Detrimental impacts of toxic Microcystis aeruginosa from Vietnam on life history traits of Daphnia magna

Anh hướng tiêu cực của loài Microcystis aeruginosa có độc ở Việt Nam lên các đặc điểm vòng đời của Daphnia magna

Research article

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In this study, we tested the long-term and negative effects of microcystin-producing cyanobacterium Microcystis aeruginosa from Vietnam on Daphnia magna under the laboratory conditions. The test organisms were fed with mixtures of green algae Scenedesmus armatus and toxic M. aeruginosa at different ratios (10% Microcystis + 90% Scenedesmus, 50% Microcystis + 50% Scenedesmus, 100% Microcystis, and 100% Scenedesmus) for over a period of 21 days. The life history traits of the organisms such as, survival, maturation, fecundity were daily recorded. Besides, the intrinsic population rate of D. magna in each treatment was also calculated based on the survivorship, the reproductive age and the clutch size of the animals. The results showed that survival, maturation and reproduction of the D. magna fed with 10, 50 and 100% M. aeruginosa was impaired. Additionally, the intrinsic population rate of the exposed D. magna was lower than that of the control. This study evidenced the adverse effects of toxic M. aeruginosa on both the individual and intrinsic population levels of D. magna. To our knowledge, this is the first report on the chronically detrimental impacts of toxic M. aeruginosa isolated from Vietnam on D. magna and contributed the scientific information on the severe influences of toxic cyanobacteria world wide.

Trong bài viết này, chúng tôi nghiên cứu ảnh hưởng xâm lấn của loàiMicrocystis aeruginosa có độc ở Việt Nam lên Daphnia magna trong điều kiện phòng thí nghiệm. Sinh vật thí nghiệm được cho ăn với hỗn hợp tảo lục Scenedesmus armatus và M. aeruginosa có độc ở các tỷ lệ khác nhau (10% Microcystis + 90% Scenedesmus, 50% Microcystis + 50% Scenedesmus, 100% Microcystis, và 100% Scenedesmus) trong thời gian 21 ngày. Các đặc điểm vòng đời của sinh vật bao gồm số sống, sự thành thục, số sinh sản được theo dõi hàng ngày. Bên cạnh đó, tỷ lệ phát triển quần thể của D. magna trong từng lô thí nghiệm cũng được tính toán dựa vào số sống, tuổi sinh sản và kích cỡ sinh sản của sinh vật. Kết quả cho thấy, số sống, tuổi thành thục và số sinh sản của D. magna cho ăn với 10, 50 và 100% M. aeruginosa bị ảnh hưởng xấu. Bên cạnh đó, tỷ lệ phát triển quần thể của D. magna trong lô phối nhiễm thấp hơn so với đối chứng. Nghiên cứu này chứng minh ảnh hưởng xâm lấn của M. aeruginosa có độc lên cả hai mức độ cá thể và quần thể của D. magna. Theo hiểu biết của chúng tôi, đây là báo cáo đầu tiên về ảnh hưởng xâm lấn của M. aeruginosa có độc phân lập từ Việt Nam lên D. magna and đóng góp thêm thông tin khoa học cho những ấn tượng nghiêm trọng của vi khuẩn làm có độc trên khắp thế giới.

Keywords: life history traits, microcystins, Daphnia magna, Microcystis aeruginosa, negative effects

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1. Introduction

Eutrophication is known to cause and enhance the cyanobacterial mass development. Seriously, cyanobacteria are capable of producing toxic metabolites and bioactive compounds such as microcystins (MCs), anatoxin-a, cylindrospermin, among others (Sinoven et al., 1999; Banker et al., 1999; Rohrlack et al., 2003) of which MCs are the most common and potent cyanobacterial toxins in freshwater bodies (Doekel et al., 2001).

Many investigations have showed that cyanobacteria and their toxins are extremely toxic to aquatic organisms, and the toxicity investigations have focused on the effects of Microcystis on Daphnia over the last few decades. For example, in the laboratory condition, the mortality of Daphnia increased when fed on MCs-producing cyanobacteria and purified microcystin-LR. Additionally, the effective rate strongly depended on the concentration of exposure and the sensitivity of species (Demott and Moxter, 1991; Trubetskova and Haney, 2006). Also, there have been evidences that the feeding rate was inhibited, and the growth rate and reproduction were decreased when the daphnids were priorly fed with toxic Microcystis (DeMott, 1999). In spite of not including any toxic impacts, the negative effects of some Microcystis strains on body length, fecundity (number of new born per female) and clearance rate of Daphnia were recorded (Lürling and Van der Grinten, 2003; Lürling, 2003).

