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The Role of Microflora and Plant Supplements in *Daphnia magna* Resistance to a Toxicant

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Abstract

Current risk assessment of various pollutants in aquatic environments is largely based on results from single-species laboratory studies. At the same time, there are very complex interactions between microflora, algae and zooplankton in natural waters, which are able to alter the direct effects of some toxicants. This study was done to investigate the effect of toxicants on complex aquasystems. To explore it, we treated laboratory invertebrates Daphnia magna with deltamethrin with and without addition of plant excreta.In this study we found that the excreta of *Typha angustifolia* and *Chlorella vulgaris* may play a significant role in the regulation of the invertebrate populations to the action of pollutants and we observed a 2-fold to 4-fold increase in survivability and reproduction due to its addition. The role of microflora in *Daphnia magna*, adaptation to a toxicant was found less significant.

Keywords: Chlorella vulgaris, Daphnia magna, *deltamethrin, water contamination.*

INTRODUCTION

The present-day risk assessments of various pollutants in aquatic environments is largely based on results from single-species laboratory studies. However, there are very complex interactions between microflora, algae and zooplankton in natural waters, which, of course, alter in ways the direct effects of some toxicants. Several studies showed that cooperation between various aquatic organisms might play a significant role in the withstanding of zooplanktons to the adverse effects of toxicants. Namely, the reduction of pesticide toxicity due to supplementation of algal food was shown in experiments with *Daphnia carinata* King (Barry et al. 1995). Mangas-Ramirez and coworkers showed for *Ceriodaphnia dubia* Richard, 1894, *Moina macrocopa* Straus, 1820, and *D. pulex* De Geer, 1778, that toxicity of ammonium chloride varied in dependence of the density of *Chlorella vulgaris* Beijer (Mangas-Ramirez et al. 2001, 2002). Sarma and collaborators in investigations with rotifer *Brachionus patulus* O.F.M., 1786, detected analogous effects: in acute and chronic tests, resistance of the organism to methyl parathion was increased due to supplementation with *C. vulgaris* (Sarma et al. 2001). Friberg-Jensen and coauthors described more complex interactions between crustaceans, rotifers, algae and bacteria under cypermethrin action (Friberg-Jensen et al. 2003).

The aim of the present work was to study the survivability and reproduction of some freshwater invertebrates (*D. magna* Straus, 1820) exposed to deltamethrin under varying densities of the microalga *C. vulgaris* as well as under the influence of microflora and excretas of *Typha angustifolia* L.

MATERIALS AND METHODS

Daphnia magna Straus, 1820, were obtained from the Department of Zoology (Kazan State University, Kazan, Russia). The culture of genetically homogeneous organisms were reproduced from a single ancestor and was maintained in a 10 L aquaria with dechlorinated aerated tap water at room temperature (22±2°C).

Deltamethrin ((S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate) was dissolved in 1 mL of 96% ethanol, and then diluted to the necessary concentration (1 μg/L) as described earlier (Ratushnyak et al. 2005).

Test animals (10 adult *D. magna*, females taken from cultures 3-5 week old) were exposed to a toxicant for 15 days. 500 mL glass flasks with 400 mL of tap water or with 400 mL of tap water plus below-mentioned supplements were used for the cultivation of test organisms. There were two groups with test animals: in the first group, various supplements (see below) were added along with toxicant (parts 1 at Figs. 1, 2) while in the second group supplements were added after 4 days from the beginning of the experiment (part 2 at Figs. 1, 2). Survivability (in %) and reproduction (number of offsprings per one female) were assessed after 15 days. In parallel, the amount of microflora was also analyzed. For this purpose, an aliquot (1 mL) was

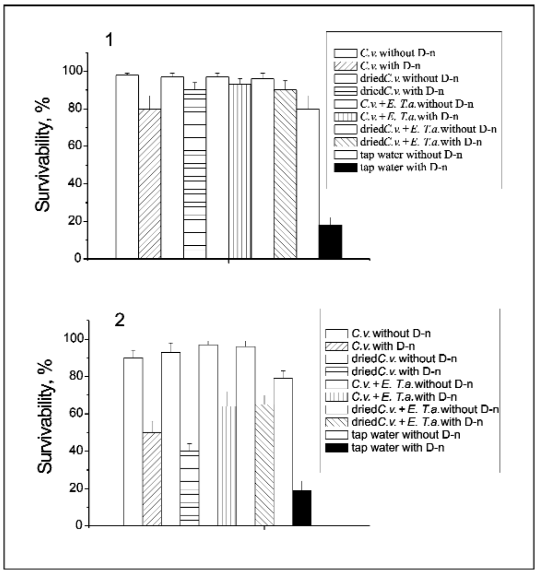

Figure 1. Effects of various supplements on survivability of deltamethrin-treated *D. magna*. Abbreviations: D-n - deltamethrin, C.v. - *Chlorella vulgaris*, E.T.a. - excretas of *T. angustifolia*

