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EFFECTS OF CRUDE EXTRACT OF *MICROCYSTIS AERUGINOSA* KÜTZ. ON GERMINATION, GROWTH AND CHLOROPHYLL CONTENT OF *ZEA MAYS* L.

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Abstract

Effects of crude extract of *Microcystis aeruginosa* containing microcystin-LR on the germination, growth and chlorophyll content of *Zea mays* was studied. Soaking of *Z. mays* seeds 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. Soaking of *Z. mays* seeds for 24 hours in different concentrations of crude extracts of *M. aeruginosa* (100, 200, 300, 500 and 800 µg dry cells/ml) from log phase showed inhibitory effect of growth parameters and germination.

Introduction

Toxic cyanobacteria produce cyanotoxins at high levels that can cause chronic and sub-chronic toxicities to animals, plants and human. Cyanotoxicity in eukaryotes was mainly focused on animals. However, the number of studies related to the impact of cyanotoxin on aquatic and terrestrial crop plants irrigated by water containing these toxins, was increased during last few years. The use of this contaminated irrigation water can also have an economical impact which is caused by the reduction of the germination rate of seeds, and alteration of the quality and the productivity of crop plants (Peuthert *et al.* 2007). In addition to crop and vegetable plants might accumulate microcystins in their edible tissues (Mohamed and Al-Shehri 2009), and therefore, these plants might contribute directly or indirectly to cyanotoxin transfer through the food chain, and thus constitute a potent health risk source (Saqrane *et al*. 2009).

Cyanotoxins contaminant as microcystins (MCs) in water utilized to irrigate food crop plants have not yet been considered within any official monitoring program on water quality. Previous studies clearly indicated that irrigation with water containing MCs can be a threat for both the quality and yield of crop plants. This fact highlights the need to examine the MCs threshold which may be detrimental to crops (Pflugmacher *et al.* 2006). 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 (Chen *et al.* 2004).

MCs can also affect seed germination, early state development, and chlorophyll content, Pflugmacher *et al.* (2006) reported that germination of alfalfa seeds was inhibited by both purified MCs and anatoxin-a from a toxic cyanobacteria bloom, and by a cell-free crude extract from the same bloom. Peuthert *et al.* (2007) reported the uptake of MC-LR and MC-LF by roots of seedling of 11 agricultural plants, and their translocation to shoots. Spray irrigation of commercial lettuce (*Lactuca sativa*) plants with water containing *Microcystis* resulted in colonies and single cells of

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the Cyanobacterium when *Microcystis* was lodged on the leaves 10 days after the last irrigation (Codd *et al.* 1999). The use of this contaminated irrigation water have an economical impact which occurred by a reduction of the germination rate of seeds, and alteration of the quality and the productivity of crop plants (Saqrane *et al.* 2009).

The main objective of this work was to find out the eventual response to toxicity of cyanotoxins as the major agricultural impacts induced by the use of contaminated water for plant irrigation and effects of different concentrations of microcystin crude extract of *Microcystis aeruginosa* on the germination, growth and chlorophyll content of *Zea mays*.

Materials and Methods

Microcystis aeruginosa was isolated from River Nile channel near Tanta city, purified and identified according to Prescott (1978). *M. aeruginosa* was grown in medium Allen's and Stanier (1968) under continuous fluorescent illumination (80 µmol/m^2/s) at 25 ± 2ºC.

Microcystin crude extract was prepared according to Harada *et al.* (1988). Microcystin-LR was estimated using high performance liquid chromatography (HPLC) according to Shen *et al.* (2003).

The dried material from extract was re-dissolved in minimal volume of sterilized distilled water to obtain the following concentrations of microcystin crude extract (100, 200, 300, 500, 800 µg/ml), distilled water was used as control. Growth parameters including percentage of germination, root and shoot lengths and number of lateral roots of seedling were measured after 4 days of germination.