In recent years, researchers have cared about the maternal effects of cyanobacteria and their toxins on zooplankton. If cyanobacteria and their toxins reside inside the body of Daphnids for a longer period of time, it can be transferred to their offspring. Guo and Xie (2006) proved the tolerance of Daphnia to toxic microcystins (MCs) in the laboratory conditions of 22 ±1ºC, dim light and light dark cycle of 14h:10h. Microcystis aeruginosa was used for exposure to D. magna. The cyanobacterium M. aeruginosa (Fig. 1) was isolated from Dau Tieng Reservoir, a drinking water supply 120 km western Hochiminh City, Vietnam. Both cyanobacterium and green alga were cultivated in Z8 medium (Kotai, 1972) with continuous aeration and under the laboratory conditions of 25 ±1ºC, light intensity of around 3000 Lux, and light dark cycle of 12h:12h.

![Figure 1. The organisms for the toxicity test. a, Microcystis aeruginosa; b, newly born Daphnia magna. Scale bar of a = 20 µm, and b = 300 µm.](image)

2. Materials and methods

2.1 The test organisms

*Daphnia magna* Straus was purchased from the MicroBioTests Inc, Belgium (Fig. 1). The animal has been fed with green alga *Scenedesmus armatus* and maintained in the laboratory conditions of 22 ±1ºC, dim light and light dark cycle of 14h:10h. *Microcystis aeruginosa* was used for exposure to D. magna. The cyanobacterium *M. aeruginosa* (Fig. 1) was isolated from Dau Tieng Reservoir, a drinking water supply 120 km western Hochiminh City, Vietnam. Both cyanobacterium and green alga were cultivated in Z8 medium (Kotai, 1972) with continuous aeration and under the laboratory conditions of 25 ±1ºC, light intensity of around 3000 Lux, and light dark cycle of 12h:12h.

2.2 Toxin analysis

The culture of *M. aeruginosa* was harvested during the exponential growth phase and filtered onto GF/A filters (Fiore, France), dried at 50°C over night and stored at -70ºC prior to toxin determination. For MCs determination, the filters containing microbes were cut into small pieces with scissors. Extraction of MCs was conducted according to Barco et al. (2005) with minor modification. Briefly MCs were firstly extracted in 5 mL of 100% (vol/vol) aqueous methanol by shaken for 60 min followed by 2 × 60 min of extraction in 3 mL of 75% aqueous methanol. Each extraction step was followed by centrifugation (4.500 rpm, 30 min, 4ºC). The supernatants of all extractions from each sample were pooled, dried at room temperature, re-dissolved in 0.5 mL MeOH (100%) and centrifuged at 8.000 rpm, 4ºC for 5 minutes. The supernatant was passed through a Minisart RC 4 filter membrane (0.2 µm pore size, Sartorius SediM Biotech, Germany), and kept at -20ºC prior to reversed phase HPLC for analysis. Reverse phase HPLC (Shimadzu 10A series, Shimadzu, Kyoto, Japan) equipped with a silica based reverse phase C18 column (Waters SunFire™ 5 µm, 3.0×250 mm, Ireland), main- tained at 40ºC. A 0.05 M phosphate buffer (pH 2.5) in methanol (50/50, v/v) was used as mobile phase, at a flow rate of 0.58 mL min⁻¹. MC congeners were detected by the
UV detection at 238 nm with a photodiode UV-visible array detector. Microcystin-LR, -RR and -YR purchased from Wako chemicals company (Osaka, Japan) were used as standards. The HPLC system had a detection limit of 0.01 μg L⁻¹.

2.3 Experimental setup

Fifteen neonates (< 24h old) were used for each chronic experiment (Adema, 1978) and individually raised in 50 mL beakers containing 20 mL of medium (Dao et al., 2010). In the control experiment, the Daphnia was fed with 100% of green alga S. armatus at the concentration of 1 mg C L⁻¹ day⁻¹ (Gustafsson et al., 2005). In exposures, Daphnia was fed with a mixture of Scenedesmus and cyanobacterium Microcystis aeruginosa with total concentration of 1 mg C L⁻¹ day⁻¹, at three different regimes (1) 10% Scenedesmus + 90% Microcystis; (2) 50% Scenedesmus + Microcystis; and (3) 100% Microcystis (Table 1). In total, four incubations including one control and three different exposures (Table 1) were run under the temperature of 22 ± 1°C, dim light and light dark cycle of 14h: 10h.