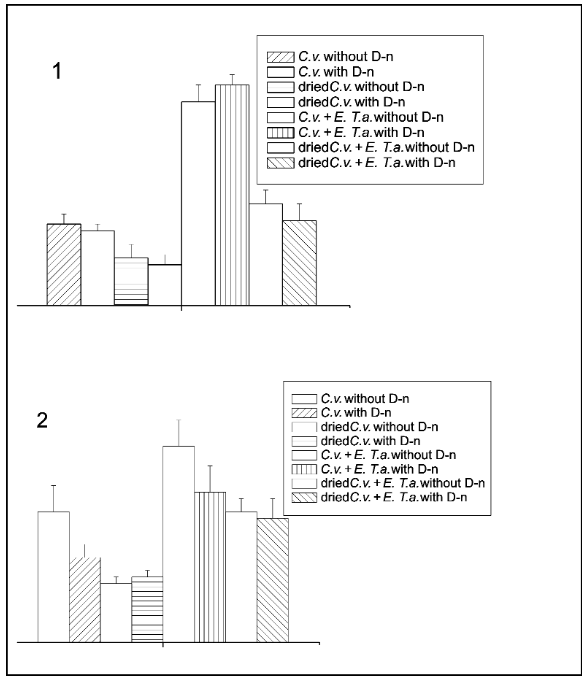

Figure 2. Effects of various supplements on reproduction of deltamethrin-treated *D. magna*. Abbreviations: D-n - deltamethrin, C.v. - *Chlorella vulgaris*, E.T.a. - excretas of *T. angustifolia.*

taken from the *D. magna*, cultivation medium, diluted gradually and plated on Petri dishes with nutrient agar. A number of colony-forming units were calculated.

The amount of *Chlorella* was assessed microscopically. Concentration of *Chlorella* in various variants of experiment was equal to 600.000 per mL of the *D. magna* cultivation medium. Dried *Chlorella* was obtained by exiccation (at 50°C, 3 h) of the liquid culture on Petri dishes and washing-off the cells into glasses with *D. magna*. The concentration of dried *Chlorella* was the same.

Intravital excretas of *T. angustifolia* L. were obtained by the following method. 250 g of *T. angustifolia* roots of were purified from alluvium and soil and placed for 24 h in 400 mL of water that was used then for *D. magna*, cultivation.

Experiments were performed in triplicate. The data on figures are presented as mean±standard deviation.

RESULTS

It was found that reactivity of *D. magna*, to deltamethrin depended on addition of various supplements. It is clear from Fig. 1 that survivability of *D. magna*, was increased significantly when *Chlorella*, dried *Chlorella* as well as intravital excreta of reed mace (*T. angustifolia*) were added to the medium for the cultivation of test organism (in comparison with control -tap water). It should be noted here that there were not significant differences between variants "*Chlorella*", "dried *Chlorella*", "*Chlorella* plus excreta of *T. angustifolia*" and "dried *Chlorella* plus excreta of *T. angustifolia*" protective effect of the supplements was roughly comparable (part 1 at Fig. 1). The observed antitoxic effect was detected in both experimental groups, i.e. when supplements were added at the first and fourth day (see part 1 and 2 at Fig. 1). However, after 4 days, the antitoxic effects in variants of "*Chlorella*" and "dried *Chlorella*" were less pronounced in comparison with variants of "*Chlorella* plus excreta of *T. angustifolia*" and "dried *Chlorella* plus excreta of *T. angustifolia*" (part 2 at Fig. 1).

The similar antitoxic effects were observed in relation to *D. magna*, reproduction (see part 1 and 2 at Fig. 2). In tap water without supplements, reproduction was absent (therefore, variant "tap water" is not presented at Fig. 2). However, our data shows that the most significant antitoxic effect of supplements was observed in the variant of "*Chlorella* plus excreta of *T. angustifolia*" while the variant of "dried *Chlorella*" demonstrated the less protective effect.

Although the above-mentioned supplements showed a protective effect against the toxicity of deltamethrin, the general tendency was a decrease both in the survivability and reproduction rate when the supplements were added after four days. This indicates the importance of early supplementation with *Chlorella* and excreta of *T. angustifolia*.

