HPLC) [3, 4].

Hepatotoxic microcystins (MCs) have been categorized as carcinogenic agents due to their capacity to hinder Protein Phosphatase (PP) enzyme activity, particularly in eukaryotic organisms [5-

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7]. The interaction between MCs and phosphatase enzymes results in a synergistic effect, leading to the inhibition of enzymatic function. This inhibition disrupts the dephosphorylation process essential for protein synthesis, ultimately impacting cytoskeletal proteins and culminating in cell death characterized by haemorrhage and significant blood loss upon acute exposure to hepatotoxic MCs [8, 9]

The precise function of hepatotoxic microcystins (MCs) in cyanobacteria remains incompletely elucidated. Nonetheless, their synthesis is partly governed by diverse cellular and environmental factors like light intensity, nutrient availability, pH levels, and precipitation patterns [10, 11]. These factors can exert a direct or indirect influence on cell proliferation and MC production [12, 13].

The specific role of these toxins is a topic of ongoing contention. Nonetheless, multiple hypotheses have surfaced to elucidate the stimuli triggering cyanobacteria to produce these substances. Some conjectures propose that MCs may function as a defence mechanism against herbivores such as zooplankton and fish, or as a competitive strategy against other cyanobacterial species for spatial and resource advantage. Notably, nutrient enrichment stemming from eutrophication significantly drives cyanobacterial blooms in aquatic environments. Furthermore, recent studies have associated the proliferation of cyanobacteria with various factors, including the impact of heightened agricultural fertilizers containing phosphorus and nitrogen. Additionally, cellular stress or senescence could potentially contribute to MC production [14-17].

2. Material and Methods

2.1 Sample collection and isolation

Samples of cyanobacteria were obtained from the water of the Shatt al-Arab River in Basrah Governorate, located in southern Iraq, at a depth of 30 cm beneath the surface. These specimens were acquired in pristine, airtight plastic receptacles and expeditiously conveyed to the laboratory for the purpose of isolating cyanobacterial species. The procedure for isolation adhered to the protocol delineated by Stein [18].

Glass slides were prepared from the collected samples to identify cyanobacteria, and they were examined using an Olympus optical microscope of the CX21 model. A 10 ml portion of the cyanobacterial solution was then subjected to multiple washes with distilled water using a TLE-Danger centrifuge operating at 3000 rpm for 5 minutes. Subsequently, 2 ml of the washed samples were transferred into test tubes and diluted to a total volume of 10 ml using sterile liquid media (BG-11).

The cyanobacterial cultures were subsequently placed in a growth chamber for 7 to 10 days, where they experienced alternating cycles of light and darkness following a 12-hour light and 12-hour dark illumination regimen. During this incubation period, the light levels varied between 130 to 150μE2/Sec. Following this incubation phase, the uni cyanobacterial cultures were established using the procedure outlined by Stein [18]

2.2 Purification of cultures

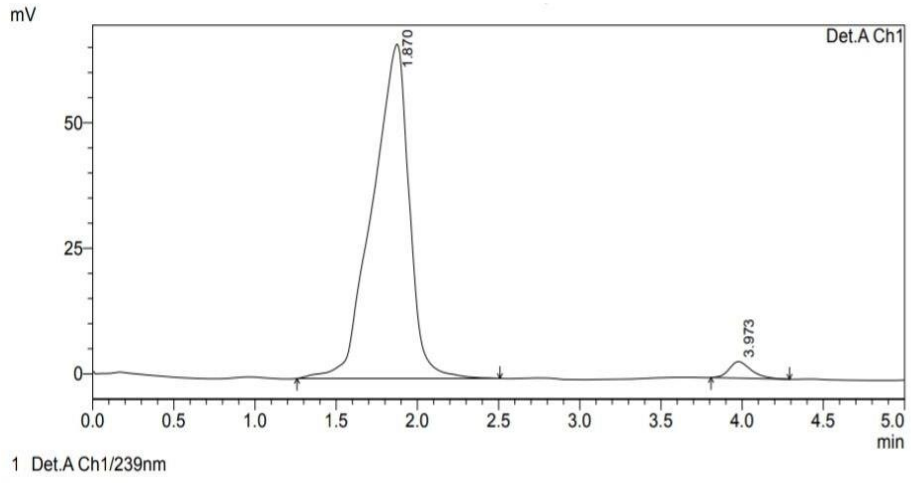
The technique described by Wiedeman [19] was employed in order to achieve the axenic cultivation of cyanobacteria. Following this, the monocultures of cyanobacteria underwent a series of washes using sterile distilled water, with centrifugation at 3500 rpm for 3 minutes. The resulting sediment was subjected to 12 additional washes with sterilized distilled water. Procedures outlined in reference [18] were implemented to prevent the proliferation of bacteria and fungi.

