Short Communication (NS)

TOXIC INFLUENCE OF CYANOTOXINS IN EMBRYO-LARVAL DEVELOPMENT OF
Carassius carassius

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ABSTRACT

Cyanobacteria and their toxic products represent a serious problem in many waters. The aim of this study was to find out how crude extract of cyanobacteria can influence the embryo-larval development of carassius on the basis of embryo-larval toxicity test. Crude extract of cyanobacteria containing the known amount of microcystins LR, YR and RR (90, 9.0 and 0.9 μgL-1, i.e. high, medium and low concentration of the extract), was administered to carassius eggs. The experiments were finished after 8 days short-term exposure. Evaluation of the tests was based on the OECD guideline for testing chemicals, direction 210 from 1992. The extract with high concentration caused 93.5% embryonic mortality, prolonged hatching, increased numbers of malformed and dead larvae and a decrease in average total length. Yolk sac dropsy were observed. Tissue sections were stained with haematoxylin-eosin and examined using light microscopy. Changes of liver, in particular, were examined. No histopathological changes were found in control or experimental groups after the short-term exposure except for non-resorbed yolk sacks in the group exposed to the high concentration of the extract. The development had been retarded in this group.

Key Words: Cyanobacteria, Malformations, Fish, Toxicity test, Crude extract, OECD guidelines

INTRODUCTION

Cyanotoxins produced by cyanobacteria pose an environmental problem and influence the health status of both human and aquatic organisms. Many studies described health damage or even intoxication in animals and humans.13 There are a number of different hepatotoxins produced by species and strains within the genera Anabaena, Cylindrospermopsis, Microcystis, Nodularia, Oscillatoria, Nostoc, Aphanizomenon, Gloeotrichia and Coelosphaerium.1 There are 28 microcystins known presently. Microcystins are cyclic heptapeptides. They have hepatotoxic effect. Microcystins- LR is the most common and the most often studied hepatotoxin. The mechanism of its influence is the same in humans as well as in fish. The effect of microcystins and the crude extract of cyanobacteria on the development of fish were studied.45 Found higher MCs retarded egg development and larval growth, reduced hatching rate and caused high malformation rate on southern catfish with description of histopathology. Ultrastructural changes in hepatocytes of posthatching loach larvae after exposure to microcystin LR show focal necroses and dystrophic changes of hepatocytes with vacuolization and nuclei damage (pyknosis, karyolysis).4 MCLR caused a noticeable damage to liver ultrastructure, a widespread swelling in the rough endoplasmatic reticulum and mitochondria.6

AIMS AND OBJECTIVES

The research into this area has been aimed at investigation of effects of cyanotoxins on early life stages of organisms on the basis of embryo-larval toxicity test.

MATERIAL AND METHODS

The carassius eggs were obtained by artificial reproduction at the fishery in Laknas (Albania). Fertilised and unsticked carassius eggs were
The crude extract of cyanobacteria to keep the concentration of crude extract of cyanobacteria. The conditions in baths were as follows water temperature 21-22 °C, dissolved oxygen 75-100%, i.e. 5.5-10 mgL⁻¹ and pH was 8-9. The larvae were fed by commercial food Artemia Premium since the 5th day. Feeding was performed before 20-30 min of every water changing intervals. Tests were performed with the crude cell extract obtained from field samples of water bloom (Shkodra lake). Water samples contained the planktonic species _M. aeruginosa_ (90%), _Microcystis ichtyoblabe_ (4%) and _Aphanizomenon flos-aquae_ (2%). The samples were collected from surface water bloom (0.3 m depth) and concentrated by plankton net 25 μm. The samples were stored frozen at -20 °C. The concentration of microcystins was determined by HPLC according to the method described by. Total Microcystin Concentration (MC) was 1056.2 μgg⁻¹ dry weight in the biomass. To obtain the crude extract, the material was ultrasonicated for 7 min. and was centrifuged for 20 min at 4500 rpm. Re-extraction was done twice by standard water. The final concentration of hepatotoxic microcystins in the crude extract used for exposure was 17.3 μgL⁻¹ (4.8 μgL⁻¹ of microcystin YR, 9.59 μgL⁻¹ of microcystin LR, 2.9 μgL⁻¹ of microcystin RR).

**Experimental treatments**

The crude extract of cyanobacteria with known amount of microcystin LR (50, 5 and 0.5 μgL⁻¹) was added to the eggs in three concentrations: the first with 0.5 μgL⁻¹ of microcystin LR (low concentration of the extract) the second with 5 μgL⁻¹ of microcystin LR (medium concentration of the extract), the third with 50 μgL⁻¹ of microcystin LR (high concentration of the extract). The controlled eggs were incubated in toxic free water. The cumulative amount of microcystins was 90, 9 and 0.9 μgL⁻¹, respectively. Tests were finished after 8 days. Evaluation of the tests was based on the OECD. During the test we observed:

1. The time of start and the end of hatching
2. Numbers of larvae hatching each day
3. Numbers of malformed larvae
4. After finishing the tests, we evaluated cumulative mortality
5. Numbers of healthy fish at the end of the test
6. Average total length and body mass (the average total length was determined in 10 larvae and body mass in 20 larvae).