DISCUSSION

The presented data shows that some environmental factors may change resistance of test organism to a toxicant. It is possible to suggest that absence of survivability differences between variants "dried *Chlorella*", "*Chlorella* plus excreta of *T. angustifolia*" and "dried *Chlorella* plus excretas of *T. angustifolia*" (part 1 at Fig. 1) can be explained by the increased feeding activity of *D. magna*, during the first hours and days of the deltamethrin action. So, the initial antitoxic effect can be mediated by action of substances contained in *Chlorella*; this is in agreement with previously published data (Sarma et al. 2001). Moreover, our suggestion about a pivotal role of chlorella-mediated antitoxic effect can be supported by the fact that the amount of microflora varied significantly in variants of"dried *Chlorella*", "*Chlorella* plus excreta of *T. angustifolia*" and "dried *Chlorella* plus excreta of *T. angustifolia*" while the antotoxic effect was comparable (Table 1). On the other hand, it is possible to assume that the general amount of microflora seems unimportant when supplements were added along with deltamethrin: variants of "dried *Chlorella*" and "*Chlorella* plus excreta of *T. angustifolia*" are differed significantly on microflora content while their antitoxic effect is almost equal (Table 1, part 1 at Fig. 1). The role of the excreta of *T. angustifolia* in maintenance of the antitoxic effect is probably also minor when this supplement was added without *Chlorella*: results were undistinguishable from the control variant (tap water) (data not shown). However, when supplements were added at the forth day, the role of *Chlorella* was probably not so evident: one can compare variants of "dried *Chlorella* and "dried *Chlorella* plus excreta of *T. angustifolia*". In this case, the antitoxic effect can be mediated by either the synergetic effect of dried *Chlorella* and excreta of *T. angustifolia* or by the favorable effect of microflora: when the total amount of microflora in variants of "dried *Chlorella* plus excretas of *T. angustifolia*" and "*Chlorella* plus excreta of *T. angustifolia*" was increased (Table 1).

Concerning reproduction, it is reasonable to suggest that exiccation of *Chlorella* decreases its protective effect: it was minimal in comparison with other variants (parts 1 and 2 at Fig. 2). However, a combination of dried *Chlorella* with excreta of *T. angustifolia* restored the antitoxic effect: it was comparable with the variants of "*Chlorella*" (part 1 at Fig. 2) and even exceeded it (part 2 at Fig. 2). The observed data cannot be explained by the positive action of microflora because its content was increased in the variants of "*Chlorella*" in comparison with the variants of "dried *Chlorella* plus excretas of *T. angustifolia*" (Table 1). Finally, the combination of *Chlorella* with excreta of *T. angustifolia* was probably the most favorable for reproduction of *D. magna*. It is very likely that excreta of *T. angustifolia* have some reproduction-promoting chemical agents that act only in the presence of *Chlorella*. The observed effect was probably not mediated by microflora because its content varied significantly in groups 1 and 2 (Table 1, variant "*Chlorella* plus excreta of *T. angustifolia*) while its antitoxic effect was very evident in this variant despite the time of the supplement addition (part 1 and 2 at Fig. 2).

The obtained data suggests the presence of complex interactions between *D. magna*, and autotrophic organisms and their metabolites. It is probably a common phenomenon since the similar effects were observed not only in *D. magna*, but also

Table 1. Amount of spontaneous microflora (cell/mL) in various variants of experiment with *D. magna*.

Variant of experiment	Group 1 (addition since first day)		Group 2 (addition since forthday)	
	Without deltamethrin	With deltamethrin	Without deltamethrin	With deltamethrin
Chlorella	4.6×10^6	4.2×10^7	2.0×10^2	8.0×10^3
Dried chlorella	4.2×10^5	4.2×10^7	3.4×10^3	8.0×10^2
Chlorella+ exometabolites of *Typha angustifolia*	4.6×10^{11}	4.2×10^{11}	8.0×10^5	8.0×10^5
Dried chlorella+ exometabolites of *Typha angustifolia*	1.2×10^{12}	2.6×10^{11}	8.0×10^5	8.0×10^5
Tap water	6.0×10^5	5.0×10^4	1.0×10^3	4.0×10^3

in other invertebrate species including *D. carinata* (Barry et al. 1995), *C. dubia, M. macrocopa,* and *D. pulex*, (Mangas-Ramirez et al. 2001, 2002) and rotifer B. patulus (Sarma et al. 2001).

CONCLUSION

At the biocenotic level, the consortium of macrophytes and autotrophs may play a significant role in the adaptation of invertebrate populations to action of pollutants. Our experiments showed that autotrophic organisms like *Chlorella* and their combination together with macrophytes (*T. angustifol*) may act as antitoxic factors on deltamethrin-treated *D. magna.* The role of concomitant microflora is probably not so important in comparison with algal and plant components.

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