2.3 Extraction and purification of Microcystins

The method described by Luukkainen [20] was utilized for the extraction of microcystins from the cyanobacterial isolates. Initially, 50 mg of freeze-dried cyanobacterial biomass was combined with a solution containing MBW (Methanol: n-Butanol: Water) in a ratio of 15:1:4 ml, respectively, in 100 mL conical flasks. The resulting mixture was agitated using a magnetic stirrer for one hour.

Subsequently, the mixture underwent centrifugation at 3000 rpm for 10 minutes, a process that was iterated three times. Finally, the combined supernatant was gathered and evaporated to a volume of 5 mL using a stream of dry air.

The procedure for purifying microcystins was conducted by Namikoshi's method [21], with certain adjustments as specified. A 15 x 2 cm glass column packed with silica gel (mesh size 200-100 μ) was utilized for this purpose. The concentrated extract obtained previously was introduced into the column and underwent a washing process involving three distinct solvents: initially, 20 ml of ion-free distilled water, followed by 20% and 80% methanol, flowing at a rate of 3 ml/min. Subsequently, the resulting eluent was concentrated and preserved at a temperature of (4°C) in a refrigerator until the time of analysis. HPLC technique was employed for the analysis, using a standard provided by Abraxis Company (United States) as depicted in Figure (1).



PeakTable

Detector A Ch1 239nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.870	1044010	66590	97.106	95.299
2	3.973	31119	3285	2.894	4.701
Total		1075129	69875	100.000	100.000

Fig.1. High-Performance Liquid Chromatography HPLC of MCs Standard

3.Results and discussion

3.1 growth curve

A growth curve was drawn based on the dry weight of cyanobacterial *Ch. cubana*. The results indicated that the lag phase extended for five days before entering the exponential growth phase, which lasted for 22 days. Subsequently, it reached the stationary phase, lasting for 16 days, and the cyanobacteria were collected. The growth constant was registered as follows: K = 1.3, and the generation time (G) = 0.5 days figure (2,3).

Growth curves play a crucial role in the examination of cyanobacteria, as they document essential data regarding growth rate, reproduction, and reaction to environmental elements like temperature, light, and nutrient levels. Additionally, they have the potential to forecast cyanobacterial growth under various circumstances, aiding in the formulation of strategies to counteract harmful algal blooms or enhance ecosystem efficiency. The cyanobacterium *Ch. cubana* exhibited a growth constant of K = 1.3 and a generation doubling time of G = 0.5 hours. This outcome differed from Aubaeed's findings, where the growth curve was constructed based on dry weight measurements over two months, indicating a convergence in growth constants for cyanobacteria prone to colony formation: the unicellular cyanobacteria *M. flos-aquae* and *Merismopedia glauca* displayed growth constants of K = 0.195 and K = 0.197, respectively, with generation times of G = 1.543 and G = 1.527 hours. These specimens were sourced from the same habitat as the unicellular alga *Ch. cubana* found

in the Al-Ashar River. Discrepancies could be attributed to variations in growth media, as BG-11 medium was utilized in the present research, whereas Chu10 medium was employed in Aubaeed's study [22], or potentially to genetic, environmental, or laboratory conditions.

