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Assessment the Toxic Effects of Dimethoate to Rotifer Using Swimming Behavior

Ruixin Guo · Xinkun Ren · Hongqiang Ren

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Abstract The toxic effects of the common organophosphorus pesticide dimethoate on freshwater zooplankton *Brachionus calyciflorus* (rotifer) were tested. Because of the advantages of behavioral response in environmental monitoring, swimming behavior was used as the endpoint in this research. After exposure 6 h at five dimethoate concentrations (0.18, 0.53, 0.88, 1.23 and 1.59 $mg{\cdot}L^{-1}$), the pesticide disrupted the balance in rotifer swimming direction and caused an obvious direction preference. It also inhibited significantly the swimming angular and linear speed. Our results showed that dimethoate has a sublethal toxic effect on this aquatic invertebrate.

Keywords Dimethoate · Rotifer · Aquatic toxicity · Swimming behavior

Dimethoate is a broad use organophosphorus pesticide, which has been in use since 1956 against insects and mites on agricultural crops and ornamental plants (Dogan and Can 2011). It mostly entered aquatic environments after spraying or rainfall, possibly affecting non-target organisms, disrupting the food chain, modifying the food web, and causing an imbalance in the entire ecosystem. Most investigations of dimethoate aquatic toxic effects have focused on fish (Dogan and Can 2011), snails (Tripathi and Singh 2003), and crabs (Lundebye et al. 1997). However, studies on zooplankton are limited (Andersen et al. 2006). Rotifers are an important group of zooplankton in freshwaters which play an important role in the transfer of energy from primary producers to secondary and tertiary consumers in aquatic food webs. Although rotifers have a noteworthy position in aquatic ecosystem, the toxic effect of the commonly used pesticides dimethoate on rotifers is not known. Thus, it is critical and necessary to examine the toxic impact of dimethoate on rotifers.

As the index of the toxic stress, the aquatic animal behavioral responses were used to assess the impact of environment contaminants (Janssen et al. 1994; Charoy et al. 1995; Charoy and Janssen 1999; Cailleaud et al. 2011). The present study aimed to examine the toxic stress of different concentrations of dimethoate on rotifer *Brachionus calyciflorus*, using the swimming alteration as the behavioral response. Our results will indicate that the pesticide dimethoate has toxic effects in environmental safety and health.

Materials and Methods

The test rotifer *Brachionus calyciflorus* has been extensively used in ecotoxicological tests reviewed by Snell and Janssen (1995). It was originally isolated from the Sheshan Reservoir in the southern suburb of Nanjing, China (31°58′28″N and 118°55′06″E). Cultures were established as clones from individual female and maintained for more than 3 months before the experimentation. The test animals were cultured in artificial freshwater medium (EPA medium contains $NaHCO_3$, 1.14×10^{-3} $mol{\cdot}L^{-1}$, $CaSO_4$, 0.44×10^{-3} $mol{\cdot}L^{-1}$, $MgSO_4$, 0.5×10^{-3} $mol{\cdot}L^{-1}$ and KCl, 0.05×10^{-3} $mol{\cdot}L^{-1}$, the pH was adjusted to 7.5) at 25 ± 1 °C on the photoperiod 12:12 (L:D) with 4,000 lx light and was provided with the single-cell green alga *Chlorella pyrenoidosa* as the diet.

R. Guo · X. Ren · H. Ren (✉)
State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210046, People's Republic of China
e-mail: hqren@nju.edu.cn

Table 1 Actual dimethoate concentrations (mean with ± standard error) measured at t = 0 and 6 h and their corresponding nominal concentrations

Nominal concentration	Actual concentration	
	0 h	6 h
0.2	0.18 ± 0.005	0.16 ± 0.005
0.6	0.53 ± 0.02	0.48 ± 0.02
1.0	0.88 ± 0.03	0.80 ± 0.02
1.4	1.23 ± 0.04	1.11 ± 0.03
1.8	1.59 ± 0.04	1.43 ± 0.04

The organophosphorus pesticides dimethoate (*O*, O-dimethyl S-methylcarbamoylmethyl phosphorodithioate, CAS Registry NO.: 60-51-5) was obtained from Jiangsu Pesticides Research Institute. Toxicant test solutions were prepared by diluting specific volumes of a dimethoate stock solution with EPA medium and adjusting the pH to 7.5. The actual concentrations were analyzed using high-performance liquid chromatography (HPLC) technique. Dimethoate analytical standard sample was obtained from Sigma-Aldrich, Germany. The results of chemical analysis are shown in Table 1. All exposure concentrations in the following graphs and texts were given as the measured values.

