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# Allelopathic Effect of Cell-Free Medium of *Microcystis aeruginosa* Kützing on the Chromosomal Changes in *Allium cepa* Root Tips and Plumule Formation of *Zea mays* Seedling

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors MMES and HMK wrote the protocol supervised the experimental work and wrote the first draft of the manuscript and corrected the manuscript according to reviewers comments. Author RES carried out the experiments of this work and managed the literature searches. All authors read and approve the final manuscript*

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## ABSTRACT

This work confirm the allelopathic toxic effect of both cell-free medium in log and death phase of growth of *Microcystis aeruginosa* on chromosomal changes of *Allium cepa* root tips and the plumule formation of *Zea mays* seedlings. The results showed high significant inhibition of mitotic index in *Allium cepa* root tips in addition, chromosomal abnormalities were also detected. There were morphological change occurred in the plumule formation of *Zea mays* seedlings. The results revealed that, seed soaked in log phase cell-free medium had less inhibitory effect on plumule formation than that the seed soaked in death phase cell-free medium of *M. aeruginosa*. This was confirmed by the allelopathic effect of toxins released during death phase of growth was more than log phase.

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## 1. INTRODUCTION

Some species of cyanobacteria can produce multiple types and variants of cyanotoxins. Quantitative cyanotoxin analysis is needed to determine if the cyanobacteria are actually producing the toxin, and it is impossible to tell if a species is toxic or not toxic by looking at it. Also, even when toxin-producing cyanobacteria are present, they may not actually produce toxins. Furthermore, water contaminated with cyanobacteria can occur without associated taste and odor problems.

*Microcystis aeruginosa* is a common bloom-forming and toxin-producing cyanobacteria in Egypt. EL-Sheekh et al. [1] estimated the concentrations of microcystin-LR inside the cells of *M. aeruginosa* isolated from Egyptian water by using HPLC during log, stationary and retardation phases of growth and the toxicity was determined in *Artemia salina* bioassay. The HPLC results showed that microcystin-LR concentrations were 65.6, 1.2 and 0.8 $\mu g.g^{-1}$ during the log, stationary and retardation phases of growth, respectively. EL-Sheekh et al. [2] studied the effects of crude extract of *Microcystis aeruginosa* containing microcystin-LR on the germination, growth and chlorophyll content of *Zea mays*.

Cyanotoxins in water utilized to irrigate food plant crops have not yet been considered within any official monitoring program on water quality. Previous studies clearly indicate that irrigation with water containing MCs can be a threat for both the quality and yield of crop plants [3]. However, the number of studies related to the impact of cyanotoxin on aquatic and terrestrial crop plants irrigated by water containing these toxins, have increased during last few years. In addition crop and vegetable plants can accumulate microcystins in their edible tissues [4], and therefore these plants could contribute directly or indirectly to cyanotoxin transfer through the food chain, and thus constitute a potential potent health risk source [5].

Since then, the research interest on phytotoxic effects of cyanobacteria on terrestrial plants has increased, demonstrating morphological and physiological alterations by cyanotoxins in a range of terrestrial plants, including *Brassica napus*, *Lolium perenne*, *Oryza sativa*, *Sinapis alba*, *Solanum tuberosum* and *Trifolium repens* [6]. Máthé et al. [7] focusing mainly on cyanotoxin-induced changes of chromatin organization and their possible cellular mechanisms. They concluded that, microcystin probably inducing alterations of chromosome number.

Spray irrigation of commercial lettuce (*Lactuca sativa*) plants with water containing *Microcystis* resulted in colonies and single cells of the Cyanobacterium *Microcystis* being lodged on the leaves 10 days after the last irrigation [8]. The use of this contaminated irrigation water have an economical impact which appears by a reduction of the germination rate of seeds, and alteration of the quality and the productivity of crop plants [5].

