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Toxic pesticides effects on the respiration rate in *Dreissena polymorpha* (Pallas, 1771)

VESELA YANCHEVA[1], IVELIN MOLLOV[1*], STELA STOYANOVA[2], BORISLAVA TODOROVA[1], ILIANA VELCHEVA[1], ELENKA GEORGIEVA[2]

[1]University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Ecology and Environmental Conservation, 24, Tzar Assen Str., BG-4000 Plovdiv, Bulgaria, [*]E-mail: mollov_i@uni-plovdiv.bg
[2]University of Plovdiv "Paisii Hilendarski", Faculty of Biology, Department of Developmental Biology, 24, Tzar Assen Str., BG-4000 Plovdiv, Bulgaria*

Abstract. With the present experiment we aimed to study the possible negative effects of two commonly used insecticides – cypermethrin (CYP) and chlorpyrifos (CPF) on the respiration rate of Zebra Mussel (*Dreissena polymorpha* Pallas, 1771) after 96 hours and 30 days. We found that both chemicals altered the respiration process; however CYP was determined to be more toxic regarding this particular biological measurement.

Key words: Zebra Mussel, pesticides, water contamination.

Introduction

The increasing demand on agricultural productivity, associated with growing world population, global climate change, trends towards monoculture and resistance of pests towards many plant protection chemicals, has contributed to the widespread use and invention of new effective pesticides (Santana *et al.*, 2021). Thus, the natural ecosystems have been increasingly polluted and the water bodies particularly turn into the ultimate precipitator of all different types of anthropogenic toxicants, including persistent organic pollutants, such as pesticides (Thoré *et al.*, 2021).

As one of the first animals used to assess the environmental quality of seawater (Goldberg, 1975), mussels have been utilized extensively as ideal biological indicators in monitoring of anthropogenic pollution (Beyer *et al.*, 2017; Li *et al.*, 2019). In addition, to assess the environmental effects of waterborne contaminants, a battery of standard biological tools is applied. Therefore, biomarkers are described as early warning signals at all biological levels used for the purposes of ecological monitoring and the related risk assessment. In this sense, the measurement of respiration rate, which is relatively inexpensive, but sensitive biomarker could be also commonly applied in terms of the multi-biomarker approach (Sárkány-Kiss *et al.*, 2012).

In the present study we aimed to study the possible negative effects of two generally used insecticides on the Zebra Mussel (*Dreissena polymorpha* Pallas, 1771). For this purpose, we applied different decreasing (50% and 30%) concentrations of cypermethrin (CYP) and chlorpyrifos (CPF), based on the maximum allowable concentrations set in the

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Directive 2013/39/EU (EC, 2013) (MAC-EQS = 100%) and followed the respiration rate changes in laboratory conditions after a short (96 hours) and long-term (30 days) exposure.

Material and Methods

The tested species, according to Karatayev *et al.* (2006) is a native of the Black and Caspian Seas region in south-eastern Europe, is one of the most aggressive and successful aquatic invaders of freshwaters in the northern hemisphere. However, according to Binelli *et al.* (2015) the Zebra Mussel, *Dreissena polymorpha* represents the freshwater counterpart of the *Mytilis* species in toxicological studies because of its specific characteristics, such as wide distribution, continuous availability throughout the year, adequate body size, easily sampled, high longevity (3-5 years), great resistance in laboratory conditions, etc. The individuals for this study were collected from a presumed for relatively clean reservoir in Bulgaria, which is used mainly for recreation fishing (Jrebchevo Reservoir, Bulgaria). The tested pesticides - pyrethroid and organophosphorous insecticides are among the most frequently used classes of pesticides, overlapping in some crops for better results. Due to their effects, both insecticides are considered highly toxic to fish and aquatic invertebrates (Bachetta *et al.*, 2014). The tested concentrations of both, CYP and CPF were prepared by experts from the regional accredited laboratory of the Ministry of Environment and Waters in Plovdiv, Bulgaria based on the EU legislation. The biomarker – respiration rate was calculated by determining the difference in the dissolved oxygen levels before and after the tested time, following Tsekov (1989): I = Q2/G, where: I – respiration rate index; G – weight of the mussels, in grams, Q2 – oxygen consumed by the mussels between the two measurements (the difference between the oxygen levels before and after the 1-hour $Q2=Q_{Start}-Q_{1hour}$). Q was calculated by the following formula: Q = V x q, where: Q – total oxygen level in the water tank; V – volume of the water in the tank, in litres; q – level of dissolved oxygen in 1 litre of water (mg/L). The entire experimental set up was followed after Yancheva *et al.* (2016).