Newly hatched rotifers from amictic eggs (age about 2 h) were introduced into 40 mL fresh media for 10 h pre-culture before the experiments. We provided *C. pyrenoidosa* as the exclusive diet during the pre-experiment time until 2 h before the experiments. The experimental set up used to measure the rotifer swimming behavior consisted of a video camera-MBR system (Swimming behavior recorder system, developed by Nikon, Japan) with 24-well cell culture cluster (Corning Inc. USA) that had a transparent side. After being exposed for 6 h, the rotifer swimming behavior was digitally recorded for 4 min per individual in every group (10 individuals per group). Films were transferred to computer and trajectories were manually extracted using the image processing software NIS-Elements V 3.1 (Nikon, Japan). One test included of a control group and five test concentrations (0.18, 0.53, 0.88, 1.23 and 1.59 $mg{\cdot}L^{-1}$), each with six replicates. The experiments were conducted under same environmental conditions (light and temperature) as that in the pre-culture time (fixed by preliminary tests in order to reach steady swimming speed and uniform spatial distribution) to avoid light and temperature effects, and in the absence of food. The indexes characterizing the swimming behavior were then computed: the percentage of angles turn left (%), the percentage of angles turn right (%), the swimming angular speed ($°s^{-1}$) and the swimming linear speed ($mm{\cdot}s^{-1}$), as described by Mimouni et al. (1993) and Cailleaud et al. (2011).

All the statistical analyses were carried out with the SPSS analytic package16.0. The data were first tested for homoscedasticity (Levene's test for ANOVA) and normality (Kolmogorov-Smirnoff test). Analysis of variance (ANOVA) was used to quantify the difference in the indexes of the rotifer swimming behavior in relation to different dimethoate concentrations. The data that were non-normal or heteroscedastic were analyzed using the Kruskal–Wallis tests. All of the figures were produced using SigmaPlot version 11.0.

Results and Discussion

The indexes of the swimming behavior of the test rotifer *Brachionus calyciflorus* exposed to five concentrations of dimethoate after 6 h are presented in Fig. 1. In general, rotifers exhibited significant modifications in the swimming behavior under dimethoate stress. Firstly, the percentage of angles turn left (PAL) varied at any given toxicant concentration (Fig. 1a). The PAL were higher than 50 % as the concentrations of dimethoate increased from 0.18 to 1.23 $mg{\cdot}L^{-1}$ (Fig. 1a). In addition, rotifers obtained the maximum in PAL (80.00 % in average) at 0.53 $mg{\cdot}L^{-1}$. When rotifers exposed to dimethoate at 0.18 and 0.35 $mg{\cdot}L^{-1}$, PAL was 31.77 % and 62.17 % higher than that of the control, respectively, whereas at 1.59 $mg{\cdot}L^{-1}$, it decreased 12.16 % as the control. The percentages of angles turn right (PAR) of rotifers when exposed to dimethoate are shown in Fig. 1b. The control rotifers that were unexposed obtained 51 % in PAR, whereas the mean value decreased 30.93 % and 60.53 % at 0.18 and 0.53 $mg{\cdot}L^{-1}$, respectively. However, a significant increase of 11.84 % was observed at 1.59 $mg{\cdot}L^{-1}$. Statistically, dimethoate had a significant influence on the rotifer swimming behavior in PAL and PAR ($p < 0.01$, F test, respectively).