The objective of this work was to discuss the agricultural impacts induced by cyanotoxin. We demonstrate the possible effects of cell free medium of *Microcystis aeruginosa* in both log and death phase on cytology of *Alium cepa* root tips and the morphological changes of *Zea mays.*

## 2. MATERIALS AND METHODS

*Microcystis aeruginosa* was isolated from River Nile channel near Tanta city, Egypt [1]. It was purified and identified according to Prescott [9]. It was grown on medium Allen's and Stanier [10] under continuous fluorescent illumination (80 $\mu mol.m^{-2}.s^{-1}$) and temperature 25±2ºC.

Microcystin-LR in *M. aeruginosa* was estimated by using High performance liquid chromatography (HPLC) according to Shen et al. [11], and the toxicity of this species was tested previously [1].

*Microcystis aeruginosa* were cultured in sterile Allen's and Stanier [10] in 250 ml Erlenmeyer flasks, and during log and death phase of growth, the cells were removed from the culture medium by filtration through a Whattman glass fiber filters (GF/C nominal pore size 47 µm), and cell filtrate in both growth phase of *M. aeruginosa* were obtained.

*Allium cepa* was germinated on death phase cell free media of *M. aeruginosa,* and the cytological preparations of the *Allium cepa* root tips were carried out using the Feulgen squash technique [12]. Cells with good spreading of chromosomes were photographed using a Zeiss Ultraphoto microscope with automatic camera.

The mitotic index of both control and treated *Allium cepa* root tip were determined according to:

$$\textbf{Mitotic} - \textbf{index} = \left\{\frac{\text{Number of dividing cells in all stages}}{\text{Number of total cells (dividing + non dividing)}}\right\} X100\}$$

*Zea mays* seeds were surface sterilized in 3.5% sodium hypochlorite for 20 min, rinsed several times with distilled water. Some seeds were soaked in the cell free medium of *M. aeruginosa* at two different growth phases (lag and death phase) for 24 hours and the same number of seeds were soaked in distilled water as control.

### 2.1 Statistical Analysis

Results were presented as mean ± SD for three replicates. The statistical analysis were carried out using SAS program [13] version 6.12. Data obtained were analyzed statistically to determine the degree of significance between treatments using one way analysis of variance (ANOVA) at $P \leq 0.001$.

## 3. RESULTS

Table 1 shows the effects of cell-free medium (CFM) of *M. aeruginosa* in both log and death phase on the mitotic index (MI) of *Allium cepa* root tips. The results obtained showed that, the both CFM show high significant inhibition of MI at ($P \leq 0.001$), and the inhibition were more pronounced in death phase CFM than in log phase. The mitotic inhibition in log phase CFM was amounted by 30.7% while in death phase CFM was 60.5% below the control level.

**Table 1. Effects of cell-free medium of *Microcystis aeruginosa* in both log and death phase on mitotic index (MI) of *Allium cepa* root tips**

| Growth phases | No. of total cells | No. of dividing cells | Mitotic index | F-value | P – value |
|---|---|---|---|---|---|
| Control | 4325 | 494 | 11.4±0.2 | | |
| Log phase | 4440 | 355 | 7.9±2.0*** | 188.15 | 0.0001 |
| Death phase | 5050 | 230 | 4.5±0.2*** | | |

*Each value is the mean of five readings ±standard deviation.*
*** *Highly significant at P ≤ 0.001 using one way analysis of variance (ANOVA)*

The results in Fig. 1. showed four chromosomal abnormalities in root tip of *Allium cepa* treated with death phase CFM: 1A) stickiness metaphase, 1B) chromosome fragment at C metaphase stage, 1C) chromosome bridges and 1D) micronucleus at interphase.

