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Germination and Early Life Stage Development of Lettuce and Carrot upon Exposures to Dissolved Microcystins

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Abstract

Cyanobacterial mass development and their toxins have become an environmental issue recently. In this study, we tested the effects of two crude extracts contained microcystins (MC), including an isolated *Microcystis aeruginosa* and a water blooms sample collected from Dau Tieng Reservoir, Vietnam, on the seed germination, root and shoot prolongation and wet weight (WW) during the seedling of lettuce and carrot. The MC concentrations were environmentally relevant, 1-100 µg/L and the experimental period lasted for 7 days. We found that both lettuce and carrot seeds were suffered from the MC impact with a lower germination rate compared to control. After 4 and 7 days of the experiment, the root and shoot of both plants were shorter upon MC incubation and the higher MC concentration applied, the shorter length of root and shoot was. The WW of lettuce and carrot seedling was significantly reduced in exposures to MC at the concentrations of 10 and 100 µg/L. This study confirmed the potent toxicity of MC from Vietnam waters at the environmental concentrations to lettuce and carrot. Bioaccumulation and distribution of MC in the plants are suggested to investigate in the future.

Keywords: Cyanobacterial toxins, Seedling, Exposure, Impacts

1. Introduction

Freshwater bodies in the world have commonly been under eutrophic conditions (Chorus & Bartram, 1999) which would be consequently on the mass development of cyanobacteria. Besides, bloom forming of cyanobacteria has been enhanced frequently and intensity upon the climate change context (Paerl & Huisman, 2009). Cyanobacteria could gain nearly 100% of phytoplankton biomass during their blooms (Vasconcelos & Pereira, 2001) and they are usually associated with cyanobacterial toxin occurrence (Zurawell, Chen, Burke, & Prepas, 2005). Microcystins (MC) are among the most common and potent cyanobacterial toxins (e.g. MC, anatoxin-a, cylindrospermopsin, saxitoxins) in inland aquatic ecosystems. Their concentrations in water could be very high, some hundred µg MC/L in dissolved form or even up to several mg/L in bloom materials. The toxins could induce a wide range of impacts on aquatic plants, animals and human beings (Chorus & Bartram, 1999).

Surface water from rivers, lakes, and reservoirs could be used for multi-purposes such as drinking water supplies, aquaculture and agricultural activities. Codd (1999) reported that the cyanobacterium *Microcystis aeruginosa* and its toxin (MC) were retained by lettuce after spraying with irrigation water containing the toxic cyanobacterium. In line with this study, McElhiney Lawton, and Leifert (2001) found the plant growth was inhibited when watered with cyanobacterial extract containing MC. Similarly, the growth and development of different plants such as rice, rapeseed, mustard, duckweed were negatively affected by MC (Chen, Song, Dai, Gan, & Liu, 2004; Kurki-Helasmo & Meriluoto, 1998; Mitrovic, Allis, Furey, & James, 2005). Incubation in MC (either from purified or extracted material) resulted in a reduction of seed germination, root and shoot length and leaf shape of spinach, rapeseed and tomato (Al-Sultan & Hatem, 2019; Chen et al., 2004; Dao, Le, Pham, Do-Hong, & Nguyen, 2014; Pflugmacher, Aulhorn, & Grimm, 2007). Previous investigations noted that the fresh weight of plants (e.g. rice and rapeseed) exposed to MC was reduced (Chen et al., 2004; Dao et al., 2014). Besides, cyanobacterial toxins (MC, anatoxin-a) could cause a decrease of pigments in plants (Al-Sultan & Hatem, 2019; McElhiney et al., 2001; Weiss, Liebert, & Braune, 2000), consequently photosynthesis inhibition (Pietsch et al., 2001; Wiegand, Peuthert, Pflugmacher, & Carmeli, 2002). The cyanobacterial toxins could strongly regulate the antioxidant and biotransformation enzyme activities in plants (Ha & Pflugmacher, 2013; Pflugmacher et al., 2007).