All medium and food were renewed every two days. The life history traits of Daphnia such as survival, maturity age, reproduction were daily observed during 21 days. Besides, age specific survival and clutch size were used to estimate the intrinsic rate of population increase, r, as a measure of fitness. The Euler equation (Stearns, 1992) was used to calculate r:

\[
1 = \sum e^{-rx}l_xm_x
\]

Where x is age (in days), l_x is the probability of surviving and m_x is the fecundity at age x

| Table 1. Summary of the treatments in the toxicity test |
|------------------------------------------------------|
| Scenedesmus armatus | Microcystis aeruginosa |
| Control | 100% | 0% |
| 10% Ma | 90% | 10% |
| 50% Ma | 50% | 50% |
| 100% Ma | 0% | 100% |

2.4 Statistical analysis

SigmaPlot version 12 was used for the data treatment. Kruskal-Wallis test was applied for calculation on statistically significant difference of the maturation of D. magna. P-values less than 0.05 were considered statistically significant.

3. Results and discussion

3.1 Microcystins concentration in the M. aeruginosa

The results of HPLC analysis showed that the M. aeruginosa strain produced MC-LR with the concentration of 3733 μg g⁻¹ dry weight (Fig. 2). Along with MC-LR, MC-RR is the most frequent MC variant, which poses a grave threat to both environment safety and public health (Zhang et al., 2007). This concentration of MCs was comparable with the results in previous the studies (e.g. Nguyen et al., 2007; Vasconcelos et al., 1996), in which the highest MCs concentrations were up to 4120 μg g⁻¹ dry weight in culture and 1000 – 7100 μg g⁻¹ dry weight in natural lakes, reservoirs and rivers. The high MCs-producing Microcystis proposed a serious risk to local residents who daily use the water from Dau Tieng reservoir for domestic activities.

3.2 Effects of M. aeruginosa on the survivorship of D. magna

In the control, all D. magna were well alive by the end of experiment. However, Daphnia started to die within approximately 1 week in all Microcystis treatments (Fig. 3). By the end of incubation, the Daphnia exposed to 10% and 50% Microcystis decreased their survival proportion to 47% and 33%, respectively. The survivor proportion was lowest in the treatment of 100% Microcystis (27%, Fig. 3) evidencing that toxic Microcystis had a strong impact on survival of Daphnia with concentration dependence. The current record was in agreement with the investigation of Dao et al. (2010), in which survivor of D. magna was 10% and 55% in 5 and 50 μg MC L⁻¹ treatments within 2 months, respectively. With the MCs concentration of 3733 μg g⁻¹ dry weight (as mentioned above) and the concentration of M. aeruginosa used for the test (0.1 – 1 mg C L⁻¹), the MCs concentrations in the treatments of our study were not more than 0.013 μg MC L⁻¹ (100% Microcystis treatment). However, the survivorship of the exposed D. magna decreased so strong (up to 73% in 100% Microcystis treatment). This could be explained as (i) the cyanobacterium used in the experiment was live cells and cyanobacteria were considered to be low nutritional value for zooplankton, mainly due to the absence of essential polyunsaturated fatty acid (PUTA) and sterols (Brett and Muller-Navarra, 1997; Von Elert, 2002), and (ii) beside MCs, Microcystis could produce other toxic bioactive compounds which
need further investigation and chemical analysis with modern equipment (e.g. LC/MS, GC/MS) to confirm.

Figure 3. Survival of D. magna from control and exposures during 21 days of incubation. Abbreviation as in Table 1.

3.3 Effects of M. aeruginosa on the maturation of D. magna

Daphnia raised in control reached its maturity at the age of around 6 days old. However, the animals fed with toxic M. aeruginosa significantly delayed their maturation to the ages from 10 – 11 days (Fig. 4). Seriously, some organisms in the Microcystis treatments were not able to reach their maturation although they were alive during 21 days of experiment. The negative effect of MCs on maturation of the tested organisms in this study was similar to the investigation of Dao et al. (2010).

