TOXIC EFFECTS OF *ANABAENA* SP. ISOLATED FROM TRI AN RESERVOIR ON *DAPHNIA*

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ABSTRACT

Harmful cyanobacterial blooms (HCB) have become a global threat to human health and aquatic biota around the world. While the ecotoxicity of microcystins (MC) producing cyanobacteria such as *Microcystis* spp. has been studied extensively, little is known about toxic effects of others filamentus cyanobacteria such as *Anabaena* spp. In this study, several strains of *Anabaena sp.* were isolated from Tri An Reservoir and cultured under laboratory conditions. Microscopic observation was used for morphological identification. The culture biomass were collected to prepare the crude extract and used for the acute (48 h) and sub-chronic (15 day) toxicity experiments on *Daphnia magna*. The acute assay showed that crude extract from all isolated strains of *Anabaena* sp. generated toxic effects on *D. magna*. 48-h EC_{50} values of crude extracts of *Anabaena* sp. on *D. magna* ranged from 340.4–538.6 mg dry weight (dw)/l. in the sub-chronic test, no significant difference was found between the control and the 1 mg dw/l treatment. However, the survival rates, growth and reproduction of parent *D. magna* were inhibited at 10, 50 and 150 mg dw/l treatments. This finding indicated that crude extracts from filamentus cyanobacteria such as *Anabaena* spp. isolated from the Tri An Reservoir generated significant acute and chronic toxic effects on *D. magna*.

***Keywords*:** Sub-chronic, acute, Anabaena, cyanobacteria, *Daphnia*.

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1. INTRODUCTION

Harmful cyanobacterial blooms (HCB) in eutrophic freshwater bodies have become an environmental concern worldwide [1]. *Anabaena* (*Dolichospermum*) is one of the most common planktonic freshwater cyanobacterium which frequently cause bloom-forming in lentic ecosystems. *Anabaena* is known to produce various toxin such as microcystins (MC), anatoxins and other bioactive peptides which may generate toxic effects on aquatic organisms as well as human population [2].

Microcrustaceans play important roles in aquatic ecosystems, serving as both feeders and consumers. As a filter-feeder, microcrustacean *Daphnia* is potential consumers of planktonic cyanobacteria. Several studies have examined the toxic effects of cyanobacterial bloom and MCs on *D. magna* in laboratory situations [3]. Acute exposure of *Daphnia* to cyanotoxins resulted in inhibition of filtration rate, decrease in swimming movements and even death [4]. Among chronic effects, previous reports decreased fecundity and population growth rate [3]. However, little is known about the adverse effects of filamentus cyanobacteria such as *Anabaena* spp. on microcrustaceans. In this study, we isolated several strains of the *Anabaena* spp. from the Tri An Reservoirs and maintained in the laboratory condition. The crude extracts from dry biomass was prepared and used to investigate the toxic effects on *Daphnia magna* under acute and sub-chronic toxicity tests.

2. MATERIALS AND METHODS

2.1. Sample collection and isolation

Bloom samples from the Tri An Reservoirs were collected in July of 2017. Single filamentus of *Anabaena* spp. was isolated and cultured in Z8 medium. All culture were grew at a temperature of 28°C under 12h:12h light:dark cycle at an intensity of 50 µmol photons/m^2/s. Biomass of *Anabaena* spp. was collected onto GF/C fiberglass filters at stationary phase. After drying completely at 45°C, samples were kept at –20°C prior to the experiment.

2.2. Crude extract preparation and analysis

The crude extracts of *Anabaena* spp. were prepared according to the method of Pham et al. (2016) [5]. Briefly, 1.0 g dry weight (dw) biomass of *Anabaena* spp. was dissolved into 100 mL MQ water and frozen at −70°C then thawed at room temperature. Then, the samples was sonicated for 3 minutes. This freeze–thaw–sonicate cycle was repeated five times. After centrifugation at 4000 rpm for 10 minutes, the supernatant was collected and kept at −20°C.

2.3. Acute and sub-chronic bioassays

D. magna neonates (<24 h-old) were exposed with crude extracts of *Anabaena* spp. at six different concentrations of 10, 50, 200, 600, 1000 and 1500 mg/l with 10 neonates per replicate. Test containers were conducted at 25±1°C and a 14:10 h photoperiod during 48h. The 48h immobility of cladocerans was used to determine the median lethal concentrations (EC_{50}) values with the 95% confidence interval by using the SPSS software. Sub-chronic tests were performed with crude extract of *Anabaena* spp. at 4 concentrations of supernatant (equal to 1, 10, 40 and 100 mg dw/l) and a control. Each treatment contained 15 replicates (n = 15). The mortality, maturation and production of live offspring were observed.

3. RESULTS AND DISCUSSION

3.1. Isolation and morphological characteristics

Microscopic observation of the cyanobacterial bloom samples revealed the dominance of *Microcystis* and *Anabaena*, mainly *Anabaena circinalis* (Fig. 1); *A. smithii*; *A. planctonica*, and the less frequent occurrence of other genera (*Arthrospira*, *Planktothrix*, *Pseudanabaena*, and *Cylindrospermopsis*).

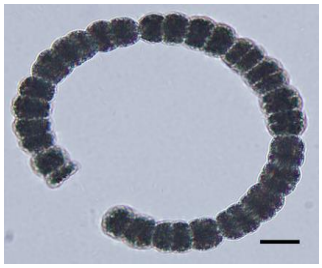

Fig. 1. Morphology of *Anabaena circinalis*. Scale bar: 10 µm.