In Vietnam, surface-water inland has been exploited for domestic use and farming. Toxic cyanobacteria, cyanobacterial blooms and their toxins (MC) have been reported widely (Dao, Cronberg, Nimptsch, Do-Hong, & Wiegand, 2010; Dao et al., 2014; Duong et al., 2013; Pham, Dao, Shimizu, Do-Hong, & Utsumi, 2015; Pham et al., 2017). Biomass of harmful cyanobacteria in water bodies (e.g. Tri An Reservoir) tends to increase spatially and temporally during the latest years (Nguyen, Ha, & Pham, 2020). Plants could be irrigated with water containing cyanobacteria and their toxins during farming. Therefore, we raised a question if the germination and seedling of the lettuce and carrot, which are commonly planted and consumed from Central Highland of Vietnam, are affected by MC at the environmental concentrations (1-100 µg/L) from Vietnam waters.

2. Materials and Methods

2.1 Materials

The seeds of lettuce (*Lactuca sativa* L.) and carrot (*Dacus carota* L.) were purchased from Trang Nong Company, located in District 6, Hochiminh City. The two cyanobacterial materials were used for experiment including (i) cyanobacterial scum (mainly *Microcystis* spp.) collected in July 2011 and (ii) the cyanobacterial isolate *Microcystis aeruginosa* from Dau Tieng Reservoir.

Crude extract from cyanobacterial scum and the isolate was prepared according to Pietsch et al. (2001) with minor modification. Briefly, 5 g of the dried biomass of cyanobacterial scum and isolate on GF/C filters was homogenized, suspended into 100 mL reversed osmosis water, sonicated, frozen at −70°C overnight and thawed at room temperature. The freeze/thaw cycle was repeated five times. After the last thawing cycle, samples were centrifuged at 4500 rpm, 4°C for 15 min. Supernatant was collected and kept at −70°C prior to experiments on the plant seedlings. The cyanobacterial scum material (Scum) contained around 687 µg MC/g dried mass (Dao et al., 2014), and the cyanobacterial isolate (*M. aeruginosa*, Ma) had a MC production of 3,733 µg/g dried mass (Vo, Pham, & Dao., 2016). Therefore, two cyanobacterial extracts, so-called mother solutions, from the scum material has a MC concentration of 34,350 µg/L, and that from the isolate has a MC concentration of 186,650 µg/L. To get the concentrations of around 1, 10 and 100 µg MC/L for the experiment, we diluted the mother solution with distilled water. We took 2.91 mL, 291 µL, and 29 µL of mother solution from scum material and filled with distilled water to a total volume of 1 liter, to make final MC concentrations of around 100 µg/L, 10 µg/L and 1 µg/L, respectively. Similarly, we took 536 µL, 54 µL, and 5.4 µL of mother solution from isolate and filled with distilled water to a total volume of 1 liter, to make final MC concentrations of around 100 µg/L, 10 µg/L and 1 µg/L, respectively.

2.2 Experimental setup

The experimental setup to test the seedling of lettuce and carrot was conducted according to Chen et al. (2004) and Dao et al. (2014) with minor modification. We did the same experimental design for seeds of carrot and lettuce. For the first experiment, the germination investigation, 50 seeds of either lettuce or carrot were placed onto paper

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tissue put in a glass petri disc (10 cm in diameter) and three replicates (n = 3) were prepared for each treatment. In the control, the seeds were watered with 10 mL of distilled water. However, in the MC exposures, the seeds were treated with 10 mL of cyanobacterial extract from either scum sample or *M. aeruginosa* (cyanobacterial isolate) containing around 1, 10 and 100 µg MC/L. Therefore, in each kind of seed (lettuce or carrot) there were 7 treatments including (1) control, (2) around 1 µg MC/L from scum sample (abbreviated as Scum-1), (3) around 10 µg MC/L from scum sample (abbreviated as Scum-10), (4) around 100 µg MC/L from scum sample (abbreviated as Scum-100), (5) around 1 µg MC/L from the isolate *M. aeruginosa* (abbreviated as Ma-1), (6) around 10 µg MC/L from the isolate *M. aeruginosa* (abbreviated as Ma-10), and (7) around 100 µg MC/L from the isolate *M. aeruginosa* (abbreviated as Ma-100). The seed germination, defined as the root appearance from the seeds, in each treatment were counted after 24 h for lettuce and 72 h for carrot. Experiments were run at 25±1ºC, with the humidity of 75%, and in the dark.

