ACUTE TOXICITY ASSESSMENT OF DEFENSE SECRETIONS OF *MEGAPHYLLUM BOSNIENSE* (VERHOEFF, 1897) AND *M. UNILINEATUM* (C. L. KOCH, 1838) (DIPLOPODA, JULIDA) ON *ARTEMIA SALINA*

<u>Jelena Milovanović^{1*}</u>, Jelena Đorđević^{2,3}, Aleksandra Stevanović³, Anastasija Malešević³, Bojan Ilić¹, Branka Vuković-Gačić³, Slobodan Makarov¹

¹University of Belgrade, Faculty of Biology, Institute of Zoology, Chair of Animal Development, Belgrade, Serbia

²University of Belgrade, Institute for Multidisciplinary Research, Belgrade, Serbia

³University of Belgrade, Institute of Botany and Botanical Garden "Jevremovac", Chair of Microbiology, Centre for Genotoxicology and Ecogenotoxicology,

Faculty of Biology, Belgrade, Serbia

*Corresponding author: jelena.milovanovic@bio.bg.ac.rs



- @ Different chemical compounds can be found in defense secretions of species of the order Julida, with the highest representation of quinone and/or esters.
- Numerous biological activities of these secretions are reported: antimicrobial, antioxidative, cytotoxic, antineurodegenerative.



Results and Discussion

- © LC50 values for extracts of defensive secretions of MBO and MUN had similar values after 24h, while LC50 of MBO was twice weaker after 48h in comparison with MUN (**Table 1**).
- Number of surviving individuals decreased with increasing concentration of tested extracts and increasing incubation time (**Figure 1**).

Materials and Methods

- @ Adult individuals of MBO and MUN were collected during April and May of 2021 on Mt. Avala and Krnjača (respectively), Belgrade, Serbia.
- The stock concentration of extracts used in ARC test was 20 mg/mL from which dilutions were made (range of concentrations 0,1 mg/mL 0,003125 mg/mL).

Objectives

To examine the toxic effects of defensive secretions of two species from the order Julida [Megaphyllum bosniense (MBO) and M. unilineatum (MUN)] using Artemia salina (ARC test).

- @ In a plate with 24 wells, 900 μl of seawater with *A. salina* larvae (10-15 per well) was placed and then 100 μL of tested extracts was added.
- Potassium dichromate (K2Cr2O7) was used as a positive control and DMSO as a solvent control.
- The experiment was done in triplicate.



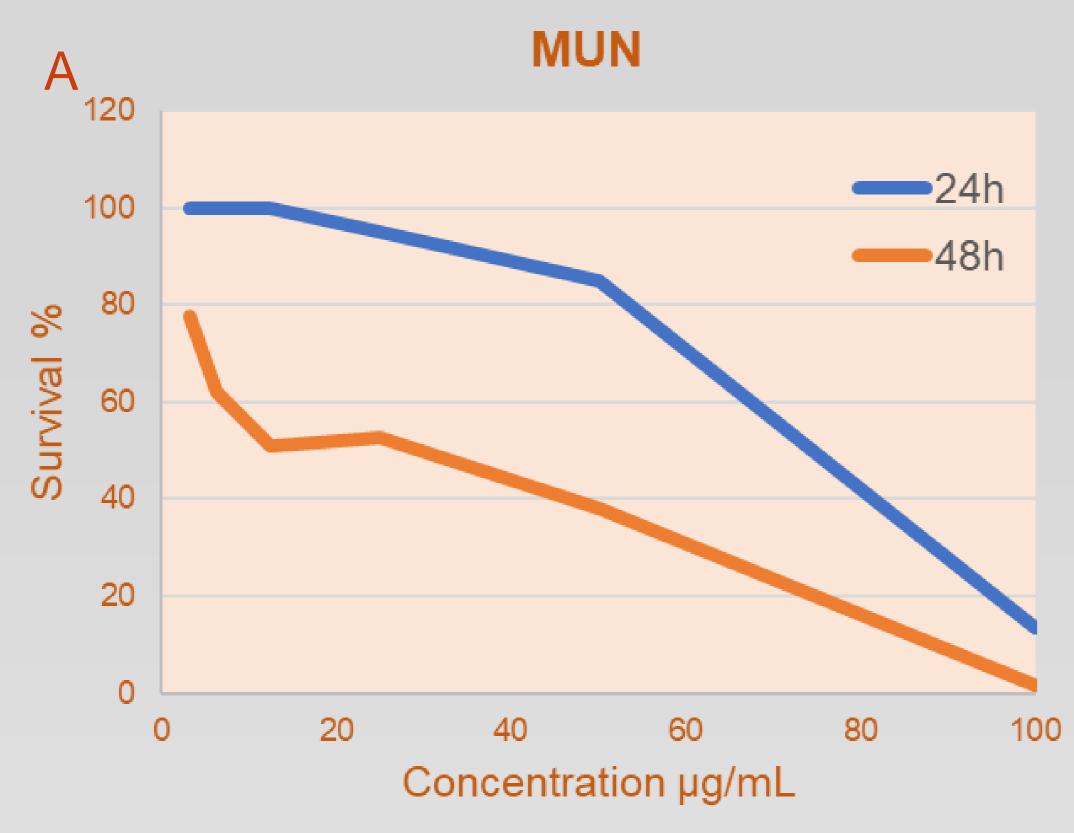
Table 1. LC50 values of defensive secretions of *M. unilineaum* and *M. bosniense* after 24h and 48h.