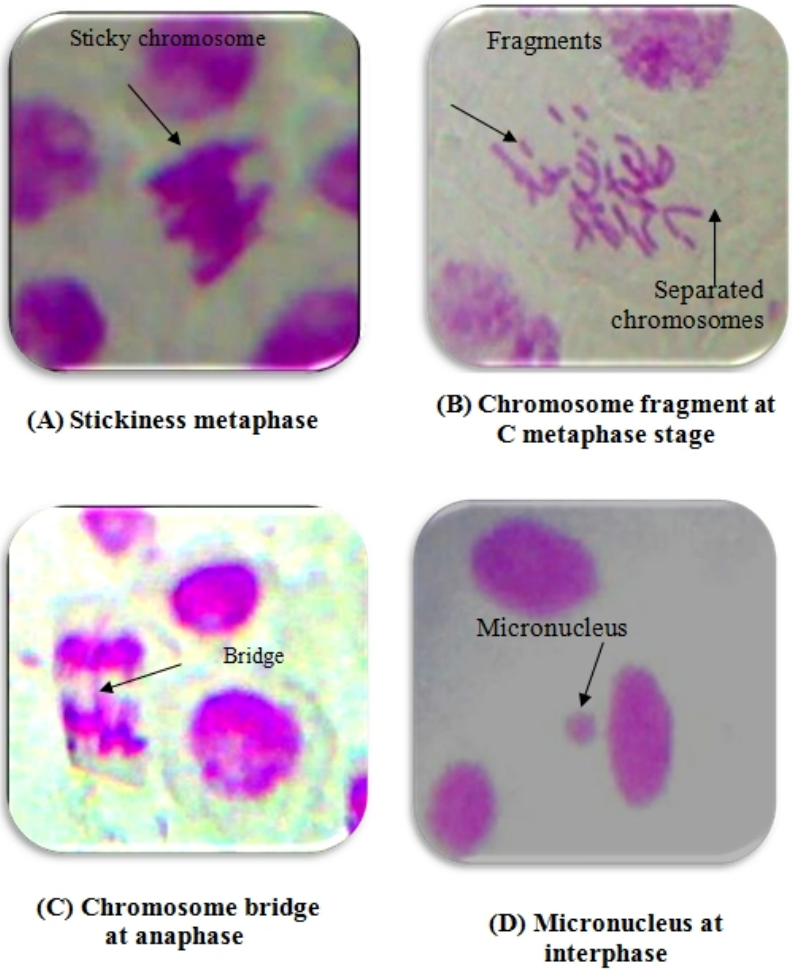


**Fig. 1. (A, B, C and D), Chromosomal abnormalities (stickiness, chromosome fragments, chromosome bridge and micronucleus) observed in *Allium cepa* root tips exposed to *Microcystis aeruginosa* cell-free medium in death phase**

Effect of the cell-free medium of *M. aeruginosa* in both log and death phase on plumule formation of *Zea mays* was represented in Fig. 2. Generally there were morphological changes occurred in the plumule formation of *Zea mays* seedlings. The present results revealed that, seed soaked in log phase cell-free medium had less inhibitory effect on plumule formation than that the seed soaked in death phase cell-free medium of *M. aeruginosa.*

**A) Control** **B) Log phase** **C) Death phase**

**Fig. 2. Effect of seed soaking in the cell-free medium of *Microcystis aeruginosae* in log and death phase of growth on the plumule formation of *Zea mays*(8-day old), (A) control, (B) log phase and (C) death phase**

## 4. DISCUSSION

The present investigation showed the effect of the cell-free medium of *M. aeruginosa* in two different growth phases (log and death phase) on the chromosomal changes of *Allium cepa* root tips and the morphology of *Zea mays* germination.

Release of microcystin into the extracellular environment has been attributed to the death and lysis of cyanobacterial blooms [14] so the amount of microcystins in culture medium in death phase is higher than in culture medium in log phase, then the cyanobacterial toxins (microcystins) could be used as allelopathic substances that have high significant negative effects.