Secondly, the rotifer swimming angular speed and linear speed were also adversely affected as a function of the toxicant concentrations. After a 6 h exposure, the mean swimming angular speeds at five concentrations were 41.52, 44.73, 41.65, 39.52 and 39.90°.s^{-1}, only 68.87 %, 74.19 %, 69.09 %, 65.56 % and 66.17 % of that of the control, respectively (Fig. 1c). The statistical analyses demonstrated that the rotifer swimming angular speed was significantly influenced by dimethoate ($p < 0.01$, F test). Additionally, the rotifer swimming linear speed decreased from 0.15 ± 0.008 $mm{\cdot}s^{-1}$ to 0.13 ± 0.004 $mm{\cdot}s^{-1}$ when the toxicant concentrations increased from 0.18 to 1.59 $mg{\cdot}L^{-1}$, which were 53.13 %, 48.67 %, 43.41 %, 29.37 % and 45.18 % of that of the control, respectively.

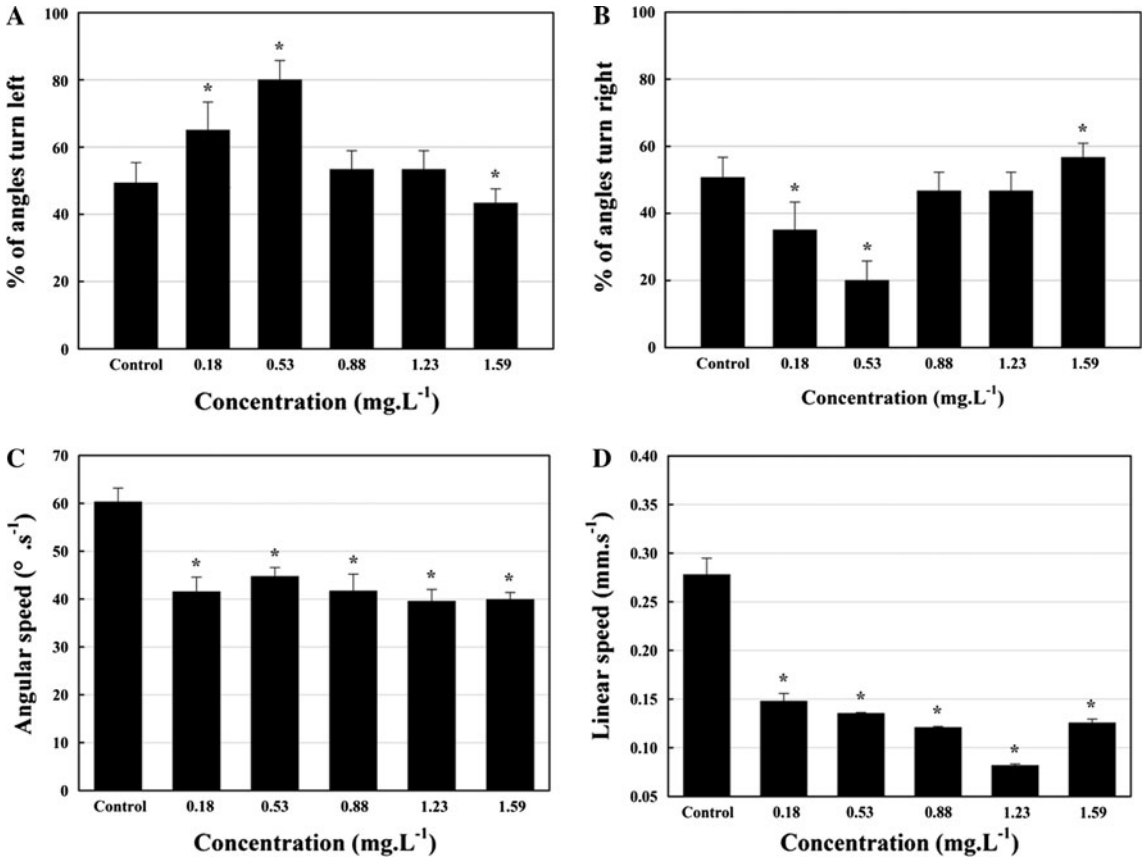

Fig. 1 The indexes of the swimming behavior of *Brachionus calyciflorus* exposed to dimethoate after 6 h, as to the control (Mean ± SE, n = 6). **a** the percentage (%) of angles turn left, **b** the percentage (%) of angles turn right, **c** the angular speed (°.s^{-1}), **d** the linear speed (mm.s^{-1}). *, a significant differences from control ($p < 0.05$)

Statistically, dimethoate had a significant influence on the rotifer swimming linear speed ($p < 0.05$, Kruskal–Wallis test). The relative lowest value under the toxic stress was observed at 1.23 mg·L^{-1}.