Seeds of *Zea mays* (maize) were surface sterilized in 3.5% sodium hypochlorite for 2.0 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 hrs and the same number of seeds were soaked in distilled water as control. Leaf areas of the seedlings were recorded using Ushikata x-plan 360d Planimeter (Featonby and Van Staden 1983). The photosynthetic pigments were estimated speectrophotometrically according to Metzner *et al.* (1965).

Results were presented as mean ± SD for three replicates. The statistical analyses were carried out using SAS program (1989-1996) version 6.12. Data obtained were analyzed statistically to determine the degree of significance between treatments using one way analysis of variance (ANOVA) at $p \leq 0.001$.

Results and Discussion

Soaking of maize seeds for 24 hrs in the cell-free medium of *M. aeruginosa* induced a significant reduction in root lengths of seedlings. The most pronounced inhibition was observed in seedlings soaked in the cell-free medium during death phase leading to 76.7% decrease as compared to the control value after 8 days of germination (Table 1). In case of seeds treated with cell-free medium of log phase, the root length was decreased by 44.6%. The shoot length was decreased by 97.6% as compared with death phase cell-free medium, while, seeds treated with cell-free medium from log phase, shoot length of maize seedlings was decreased by 25.8%. The highest reduction in number of lateral roots seedlings was recorded in seeds soaked in cell-free medium from death phase (Table 1). While, the seeds soaked in cell-free medium from log phase showed a reduction in the number of lateral roots by 20% as compared to that of control. On the other hand, the fresh weight of roots and shoots of maize seedlings was decreased by 97.7% in cell-free medium from death phase and by 37.5 and 44.6% for cell-free medium from log phase as compared to that of the control (Table 1). Cell free-medium of *M. aeruginosa* in death phase

decreased the dry weight of the root and shoot of maize seedlings by 99% as compared to that the control (Table 1). Also, cell-free medium from log phase reduced the dry weight of the root and shoot by 5.6 and 20.2% of that of the control after 8 days of germination. No leaves were formed for the seedlings from seeds that were soaked in cell-free medium from the death phase of *M. aeruginosa*. On the other hand, soaking seeds in cell-free medium from log phase reduced the leaf area by 23% as compared in that in the control (Table 1). This inhibition may be due to the presence of algal allelochemicals in the cell-free medium of *M. aeruginosa* (El-Sheekh *et al.* 2010). These allelochemicals which was 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 maize plant.

Table 1. Effects of extract of *Microcystis aeruginosa* on root and shoot lengths, number of lateral roots, fresh, dry weight of root and shoot, leaf area of 8-day-old of *Zea mays* seedlings.

Growth phases	Root length (cm)	Shoot length (cm)	No of lateral roots	Fresh weight of. root (g)	Fresh weight of shoot(g)	Dry weigh of root (g)	Dry weight of shoot (g)	Leaf area (cm^2)
Control	19.3 ± 1.5	25.2 ± 1.6	10 ± 1.00	0.72 ± 0.13	1.3 ± 0.10	0.09 ± 0.01	0.104 ± 0.01	65.5 ± 17.0
Log phase	10.7 ± 0.5***	18.7 ± 1.1***	8 ± 0.57***	0.45 ± 0.04(ns)	0.72 ± 0.09***	0.082 ± 0.008(ns)	0.083 ± 0.01(ns)	50.5 ± 11.2(ns)
Death phase	4.5 ± 0.5***	0.6 ± .03***	2 ± 0.57***	0.13 ± .0005***	0.03 ± 0.002***	0.0008 ± 0.00***	0.001 ± 0.0003***	0 ± 0***
F value	123.12	234.91	46.83	15.19	65.16	107.88	67.59	18.60
p value	0.0001	0.0001	0.0001	0.0011	0.0001	0.0001	0.0001	0.0006

Each value is the mean of three readings ± standard deviation. ***Highly significant at $p \leq 0.001$ using one way analysis of variance (ANOVA. (nsNon significant at $p \leq 0.001$ using one way analysis of variance.