**Histology**

Five fish from each group were killed, immediately fixed in Bodian solution and processed using standard methods for histology. Tissue sections were stained with haematoxylin-eosin and cells were detected with H&E test. All sections were examined using light microscopy. Liver tissues were examined.

**RESULTS AND DISCUSSION**

Larvae hatched during three days. In the control group, the majority of larvae hatched during the first and second day. In the group with low concentration of the extract, it was similar, but the amount of larvae hatched in the first day was higher. In the group with medium concentration of the extract, the majority of larvae hatched in the second day. No larvae were hatched in the group with high concentration of the extract during the first day, most of them were hatched by the second day. Total numbers of hatched larvae were 189 in the control, 188 in the group with low concentration of the extract, 188 in the group with medium concentration of the extract and 25 in the group with high concentration of the extract. Number of malformed and dead larvae are presented in **Tables 1** and **Table 2**. Three malformed larvae were found in the control group (1.58% from 189 hatched larvae), three in the group with low concentration of the extract (1.59% from 188 hatched larvae), four in the group with medium concentration of the extract (2.12% from 188 hatched larvae) and five in the group with high concentration of the extract (20% from 25 hatched larvae) during the experiment.
Table 1: Egg hatching and malformations

<table>
<thead>
<tr>
<th>Concentration of the extract</th>
<th>Start of hatching</th>
<th>End of hatching</th>
<th>Numbers of hatched larvae for a day</th>
<th>Percentage of malformed larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd day</td>
<td>4th day</td>
<td>5th day</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>98</td>
<td>102</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Medium</td>
<td>58</td>
<td>102</td>
<td>42</td>
<td>5</td>
</tr>
<tr>
<td>Low</td>
<td>57</td>
<td>102</td>
<td>130</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>58</td>
<td>102</td>
<td>98</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2: Fry measurements and survival

<table>
<thead>
<tr>
<th>Concentration of the extract</th>
<th>Cumulative mortality (%)</th>
<th>Average total length (mm ± SD)</th>
<th>Average total body mass (mg ± SD)</th>
<th>Percentage of dead larvae (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>93.5</td>
<td>5.13±0.51</td>
<td>0.81</td>
<td>48</td>
</tr>
<tr>
<td>Medium</td>
<td>13</td>
<td>6.7±1.02</td>
<td>0.84</td>
<td>7.44</td>
</tr>
<tr>
<td>Low</td>
<td>11</td>
<td>6.82±0.51</td>
<td>0.85</td>
<td>5.31</td>
</tr>
<tr>
<td>Control</td>
<td>9.5</td>
<td>6.44±0.45</td>
<td>1.12</td>
<td>4.23</td>
</tr>
</tbody>
</table>

8 larvae died during the experiment in the control group (4.23% from 189 hatched larvae), 10 in the group with low concentration of the extract (5.31% from 188 hatched larvae), 14 in the group with medium concentration of the extract (7.44% from 188 hatched larvae) and 12 in the group with high concentration of the extract (48% from 25 hatched larvae).

Cumulative mortality is presented in Table 2. In the control 181 larvae survived. In the group with medium concentration of the extract and in the group with low concentration of the extract 174 and 178 larvae survived. In the group with high concentration the extract only 13 larvae survived. Average total length and body mass of surviving larvae (average total length and body mass of surviving larvae) are presented in Table 2.

Histology

No histopathological changes were found both in fish from control and experimental groups except for non-resorbed yolk sacks in the larvae from group exposed to the high concentration of the extract. It means that the development has been retarded in this group (Fig. 1).

The results indicate that embryonic development is greatly influenced by the highest concentration of crude extract of cyanobacteria (50 μg L⁻¹ of microcystin LR). Very high cumulative mortality (93.5%) at this concentration. Hatching of this
concentration (0.5 μgL⁻¹ of microcystin LR). We can say that the highest used concentration of crude extract of cyanobacteria (50 μgL⁻¹ of microcystin LR) influenced the development of the *Carassius* larvae through acute toxicity. Used pure microcystin LR in doses of 0.5, 5 and 50 μgL⁻¹ and no acute toxicity was found. Higher mortality, retarded larval growth and decreased survival (at 5 and 50 μgL⁻¹ microcystin LR only) were observed at the end of larval period (after termination of the exposure and transfer of larvae into microcystin LR free water). High mortality and malformations were observed after exposure to various cyanobacterial crude extracts. Described more pronounced effects of cyanobacterial biomass than of pure toxins after embryonic exposure. Malformations combined with high mortalities and adverse effects on outer egg structures were observed concomitantly in all species after exposure to various aqueous crude extracts of cyanobacteria. Total concentrations of microcysts they used (30, 40 and 45 μgL⁻¹), however, were lower than the ones we used in High mortality and retarded development in tests with short-term exposure may be due to increased energy demand of detoxication processes, as described by. We detected no damage of liver in fish from tests with short term exposure.

**CONCLUSION**

The high concentrations of the extract corresponds with the level of dissolved microcystins in lake water. Then it could negatively influence the embryonic development of various fish species in natural water.

**REFERENCES**