LC50	24h	48h
MUN	68,56 µg/mL	29,12 µg/mL
MBO	73,23 µg/mL	47,18µg/mL
K2Cr2O7	24,4 µg/mL	13,5 µg/mL



Megaphyllum bosniense

- Both tested millipedes' defensive secretions extracts species showed toxic effect in ARC test.
- @ MBO extract has weaker toxic effect than the MUN extract.
- @ MUN is almost exclusively benzoquinone-based while esters are dominant in MBO extract.
 - Many esters detected in MBO are new natural products and further studies are needed to determine their toxicological potential.



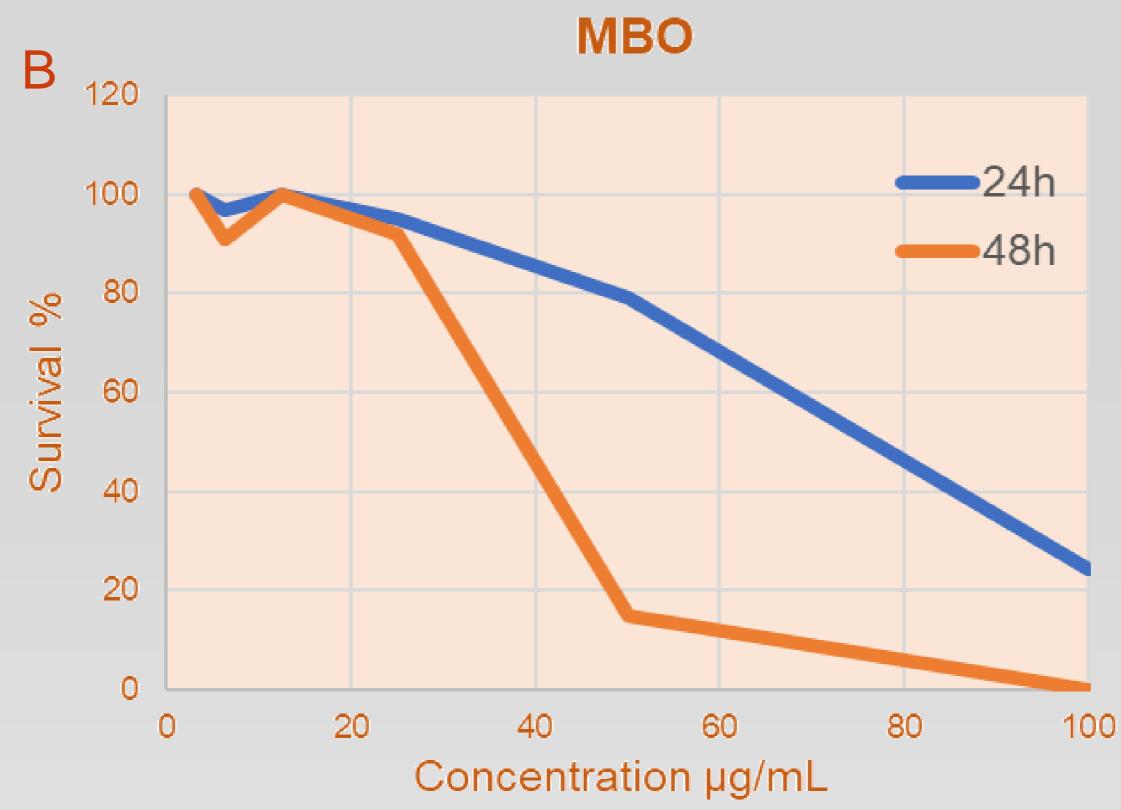




Figure 1. Survival of *A. salina* individuals after treatment with extracts of defensive secretions of A) *M. unilineaum* (MUN), B) *M. bosniense* (MBO) and C) solvent (DMSO).