The respiration rate data was normalized using arcsine transformation, due to the lack of normal distribution and Pearson correlation analysis was performed, seeking linear correlation between the insecticide concentrations and the respiration rate index. In addition, the determination index was calculated (Fowler *et al.*, 1998). All statistical processing of the data was done using the computer software "PAST" v. 3 (Hammer et al., 2001).

Results

The results on respiration rate of Zebra Mussel, *Dresissena polymorpha* treated with CYP and CPF for 96 hours and 30 days are presented in Table 1. Overall, what we found was that both tested chemicals affected this biological measurement. However, significant changes were observed on the 96th hour compared to the control. The higher two CYP concentrations, as well as the highest CPF concentration enhanced the respiration rate after the short-term exposure. On the other hand, after 30 days the respiration rate indices were similar to the control group.

We detected a strong positive correlation between the insecticide concentrations and the respiration rate index after the 96th hour (CYP - R=0.90, p=0.09; CPF - R=0.97, p=0.03). Although for CYP it was not statistically significant, the index of determination was very high (R^2=81.00%). Furthermore, after 30 days we recorded a low to moderate positive correlation between the applied concentrations and the respiration rate index, however both were not statistically significant (CYP – R=0.52, p=0.48; CPF – R=0.11, p=0.88) and with low values of the determination index (CYP – R^2=27.04%; CPF – R^2=1.21%).

Table 1. Respiration rate in Zebra Mussel, *Dresissena polymorpha* exposed to CYP and CPF for 96 hours and 30 days.

Test	Water volume, l (V)	Weight, g (G)	Oxygen level (mg/l)					Respiration rate (I)
			Start		End		Total	
			q_{Start}	Q_{Start}	q_{hour}	Q_{1h}	(Q2)	
96 hours								
Control	1.2	28.76	9.0	10.80	8.9	10.68	0.12	0.004
CYP 0.0002 ppm	1.2	38.41	9.1	10.92	8.9	10.68	0.24	0.006
CYP 0.0003 ppm	1.2	37.20	9.2	11.04	8.7	10.44	0.60	0.016
CYP 0.0006 ppm	1.2	31.26	9.1	10.92	8.6	10.32	0.60	0.019
CPF 0.03 ppm	1.2	40.83	8.8	10.56	8.6	10.32	0.24	0.006
CPF 0.05 ppm	1.2	40.21	8.8	10.56	8.5	10.20	0.36	0.009
CPF 0.1 ppm	1.2	42.60	8.8	10.56	8.4	10.08	0.48	0.011
30 days								
Control	1.2	9.90	6.9	8.28	6.8	8.16	0.12	0.012
CYP 0.0002 ppm	1.2	13.38	7.0	8.40	6.9	8.28	0.12	0.009
CYP 0.0003 ppm	1.2	17.42	6.9	8.28	6.7	8.04	0.24	0.014
CYP 0.0006 ppm	1.2	15.14	6.9	8.28	6.7	8.04	0.24	0.016
CPF 0.03 ppm	1.2	15.74	7.1	8.52	6.9	8.28	0.24	0.015
CPF 0.05 ppm	1.2	11.37	7.0	8.4	6.9	8.28	0.12	0.011
CPF 0.1 ppm	1.2	18.94	7.0	8.4	6.8	8.16	0.24	0.013

* Explanations of the abbreviations are shown in the text.

Discussion

Based on our results we can conclude that the respiration rate of Zebra Mussel, *Dreissena polymorpha* was altered due to the toxicity of CYP and CPF. Furthermore, we consider that on the 96th hour the changes in the respiration rate were linked to initial stress that the mussels experienced. What is more, after 30 days we found that the calculated indices were similar to the control group, which was probably due to adaptation mechanisms, which the mussels have developed in order to survive in chronic contaminated water. This was also confirmed by the correlation analysis data. We support the statement of Basha *et al.* (1988) and Kumar *et al.* (2012) that in most cases the respiration rate increases with the increase of the toxicant concentration and level of toxicity. We could explain the reason for this with the attempt of the organism to deliver more oxygen to all tissues and organs provoked by the stress, which is caused by the potential, exposure or concentration of the toxicant. Moreover, our results are consistent with our previous studies (Yancheva *et al.*, 2016a, b), which indicated that the respiration rate in Zebra Mussel, *Dreissena polymorpha* was increased in a dose-dependent manner.

We suggest careful application of such pesticides near water or when a drift might be a possibility in terms of the health of hydrobionts.

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