The present results demonstrated that the cell-free medium of *M. aeruginosa* in the death phase showed the highest inhibitory effects on maize than that from log growth phase. This result is in agreement with that of Pearson *et al.* (1990) which demonstrated that most of the toxin release occurred as cells age and died and was passively leaked, although active release of toxins can also occur from young growing cells. Release of microcystin into the extra cellular environment was attributed to the death and lysis of cyanobacterial blooms (Sivonen and Jones**,** 1999), 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.

As described by El-Sheekh *et al.* (2010) the amount of total phenolic compounds and alkaloids in cell-free medium in death phase is 686.97 and 0.25 mg/l, respectively which is higher than in log phase. This may explain the inhibitory effect of the algal cell-free medium. Also phenolic compounds interfere to some degree with many vital processes, mineral uptake, respiration, photosynthesis, protein and chlorophyll synthesis. Nakano *et al.* (2004) reported that alkaloids showed growth inhibition against both mono- and dicotyledonous plants. Furthermore, these alkaloids exhibited higher activity against the growth of root than that of shoot of the plant species.

Soaking of maize seeds in cell-free medium of log phase of *M. aeruginosa* decreased chlorophyll *a*, chlorophyll *b* and carotenoid content of seedlings by 67, 65.4 and 60.7%, respectively as compared to control (Table 2). Saqrane *et al.* (2009) showed that cyanobacteria aqueous extract containing various MC variants caused a 22 - 25% decrease in chlorophyll *a, b* content in maize and *Lens esculenta* following 30-day-exposure to 2.1 and 4.2 µg/ml, but no significant effect on *Triticum durum* and *Pisum sativum* was observed.

Generally, one way analysis of variance showed that, soaking of maize seeds in log and death phase cell-free medium revealed high significant effect on all growth parameters and all pigment fractions (chlorophyll *a*, chlorophyll *b* and carotenoids) at ($p \leq 0.001$), except leaf area, fresh weight of root and dry weight of both the shoot and root revealed no significant effect in the case of soaking seeds in log phase cell-free medium (Tables 1, 2).

Table 2. Effects of seed soaking in the extract of *Microcystis aeruginosa* on photosynthetic pigments contents of 8-day-old *Zea mays* seedlings.

Growth phase	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
	(mg/g/dry tissue)		
Control	1.198 ± 0.17	0.8398 ± 0.13	0.5415 ± 0.07
Log phase	0.3959 ± 0.04***	0.2903 ± 0.04***	0.2128 ± 0.02***
Death phase	0 ± 0.00***	0 ± 0.00***	0 ± 0.00***
F value	94.47	64.42	94.49
p value	0.0001	0.0001	0.0001

Each value is the mean of three readings ± standard deviation. ***Highly significant at $p \leq 0.001$ using one way analysis of variance (ANOVA. (ns)Non significant at $p \leq 0.001$ using one way analysis of variance.

The different concentrations of crude extracts of *M. aeruginosa* reduced the root length of seedlings; increasing the concentration of microcystins crude extracts caused a decreased in the root length of maize seedlings (Table 3). The most pronounced reduction was detected in seedlings produced from presoaked seeds with 500 µg/ml crude extract by 73.5% as compared to control. However, no germination was detected with 800 µg/ml crude extract. The length of maize seedlings was reduced with the increase in the concentration of the toxin extract which ranged from 20 to 88% than that of the control value (Table 3). The number of lateral roots of maize

Table 3. Effects of different concentrations of microcystin crude extract of *Microcystis aeruginosa* in log phase on shoot, root lengths, number of lateral roots and percentage of germination of 4-day-old *Zea mays* seedlings.