The germinated seeds (seedling) in the petri discs from the first experiment were used for the second experiment on the root, shoot and wet weight development of lettuce and carrot. They were transferred onto tissue paper located in 100 mL glass beakers. Three replicates (n = 3) for each treatment, and 10 germinated seeds were put into a beaker. The seedlings were daily watered with 3 mL of distilled water (in control) or water containing around 1, 10, 100 µg MC/L from either scum sample or isolate, as mentioned in detail above. The tests were incubated at 25 ± 1ºC and the humidity of 75%, light intensity of around 1500 Lux, and 12 h light followed by 12 h dark cycle. The WW, shoot length and root length of the seedlings were recorded at 4 and 7 days of incubation. The WW was determined using a balance (Sartorius BP 201S, Germany) and the length was measured with a ruler of 1 mm spacing.

2.3 Data treatment

The germination rate of lettuce and carrot seeds was calculated by the ratios of the germinated seeds over the 50 seeds used for each treatment.

Sigmaplot, version 12.0 was used for data analysis. Kruskal-Wallis test was applied to determine the statistically significant differences of the WW, shoot and root length of seedlings in control and cyanobacterial extract treatments.

3. Results and Discussion

3.1 Germination of the lettuce and carrot seeds upon treatments with MC-containing cyanobacterial extracts

The germination rate of the lettuce and carrot seeds was shown in the Table 1. The rate in two controls, lettuce and carrot seeds, was 94% and 93%, respectively. In the treatments with the cyanobacterial extract from *M. aeruginosa* (Ma-1, Ma-10 and Ma-100), the rate varied between 81-87% for lettuce, and 59-85% for carrot. Besides, the treatments with an extract from the cyanobacterial scum (Scum-1, Scum-10 and Scum-100), the germination rate of lettuce was from 80-90%, and that of carrot was from 69-79% (Table 1). The higher microcystin concentration in the treatment it was, the lower germination rate of the plants (lettuce and carrot) it was. At the same toxin concentration from two different sources of MC (scum sample and isolate), the seed germination rate was similar. However, the germination rate of carrot was lower than that of lettuce when the seeds were treated with the same kind and concentration of MC.

Chen et al. (2004) found that the germination rate of rapeseed exposed to 0.6 µg MC/L was significantly lower than that in the control. This previous finding supported our record, evidencing the germination influence by the MC from the concentration of 1 µg/L. Mackintosh et al. (1990) reported that MC could inhibit the enzyme activities (e.g. protein phosphatases 1 and 2A) in plants. During germination, many biochemical processes are happening in seeds and also cellular division. Exposure to MC would result in dephosphorylating the regulatory proteins (Mackintosh et al., 1990), hence germination of the seeds could be delayed. Our study contributes the toxicity information from the cyanobacterial toxins from Vietnam waters to the germination of the seeds of plants carrot and lettuce.

Table 1. The seed germination rate of lettuce and carrot. Numbers expressed the mean values and standard deviations of n = 30. Different letters (a- e) indicated a significant difference of $p < 0.05$ by Kruskal-Wallis test.

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Treatments	MC concentrations (μg/L)	Germination rate (%)	
		lettuce	carrot
Control	0	94 ± 1,2[e]	93 ± 1,7[e]
Extract from isolate (*M. aeruginosa*)	1	87 ± 1,7[cd]	85 ± 1,7[d]
	10	81 ± 1,7[ab]	80 ± 1,2[cd]
	100	82 ± 1,2[ab]	59 ± 1,7[a]
Extract from cyanobacterial scum (Scum)	1	90 ± 1,2[de]	79 ± 1,7[c]
	10	85 ± 1,3[bc]	77 ± 1,7[c]
	100	80 ± 1,2[a]	69 ± 0,6[b]