The results showed the cytotoxicity of the cell-free medium in death growth phase of *M. aeruginosa* on the chromosomal change of *Allium cepa* root tips. It was represented by reduced the mitotic index of *Allium cepa* root tip and induced chromosomal abnormalities at the metaphase, anaphase and interphase. Five abnormalities (stickiness; C-metaphase, bridges, fragments and micronucleus) were found in root tips and stickiness was the most common abnormality. Máthé et al. [7] reported that MC-LR induced spindle abnormalities at the metaphase–anaphase transition, the formation of asymmetric anaphase spindles and abnormal sister chromatid separation in roots of common reed aquatic macrophyte (*Phragmites australis*). In addition *M. aeruginosa* produce high amount of both alkaloids and phenolic compound in death phase cell-free medium and these compounds have adverse effect on the plant cells [1]. Generally, a mitotic poison causes disturbance of the spindle apparatus, resulting in a C-metaphase, which means the complete absence of a spindle [15]. Also results showed that treatment of *A. cepa* root tips with cell-free medium of

*M. aeruginosa* produced bridges at anaphase. The formation of anaphase bridges may be due to the extensive chromosome stickiness which observed at metaphase stage causing abnormal anaphase separation or may be due to the outcome of unequal translocation or inversion of chromosome segments. On the other hand, occurrence of micronuclei in the root tips of *A. cepa* pretreated in cell-free medium of *M. aeruginosa* may arises from fragments of chromosomes as described by [16] who reported that chromosome fragments were not incorporated into daughter nuclei but remained in the cytoplasm where they formed micronuclei. The results in the present study were in accordance with [7] which showed the formation of multipolar spindles and disrupted phragmoplasts, leading to abnormal sister chromatid segregation during mitosis. Thus, microcystin probably inducing alterations of chromosome number. Our results demonstrated that the cell-free medium of *M. aeruginosa* in the death phase showed the highest inhibitory effects on *Z. mays* seedling than that from log growth phase. This results in accordance with [17] which demonstrated that most of the toxin release occurs as cells age and die and passively leak their cellular contents, although active release of toxins can also occur from young growing cells. This inhibition may be due to the presence of algal allelochemicals in the cell-free medium of *M. aeruginosa* [1]. These allelochemicals which produced and released by *M. aeruginosa* in the cell-free medium could be considered as one of the most important components in inhibiting growth and nutrient uptake of *Zea mays* plant. Hamed et al. [18] concluded that, phenolic compounds interfere to some degree with many vital processes, including cell division, mineral uptake, respiration, photosynthesis, protein and chlorophyll synthesis, phytohormone activity and nucleic acid metabolism. EL-Sheekh et al. [2] showed that, *Z. mays* seeds soaked for 24 hrs in the cell-free medium during death phase of *M. aeruginosa* induced a significant reduction in root, shoot lengths, number of lateral roots, fresh and dry weights, leaf area and pigment contents. Materska et al. [19] reported that phenolic compounds cause multiple physiological effects like reduced leaf water potential, stomata diffusive conductance, plasma membrane perturbation and decreased photosynthetic rate in different plant species. Nakano et al. [20] reported that alkaloids showed growth inhibition against both monocotyledonous and dicotyledonous plants. The presence of short chain fatty acid (C16:0) could infiltrate the membrane lipids and change their physical properties [1]. This result was coincided with the results of the present study which demonstrated that, the seed soaked in death phase cell-free medium of *M. aeruginosa* showed more inhibitory effect on growth of *Z. mays* than log phase.

## 5. CONCLUSION

In conclusion**,** this study show the high toxic and cytotoxic influence of *M. aeruginosa* cell free media in log and death phase can affect both plant crop yield and quality, and could pose a potential risk for human and animal health, if the MC intake exceeded the recommended tolerable limits.

## 6. RECOMMENDATION

Cyanobacteria and/or their cyanotoxins are detected in the surface water supplying the water system, the treatment system operators can act to remove or inactivate them. Applying the wrong treatment process in treatment could damage cells and result in the release rather than removal of cyanotoxins, so removing extracellular dissolved toxins of the most important.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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