

EVALUATION OF TOXICOLOGICAL RISK OF CENTRAL EUROPEAN *RAPHIDIOPSIS RACIBORSKII* STRAINS USING COMMON CARP CELLS

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The adverse effects of biological active compounds produced by cyanobacteria in aquatic biota have been a subject to numerous studies. Particular attention has been paid to microcystins, anatoxin-a, saxitoxin analogues, and cylindrospermopsin (CYN). The latter compound and its two natural analogues, 7-epi-CYN and 7-deoxy-CYN are known to be produced by filamentous species belonging to *Nostocales* and *Oscillatoriales* orders. Up to date no European strain of *R. (Cylindrospermopsis) raciborskii* has been reported to produce any known cyanotoxin despite a substantial research in this regard on both analytical and molecular level. However, exudates of some of *R. raciborskii* strains isolated in Europe have been reported to reveal an *in vitro* cytotoxicity in human lymphocytes and neutrophils (Polish strains) [1].

The present study investigated whether Central European *R. raciborskii* strains can affect fish cells *in vitro*. Four strains of *R. raciborskii* isolated from freshwater lakes located in Western Poland (PL1-PL4) and three strains isolated from artificial water reservoirs in Western Ukraine (UA1-UA3) were selected for the study. The Ukrainian strains were previously shown not to produce known cyanotoxins and to reveal no *in vitro* toxicity in human platelets [2]. Polish strains studied in this study did not produce cylindrospermopsin, microcystins and anatoxin-a. All isolates were morphologically similar. The potential toxicity of cyanobacterial extracts was evaluated in common carp (*Cyprinus carpio*) hepatocytes, red blood cells (RBC), and brain homogenate. In all tests two final concentrations, 0.1% (1 $\mu\text{L mL}^{-1}$; corresponding to 6 individual per mL) and 1.0% (10 $\mu\text{L mL}^{-1}$; corresponding to 62 individual per mL) of each *R. raciborskii* extract were used. Carp hepatocytes were exposed for 2h to both concentrations of each extract. Fish RBC were exposed for 1 h to the same cyanobacterial extract concentrations to study whether a lysosomal membrane stability, a common marker of cellular well-being, is affected.

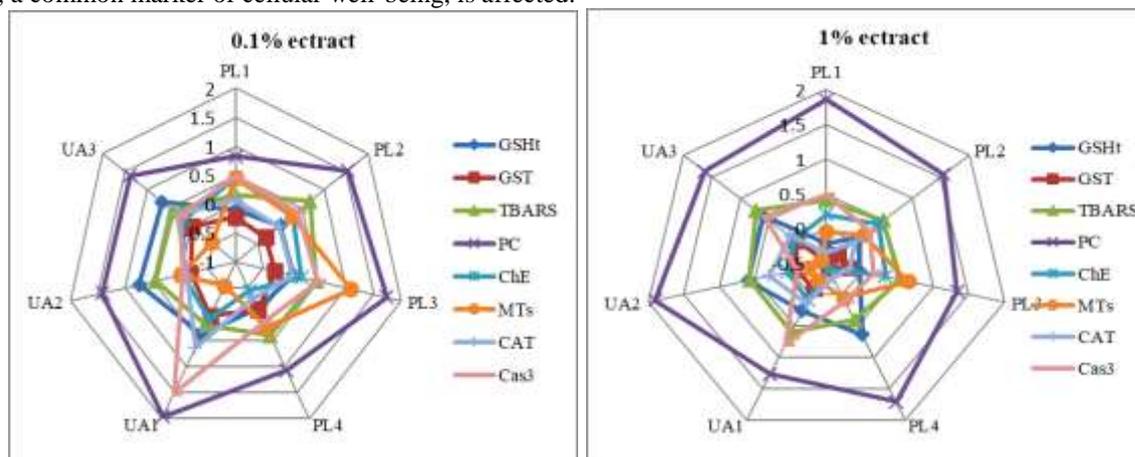


Fig. 1. Integral response index of studied biological traits of *Cyprinus carpio* cells exposed to *R. raciborskii* strains.

Studied extracts evoked different responses of catalase activity in hepatocytes with both increase and decrease observed under low and high concentrations. Cellular thiol pool was also altered with most of extracts inducing decrease in activity of glutathione-S-transferase, and Ukrainian strains leading to increase in glutathione level and decrease in metallothionein content. All studied extracts induced comparable reactive oxygen species formation, lipid peroxidation, protein carbonylation and DNA fragmentation in hepatocytes, and all but one increased activity of caspase-3. Only one extract caused lysosomal membrane destabilization as measured by neutral red retention in RBC. In contrast to extracts of Ukrainian isolates, exposure of brain homogenates to extracts of Polish strains induced increase in acetylcholinesterase activity suggesting neurotoxic action of their exudates. The results indicate that both Polish and Ukrainian strains of *R. raciborskii* may pose a toxicological risk to freshwater fish, and further that Polish strains may produce compound(s) evoking neurotoxic effects.

This work was supported by the Ministry of Education and Science of Ukraine (research grant for young fellows MV-1)

[1] B. Poniedziałek, P. Rzymiski, J. Karczewski, The role of the enzymatic antioxidant system in cylindrospermopsin-induced toxicity in human lymphocytes, *Toxicol In Vitro* **29**, 926–932 (2015).

[2] P. Rzymiski, O. Horyn, A. Budzyńska, et al., A report of *Cylindrospermopsis raciborskii* and other cyanobacteria in the water reservoirs of power plants in Ukraine, *Environ Sci Pollut Res*, **25**, 15245-15252 (2018).