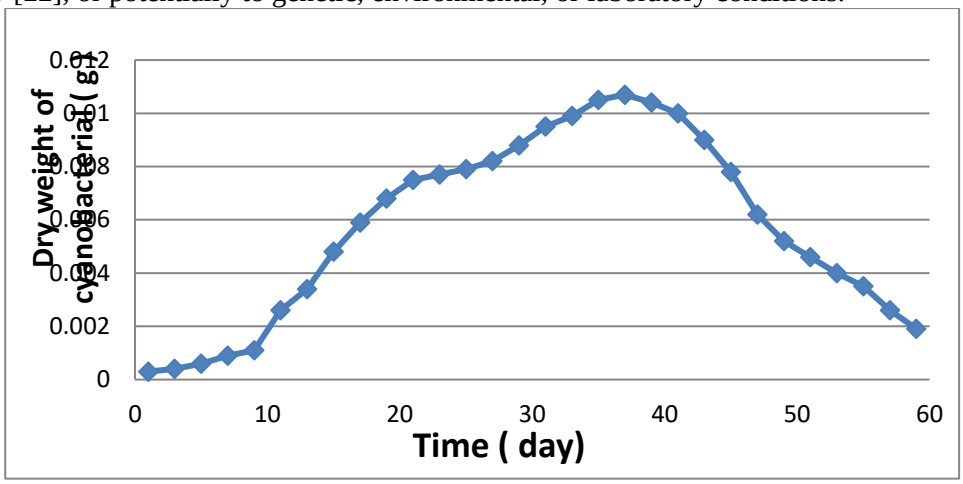


Fig.2. growth curve of cyanobacterial *Ch. cubana*

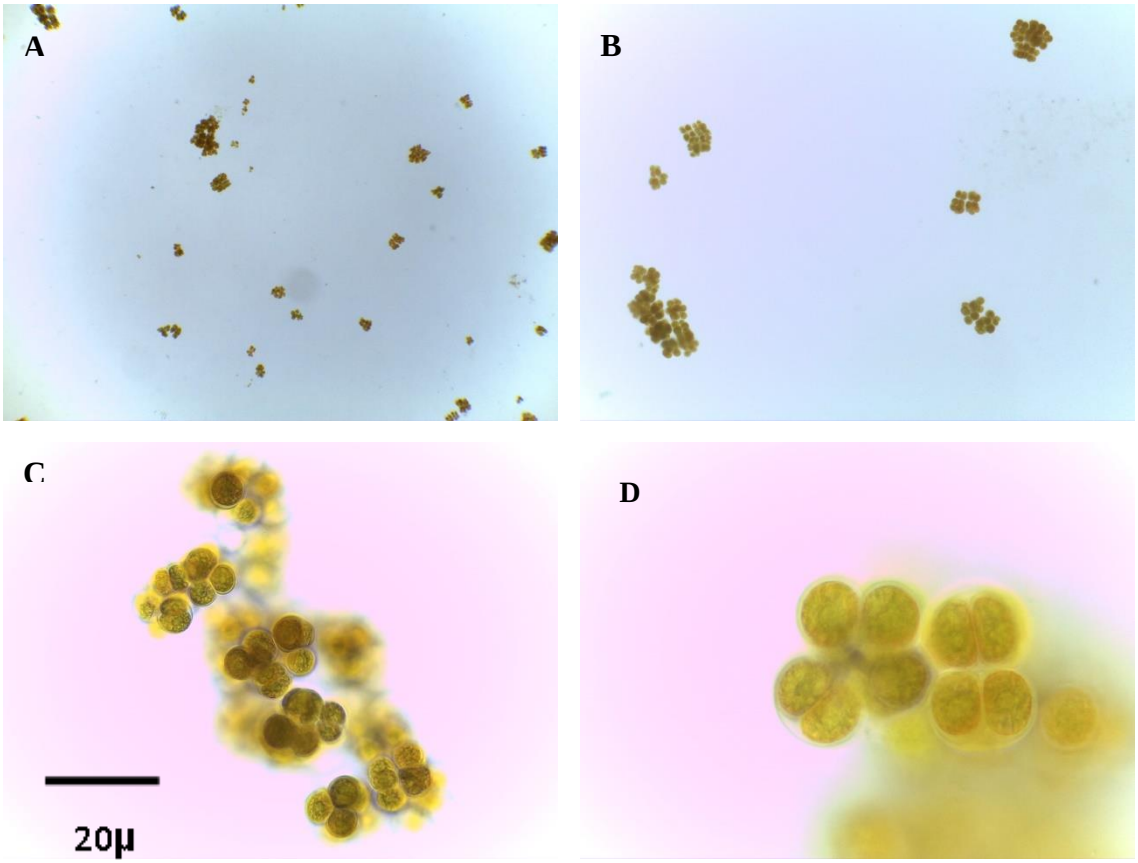


Fig.3. Light microscopy micrographs of cyanobacterial *Ch. cubana*

A- X4 B- X10 C- X40 D- X100

3.2 Quantitative and qualitative estimation of hepatotoxic toxins (MCs)

The results of the identification of hepatotoxic MCs by HPLC that cyanobacterial species *Ch. cubana* , Biomass isolate and water has the ability to produce hepatotoxins with a concentration of 4.053, 3.991 and 0.15 µg/l, Figure (4) , although studies on its toxicity are almost non-existent,