3.2. Measurement microcystins concentration from cultures

Results of HPLC analysis indicated that the water bloom samples contained two variants of MCs including (MC-RR and MC-LR) with the highest concentration ranged from 718.3 ± 14.8 µg/g dw (Table 1). But none of the isolated strains of *Anabaena* sp. produced

microcystin. From the Tri An reservoir, Dao et al. (2010) [3] reported four variants of MC, including MC-LR, MC-RR, MC-LA, MC-LY and one unknown variant in the scum samples but none were found in the cultures.

3.3. Acute bioassays with *D. magna*

The calculated 48-h EC_{50} for the crude extracts of *Anabaena circinalis* and water bloom samples were shown in Table 1. Although MCs were not detected in crude extracts in *A. circinalis*, all samples caused acute toxicity on *D. magna*. The EC_{50} values of crude extracts of *A. circinalis* on *D. magna* after 48h ranged from 340.4–538.6 mg dw/l (Table 1).

Table 1. List of samples used for acute test with MCs concentration and 48-h EC_{50} values

Strain name	Samples name	MC (µg/g dw)	48-h EC_{50} (mg dw biomass/l)
AC1	*Anabaena circinalis*	ND	458.2
AC2		ND	385.2
AC3		ND	538.6
AC4		ND	340.4
Bloom-TA	Water bloom samples	718.3 ± 14.8	297.2

ND: no detectable microcystins

3.4. Sub-chronic toxicity and reproduction bioassay

Sub-chronic toxic effects of crude extracts of *A. circinalis* (strain AC4) on *D. magna* over a period of 15 days revealed that the crude extracts of filamentous A. *circinalis* generated a dose-dependent toxic effects on the survival of *D. magna* (Fig. 2).

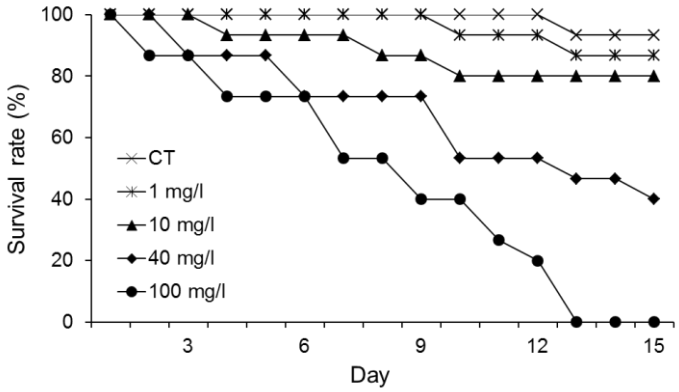

Fig. 2. Effects of crude extracts of *Anabaena circinalis* on survival of *Daphnia magna*.

During the sub-chronic test, the survival of daphnid in the control treatment was higher than 90%. Mortality rate of 13%, 20%, 60% and 100% of the exposure daphnids was recorded in the treatment with 1, 10, 40 and 100 mg/l, respectively. Results of the maturation age and average number of offspring per female of *D. magna* exposed to different concentration of crude extracts of *A. circinalis* indicated that crude extracts of *A. circinalis* at the concentration of 100 mg/l prolonged maturation ages. Treatment with 10, 40 or 100 mg dw/l declined the reproduction of parent daphnids (Fig. 3).

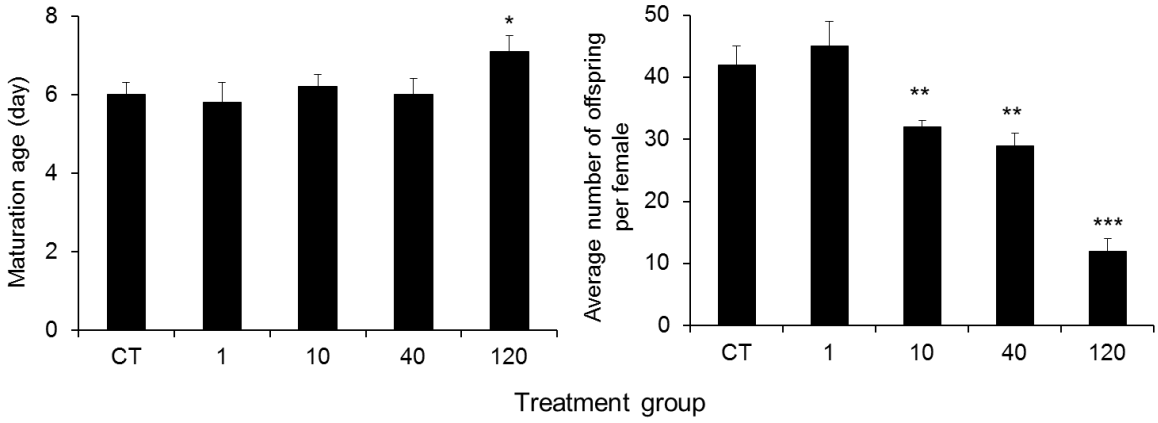

Fig. 3. Maturation age and number of offspring per female of *Daphnia magna*.

4. CONCLUSION

This study demonstrated that the crude extracts filamentous *A. circinalis* isolated from the Tri An Reservoir had significant acute and chronic toxic effects on *D. magna*. The present findings indicate that metabolites other than MC are likely to be responsible for the observed toxic effects. The toxicity mechanism of these unknown metabolites remain to be explored and need further investigation.

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