Concentration (µg dry cells/ml)	Root length (cm)	shoot length (cm)	No. of lateral roots	% germination
Control	9.44 ± 0.7	4.52 ± 0.13	6 ± 0.7	100 ± 0.00
100	5.7 ± 0.85***	3.6 ± 0.45***	5 ± 0.7**	100 ± 0.00)ns(
200	4.33 ± 0.3***	1.8 ± 0.2***	3 ± 0.6***	76.66 ± 5.8***
300	3.1 ± 0.53***	1.36 ± 0.32***	0 ± 0***	66.66 ± 5.8***
500	2.5 ± 0.5***	0.56 ± 0.05***	0 ± 0***	53.33 ± 5.8***
800	0 ± 0***	0 ± 0***	0 ± 0***	0 ± 0***
F value	101.40	148.31	87.02	249.93
p value	0,0001	0.0001	0.0001	0.0001

Each value is the mean of three readings ± standard deviation. ***Highly significant at $p \leq 0.001$ using one way analysis of variance (ANOVA. **Significant at $p \leq 0.001$ using one way analysis of variance (ANOVA). (ns)Non significant at $p \leq 0.001$ using one way analysis of variance.

seedlings treated with 100 and 200 µg/ml microcystins crude extract was reduced by 16.6 and 50%, respectively as compared to that of the control (Table 3). On the other hand, the extract at a concentration of 100 µg/ml showed the same percentage of germination of the control (without toxins) after 4 days of germination, whereas in seeds soaked in 800 µg/ml did not germinate.

Maize seeds soaked in 200, 300 and 500 µg/ml microcystins crude extract showed a reduction germination by 23, 33 and 47%, respectively as compared to that of the control (Table 3).

One way analysis of variance showed that, soaking of maize seeds in different concentrations of microcystins crude extract of *M. aeruginosa* revealed a high significant reduction in all growth parameters ($p \leq 0.001$). However, the lowest concentration of crude extract (100 µg/ml) showed a significant effect ($p \leq 0.001$) on the number of lateral roots and nonsignificant effect (p>0.001or 0.05), it is preferable to add p-value exactly) on the percentage of germination compared to control (Table 3).

The seed soaked in death phase cell-free medium of *M. aeruginosa* showed more inhibitory effect on growth of maize than log phase. Different concentrations of microcystin crude extract (200, 300 and 500 µg/ml) decreased the percentage of germination of the seeds of maize while the concentration 800 µg/ml completely inhibited the germination of seeds after 4 days of growth (Table 3). These results are in agreement with those obtained by Pflugmacher *et al.* (2006) who reported that germination of alfalfa seeds was inhibited by both purified MCs and anatoxin-a from a toxic cyanobacteria bloom, and by a cell-free crude extract from the same bloom. Kurki-Helasmo and Meriluoto (1998) showed that seed germination of *Sinapsis alba* was significantly reduced in the presence of MC-RR at concentration of 5 µg/ml.

Microcystin crude extract decreased the root length of maize plant after four days of seedlings. The reduction increased by increasing crude extract concentrations. Peuthert *et al*. (2007) used an extract with mixture of MC-LF and LR to determine the effect of microcystins on several important agricultural plant species, the present study has clearly confirmed that, exposure to MCs crude extract containing MC-LR can induce firstly highly significant effect on the inhibition of germination and elongation of the primary root of *Z. mays* and inhibition effect increased by increasing the concentration of the crude extract from 100 to 800 µg/ml. Present results are supported by Saqrane *et al.* (2008) which showed the effect of cyanbacteria aqueous extracts containing MC-LR exposure during four days strongly inhibits the germination of *Pisum sativum* seeds. Microcystins have a pronounced inhibitory effect on number of primary root, epicotyls length and the lateral root formation of maize (Table 3). Similar results were reported in white mustard seedlings by M-Hamvas *et al.* (2003).

In conclusion, this study shows the high toxic and cytotoxic influence of cyanobacterial extract containing microcystins on plant growth and supports the idea that, the use of surface water containing MC for irrigation can affect both plant crop yield and quality.

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