Figure 4. Maturation of D. magna (mean value ± SD of n as indicated in the columns) from control and exposures during 21 days of incubation. Asterisks indicate significant difference by Kruskal-Wallis test (**, p < 0.01; ***, p < 0.001). Abbreviation as in Table 1.

Some studies reported that cyanobacteria do not have enough nutrient and energy for development and maturation of the animals, mainly due to the absence of essential polyunsaturated fatty acids (PUFA) and sterols (Bret and Muller-Navarra, 1997; Von Elert, 2002). Therefore, the mal-nutrient effect should be the root for the maturation postponement of D. magna in 100% Microcystis treatment of our study. However, as green alga Scenedesmus is a good food for Daphnia, the delayed maturation in 10% and 50% Microcystis treatments could be partly because of the mal-nutrient and partly because of toxic compounds in the Microcystis cells (e.g. MC-RR, and other bioactive compounds) affecting the animal physiology. Additionally, during the experiment, we observed the smaller body size of the Microcystis exposed Daphnia compared to the control. Green (1956) and Ebert (1991) reported that smaller Daphnia took more instars to mature consequently late maturation than the larger Daphnia.

3.4 Effects of M. aeruginosa on the reproduction of D. magna

In the control, the clutch size of mother D. magna was around 10 offspring. However, the clutch size of mother D. magna was decreased by in Microcystis treatments. The average number of offspring per clutch in both 10% and 50% Microcystis treatments were approximately 5 individuals whereas that in 100% Microcystis treatment was only 1 individual (Fig. 5).

Figure 5. Fecundity of D. magna (mean value ± SD of n as indicated in the columns) from control and exposures during 3 weeks of incubation. Asterisks indicate significant difference by Kruskal-Wallis test (**, p < 0.01; ***, p < 0.001). Abbreviation as in Table 1.

The Kruskal-Wallis test again indicated the significant effects between the Microcystis treatments and control (p < 0.01, Fig. 5). In addition, during three weeks of experiment, the total accumulative offspring in the control were highest, 637 offspring. However, that in the Microcystis treatments decreased considerably. In the exposures to 10%, 50% and 100% Microcystis treatments, the total offspring were 97, 83 and 4, respectively (Table 2).

Table 2. Accumulative neonates of D. magna after three weeks of incubation. Abbreviation as in Table 1

|          | Total offspring |
|----------|-----------------|
| Control  | 637             |
| 10% Ma   | 97              |
| 50% Ma   | 83              |
| 100% Ma  | 4               |

The record in our study revealed negative effects of M. aeruginosa on Daphnia reproduction which are in line with previous investigations (Lürling and Van der Grinten, 2003; Dao et al., 2010). This result showed the seriously effects of MCs on population development for the next generations. Therefore, population of D. magna may be strongly reduced in case of cyanobacterial bloom lasting for a long time, consequently aquatic ecosystem may be unbalance, which needs further in situ investigation.

3.5 Effects of M. aeruginosa on the intrinsic population rate of D. magna
As mentioned above, the Microcystis strongly affected on survivor, maturity and reproduction of the animals, consequently effects on the intrinsic population rate. Daphnia in all Microcystis treatments had significantly lower intrinsic rate of population than the control. The intrinsic population rate of Daphnia in the control was 0.295 whereas those in 10%, 50% and 100% Microcystis treatments were 0.127, 0.041, 0.003, respectively (Fig. 4). This result is in line with results of previous investigations reporting the negative effects of Microcystis on fitness of D. magna (de Bernardi and Giussani, 1990; Gustafson et al., 2005).

4. Conclusions

The M. aeruginosa strain from the Dau Tieng Reservoir produced a high MCs concentration. Our study proved the negative effects of living cells of toxic M. aeruginosa on life history traits of D. magna including survival reduction, maturation delay, reproduction inhibition and intrinsic population rate reduction. To our knowledge, this is the first report on the chronically detrimental impacts of M. aeruginosa strain isolated from Vietnam on D. magna. The MCs or some bioactive compounds in cyanobacteria posed a serious risk to D. magna in particular and to aquatic organisms in general. Therefore, more attention to the presence, distribution in nature and impacts of cyanobacterial blooms on aquatic organisms should be paid to protect the aquatic environment quality and ecosystem balance.

5. Acknowledgement

This research is funded by Hochiminh City University of Technology – VNU-HCM and CARE-RESCIF initiative under grant number Tc-MTTN-2016-04, and Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-NN.04-2014.69.

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