3.2 Root and shoot length of the lettuce and carrot upon treatments with MC-containing cyanobacterial extracts

After 4 days of the experiment, the root length of lettuce and carrot in the control was around 17.7 and 29.6 mm, respectively. However, the root of lettuce in the treatments with the extracts from isolate and scum material ranged from 15.9-16.6 mm, and 11.3-13.6 mm, respectively (Figure 1a). The root length of carrot in the Ma and Scum treatments was from 20.9-26.6 mm and 21.4-26.2 mm, respectively (Figure 1c). The length of root in treatment was significantly shorter than that in the control ($p < 0.05$; Kruskal-Wallis test).

After 7 days of the experiment, lettuce root in MC treatments (Ma and Scum) was from 17.4-26.9 mm long which was significantly different ($p < 0.05$) from that in the control, 28.2 mm (Figure 1b; Figure 2). A similar phenomenon, significant difference ($p < 0.05$), was observed with root length of carrot in MC incubations (30.9-45.8 mm) and the control (51.1 mm; Figure 1d). Besides, we found that the higher toxin concentrations in exposure, the lower root length of the seedlings of both lettuce and carrot was observed. Hence the negative effect seemed to be concentration dependent.

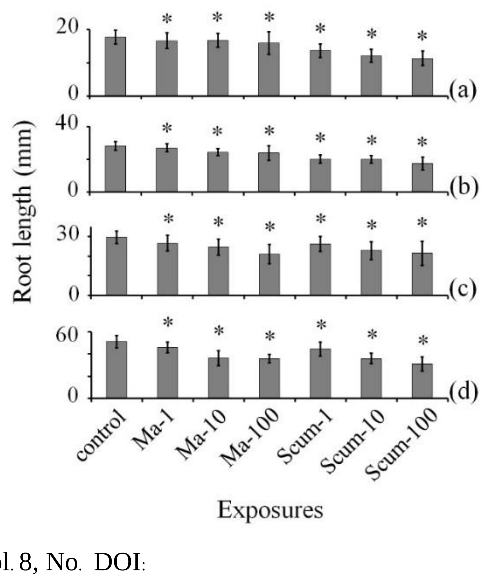

Figure 1. Root length of lettuce at 4 days (a) and 7 days (b), and of carrot at 4 days (c) and 7 days (d) of incubation. Ma and Scum are treatments with an extract from *M. aeruginosa* and cyanobacterial scum, respectively. Numbers (1, 10, 100) right after Ma and Scum revealed the MC concentrations as μg/L. The asterisks indicated the significant difference ($p < 0.05$) between control and exposures by Kruskall-Wallis test.

We found that the shoot length of lettuce in control, Ma-1 and Ma-10 was within a range of 2.9-3.1 mm after 4 days of testing. However, in Ma-100 and Scum treatments was between 2.2-2.5 mm, significantly different from the control (3.1 mm; $p < 0.05$; Figure 3a). After 7 days of incubation, the shoot length of lettuce in control was 4.7 mm, significantly longer than that in Ma and Scum treatments, ranging from 2.9-3.7 mm (Figure 3b). Shoot length of carrot was similar among the control (7.8 mm), Ma-1 (7.3 mm) and Scum-1 (7.1 mm) at the fourth day of the experiment (Fig. 3c). By the end of the experiment, the shoot length of carrot in all treatment varied between 7.5 and 9.3 mm, which was significantly different from that in control (11 mm; Figure 3d). Similar to the root development, a higher MC concentration of exposure resulted in a shorter shoot length of the carrot.