Previous research showed that the exposure of *Daphnia magna* to 30 mg·L^{-1} of dimethoate resulted in a significant reduction in the number of offspring and in the average weight of offspring (Andersen et al. 2006). The total protease activities of freshwater snail *Lymnaea acuminate* in hepatopancreas and ovotestis tissue increased when exposed to 6.0 mg·L^{-1} of dimethoate after 24 h (Tripathi and Singh 2003). Our results illustrated that different dimethoate concentrations affected the rotifer swimming behavior differently. For example, the significant increase in PAL and PAR at different concentrations implied that a significant left-turning preference was observed at 0.18 and 0.53 mg·L^{-1} and a significant right-turning preference was observed at 1.59 mg·L^{-1} (Fig. 1a, b), whereas the rotifer swimming angular speed and the rotifer swimming linear speed were inhibited under any given concentration (Fig. 1c, d). However, the 6 h-EC_{50} value for the swimming linear speed was 0.30 mg·L^{-1}, only 5.36 % of that of the swimming angular speed. This result indicated that the swimming linear speed was more sensitive than the swimming angular speed under dimethoate stress. Less concentration pesticide will reduce the rotifer swimming linear speed by 50 %. It suggested that how the toxic effects on the swimming behavior of rotifer would be high concentration dependent. The swimming behavior of rotifer is performed by the coordinated beat of the cingulum cilia and is controlled by two innerved muscles inserted on the infraciliature. Disruption in swimming can thus result from the contraction of the innerved muscles, which may also be the result of damage to the nervous system (Cailleaud et al. 2011). The action of the organophosphorus pesticides on aquatic species is probably caused by inhibition of acetylcholinesterase (AChE) activity (Roast et al. 2000). 2 mg·L^{-1} dimethoate could significantly reduce the crab *Carcinus maenas* AChE activity (Lundebye et al. 1997). Inhibition of AChE following exposure to pollutants has been observed in many aquatic species and has been shown to cause disruption in behavior (Xuereb et al. 2009). In our study, similar disruption of the toxicity was observed clearly. For one thing, the toxic compound caused an

imbalance in direction and influenced the direction change significantly. For another, it had an adverse impact on the swimming activity. Our results suggested that the result of a possible AChE inhibition might have caused an increase in acetylcholine in the synapses of cholinergic neurons in the central and peripheral system, resulting in abnormal neurotransmission duration and intensity (Pope et al. 2005) and in a possible behavior disruption (Cailleaud et al. 2011).

Behavioral response could be widely used as the biomarkers to evaluate the effect of contaminants because: (1) it is more sensitive than lethal effects under the toxic stress. As little as 1 %–5 % of LC_{50} could induce significant changes in the rotifer swimming behavior (Faimali et al. 2006), (2) it is usually rapid, occurring in hours rather than days as for traditional test (Snell and Janssen 1995). In the present study, the 6 h-EC_{50} values for the rotifer swimming angular speed and linear speed were 5.60 and 0.30 mg·L^{-1}, only 3.10 % and 0.17 % of the 24 h-LC_{50} value (180.51 mg·L^{-1}), respectively. Swimming change of zooplankton is highly related to the individual growth, food competition between interspecies and fish predation (Weis et al. 2001). As the biochemical and physiological functions of an organism, it may be taken as the first indication of the effects of an environmental perturbation (Villarroel et al. 1999). Therefore, the behavioral response provides another type of biomarker in the aquatic toxicity assessment. Additionally, it may help to explain other observed changes in the growth and reproduction in a long-term exposure.

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