except for the Magana-Arachchi study in Sri Lanka [23], which explicitly showed the ability of the genus *Chroococcidiopsis* to produce hepatotoxins (MCs), and perhaps the reason for the lack of studies on the toxicity of the genus is due to it. *Chroococcidiopsis* cannot be distinguished from the species of the genus *Microcystis* because they have the same phenotypic characteristics, as they are both single-celled with a spherical or oval shape and tend to form Pallmelloid colonies. Perhaps it was treated as a species belonging to the genus *Microcystis* due to the similarity in phenotypic characteristics, especially since some studies had results similar to what the study reached. Current concentration of hepatotoxin extracted from *Ch. cubana*, which reached around 4 µg/l, such as the study by Mitreva and Gartner [24], which showed that the concentration of hepatotoxins (MCs) extracted from species of the genus *Microcystis* ranged between 2-5 µg/l, and the study of Wang [25] that dealt with Concentrations of hepatotoxic MCs extracted from the genus *Microcystis*, which were close to the results of the current study. And the study of Mitreva and Gartner [24] which identified MCs hepatotoxins in *Microcystis aeruginosa* and *M. wesenbergii* isolated from the Danube River in Bulgaria, as it was found that the concentration of MCs hepatotoxins ranged from 2 to 5 µg/l

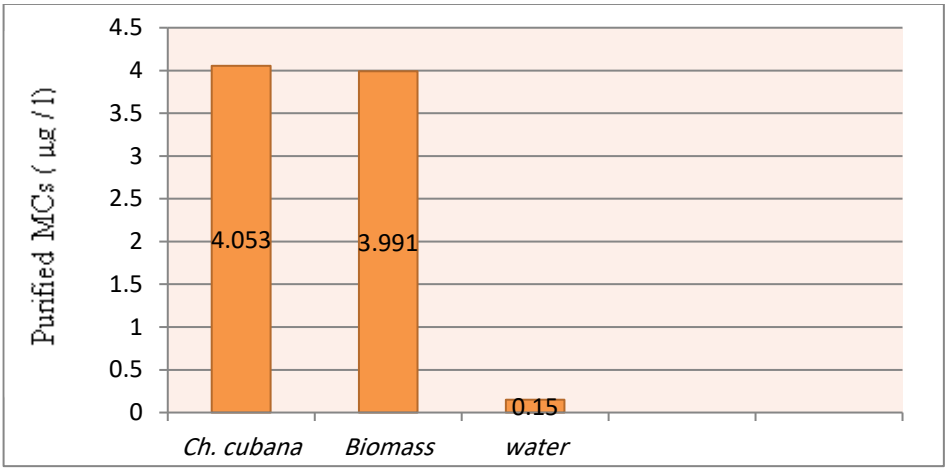


Fig. 4. The concentrations of purified MCs of cyanobacterial species, Biomass isolate and water

4.Conclusion

The cyanobacterial *Chroococcidiopsis cubana* produces the hepatotoxin microcystins in relatively high concentrations, which may cause health, environmental, and economic problems in the region where it is found.

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تقييم قابلية الطحلب الاخضر المزرق *Chroococcidiopsis cubana* المعزول من شط العرب على انتاج السموم الكبدية Microcystins

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معلومات البحث

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القبول 18 ايار 2024
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الكلمات المفتاحية

Chroococcidiopsis, HPLC, شط العرب, المايكروسستينات , سيانوباكتيريا

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الملخص

تضمنت هذه الدراسة تشخيص الطحلب الاخضر المزرق *Chroococcidiopsis cubana* وعزله وتنقيته وإكثاره من نهر شط العرب في محافظة البصرة، جنوب العراق وقياس منحنى النمو بعد استزراعه في الوسط السائل BG 11 لمدة 60 يوما ،اذ بلغ ثابت النمو (k) = 1.3 وزمن تكاثر الجيل (G) = 0.5 يوما، ومن ثم اختبرت قابليته على إنتاج السموم الكبدية (MCs) Microcystins، إذ عزلت تلك السموم وشخصت نوعاً وكماً بوساطة تقنية كروماتوغرافيا السائل عالي الأداء (HPLC) High Performance Liquid Chromatography، وبينت النتائج قدرة انتاجية عالية لتلك السموم بمعدل تركيز بلغ 4.053 مايكروغرام / لتر ويعد التسجيل الاول لذلك الطحلب محليا على صعيد النوع والجنس والقابلية على انتاج السموم الكبدية المايكروسستينات .

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