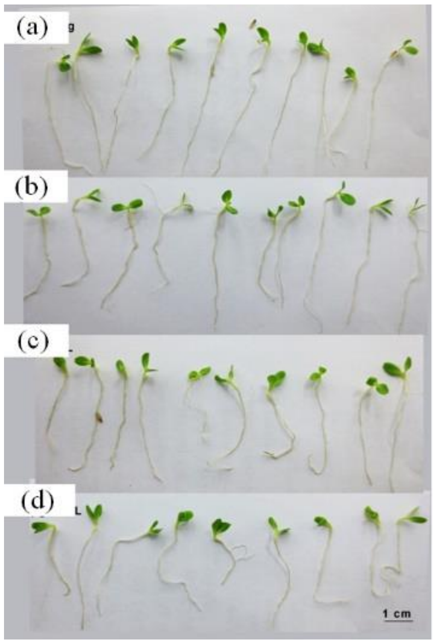

Figure 2. Shoot length of lettuce in control (a), Scum-1 (b), Scum-10 (c), and Scum-100 (d). Abbreviations as in Figure 1

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The exhibition of shorter length of root and shoot of lettuce and carrot upon MC exposures compared to that in control over time (4 and 7 days) was in line with previous studies testing with rapeseed, rice, tomato (e.g. Chen et al., 2004; Ha & Pflugmacher, 2013; Pflugmacher et al., 2007; Wiegand et al., 2002). The cyanobacterial toxin MC are known to have negative effects on the activities of functional, biotransformation, and antioxidant enzymes in plants (Chen et al., 2004; MacKintosh et al., 1990; Pflugmacher, 2004; Stuven & Pflugmacher, 2007; Wiegand et al., 2002). Besides, MC could impact on the ATP-synthetase enzyme (Mikhailov, Harmala-Brasken, Hellman, Meriluoto, & Eriksson, 2003). Therefore, under exposure to MC, the lettuce and carrot would spend energy for both seedlings and dealing with the enzyme activity alteration. This would result in energy costs and diminish for normal development, consequently slowing down the root and shoot prolongation. Also, the MC exposed seedlings would suffer from the biochemical impacts as mentioned above consequently cell development impairment which needs further studies to clarify.

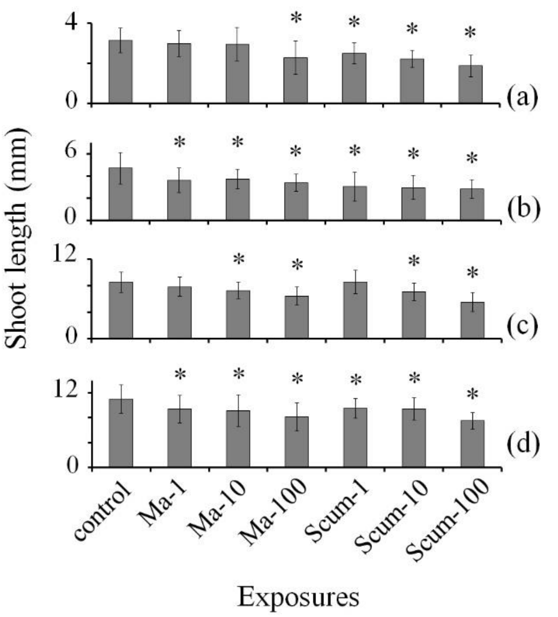

Figure 3. Shoot length of lettuce at 4 days (a) and 7 days (b), and of carrot at 4 days (c) and 7 days (d) of incubation. The asterisks indicated the significant difference ($p < 0.05$) between control and exposures by Kruskall-Wallis test. Abbreviations as in Figure 1.

In the case of shoot development, the impact of Ma-1 and Scum-1 was not shown after 4 days of incubation but clearly showed after 7 days of treatment. Maybe there was tolerance able of the shoot seedling to low MC concentration (1 µg/L) within a four-exposure day but impossible after a longer exposure time, 7 days. Also, the impacts on shoot and root length were already found at higher MC concentrations (10 and 100 µg/L) at the 4th day of experiment. Our result contributes to the understanding on the adverse effects of water containing MC on seedling of lettuce and carrot. *In situ* investigations (e.g. in farms from Dalat City, Vietnam) are suggested to clarify to impacts of the toxin on plant development and crop.

3.3 Wet weight of the lettuce and carrot upon treatments with MC-containing cyanobacterial extracts

At the 4th day of the experiment, the WW of lettuce seedlings in control, Ma-1 and Scum-1 was similar, and gained from 9.6-10.5 mg (Figure 4a). Similarly, at the 7th day of the experiment in control and the lowest MC concentration exposures (Ma-1 and Scum-1) was not significantly different, ranging from 11.9-13.5 mg (Figure 4b). However, the significant difference of lettuce seedling WW in control and other exposures (Ma-10, Ma-100, Scum-10, and Scum-100) was recorded after 4 and 7 days of the expriment ($p < 0.05$).

In the experiment with carrot, we found a similar trend with lettuce, no significant difference between WW in control (8.6 and 13.7 mg at 4th and 7th day, respectively) and lowest MC concentration incubation (Ma-1 and Scum-1) after 4 and 7 days of treatment (Figure 4c, d). We also recorded the significant difference of WW from control and higher MC exposures (Ma-10, Ma-100, Scum-10, and Scum-100) after 4 and 7 days of treatment.

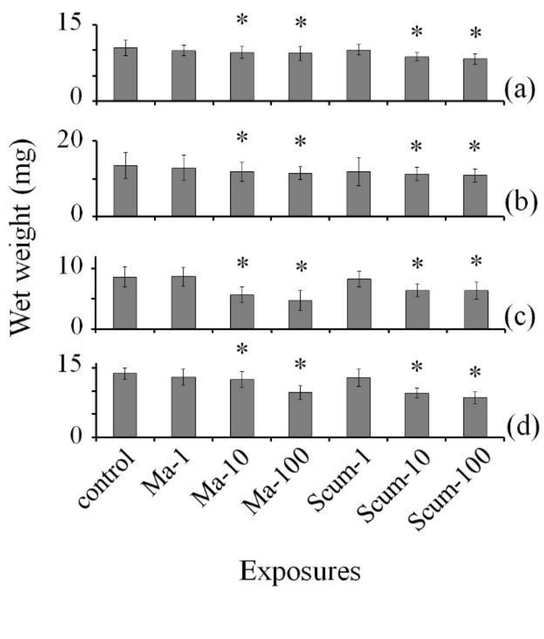

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Figure 4. The wet weight of lettuce at 4 days (a) and 7 days (b), and of carrot at 4 days (c) and 7 days (d) of incubation. The asterisks indicated the significant difference ($p < 0.05$) between control and exposures by Kruskall-Wallis test. Abbreviations as in Figure 1.

The WW reduction of lettuce and carrot in our study is supported by previous studies (Chen et al., 2004; Dao et al., 2014). As mentioned above, MC could diminish energy costs for plants and interfere with the enzyme activities in the cells of plants. The enzyme activity inhibition may lead to the decrease of water uptake into the cells. Also, the prevention of prolongation of root and shoot would consequence on the water volume reduction in the MC exposed seedlings. The influences would result in the reduction of water uptake and/or storage in cells and tissues of the plants, hence WW decreased. The similarly WW between Ma-1, Scum-1 and control revealed that the MC at the concentration of 1 µg/L was not strong enough to reduce the WW of the exposed seedlings. Furthermore, MC uptake and distribution in stems, rhizomes and leaves of aquatic macrophytes was reported (Pflugmacher, Wiegand, Beattie, Codd, & Steinberg, 1998). Other plants such as broccoli, mustard and duckweed are able to accumulate MC in their leaves and bark protein and whole plant after exposure (Jarvenpaa et al., 2007; Mitrovic et al., 2005). The uptake and accumulation of MC are not included in the current study and should be investigated with carrot and lettuce in future.

4. Conclusions

The seed germination of lettuce and carrot was negatively affected upon exposure to MC from both isolate and field samples of cyanobacteria. The effect of MC on seed germination of carrot was stronger than that of lettuce. The root and shoot development and prolongation were impaired by MC with concentration dependant. The WW of the seedlings was decreased under the MC treatment. The impacts of MC on germination and seedlings of plants in our study are believed to strongly link to the changes of enzyme activities and energy cost caused by MC during the cell processes at the early stage of the seedlings. We confirmed the toxic effects of MC from Vietnam waters at the environmental concentrations on common plants, lettuce and carrot. Bioaccumulation and distribution of MC in the plants are suggested to investigate in the future.

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