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The Influence of *Daphnia magna* Microcosm on its Resistance to Deltamethrin

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Abstract: Resistance of *Daphnia magna* to unfavorable conditions (action of deltamethrin) is regulated by interaction between components of the microenvironment (*D. magna* microcosm). At the initial stage of development of the organism, toxicoresistance is influenced by microflora. At the following stages, phytoplankton takes a significant role. Maximal antitoxic effect was detected in variants with chlorella. At 5 μg/L concentration of deltamethrin adaptation capabilities of *D.magna* seems try for a minimum.

Key words: *Daphnia magna* · Deltamethrin · Survivability · Macrophyte · Toxicoresistance

INTRODUCTION

Numerous studies confirm that a response of a living organism to a pollutant depends on the environment [1,2]. Due to this reason, the results of toxicological research obtained *in vitro* and in nature may be different. Otherwise speaking, a response of a complex biological system to a stimulus is not a sum of a particular responses of its constituents. So, investigations at the synecology level seem the most suitable approach to explore the reaction of any biosystem to some influence [1, 3].

Contamination of various waters with insecticides presents a serious problem due to a broad usage of these chemicals in agriculture [4, 5]. The investigation of their biological effects in the system "pollutant-test organism" does not seem entirely informative because, as was said before, complex interactions between organisms may influence the effect of a pollutant. Therefore, the aim of the present study was to investigate the influence of *Daphnia magna* microcosm on its resistance to deltamethrin.

MATERIALS AND METHODS

Toxicant. Deltamethrin ((*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate) was used in this study as a toxicant. It belongs to type II pyrethroid insecticides. The above-mentioned chemical was dissolved in 1 mL of 96% ethanol and then diluted to the necessary concentrations (1 and 5 μg/L).

Test organism. *Daphnia magna* Straus, 1820, organisms were obtained from continuous cultures maintained in 10L aquaria with dechlorinated and aerated tap water at room temperature (22±2 °C). Offsprings were separated at regular intervals. Test animals were 48-hr-old individuals, taken from cultures 3-5 week old.

Experimental design. Toxicoresistance of *D. magna* to a pollutant was assessed in reproduction rate and survivability coefficient. The latter was calculated as a sum of parts in regard to 100% survivability (taken for 1) for each day with subsequent cleavage on a number of days (15 days). The survivability coefficient reflects a total changes in survivability of *D. magna* in a daily dynamics.

400 mL glass flasks with 200 mL of tap water or with 200 mL of tap water plus below-mentioned supplements were used for the cultivation of test organisms (10 adult *D. magna* females). Except for tap water, natural course water (taken from the same station of Kazanka river) was used a medium for *D. magna* cultivation. Moreover, water from plankton gregarization was also involved into study. For this purpose, plankton concentrate was located into tap water for 1 day (at a room temperature) and then was eliminated during centrifugation at 3000 *g*.

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C. vulgaris was used for *D. magna* feeding. Concentration of *C. vulgaris* in various variants of experiment was equal to 600.000 per mL of the *D. magna* cultivation medium. Dried *C. vulgaris* 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 *C. vulgaris* was the same. Native or dried *C. vulgaris* (600.000 per mL of the *D. magna* cultivation medium) and concentrates of plaknton (native and dried) were added to various variants of experiment.

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

RESULTS AND DISCUSSION

It was detected that the concentrate of alive and dried plankton, chlorella and natural water favored to toxicoresistance of daphnia to deltamethrin (Figure 1 and 2).

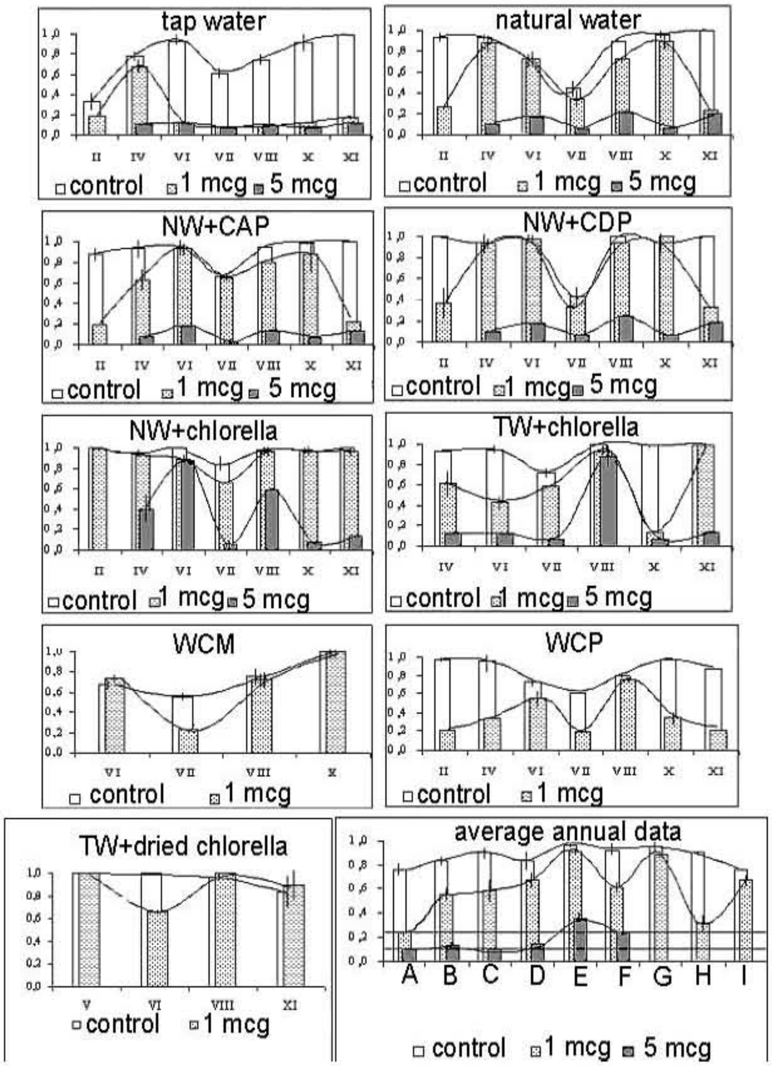

Fig. 1: The influence of deltamethrin on *D. magna* survivability coefficient at various microcosm. Note: NW=natural water; CAP=concentrate of alive plankton; CDP=concentrate of dried plankton; WCM=water from consortium of macrophytes; WCP=water from consortium of plankton; TW=tap water; A=tap water; B=natural water; C=natural water plus concentrate of alive plankton; D= natural water plus concentrate of dried plankton; E=natural water plus chlorella; F=tap water plus chlorella; G=tap water plus dried chlorella; H= water from consortium of plankton; I= water from consortium of macrophytes. Roman numerals mean month of observation. Axis Y presents coefficient of survivability.

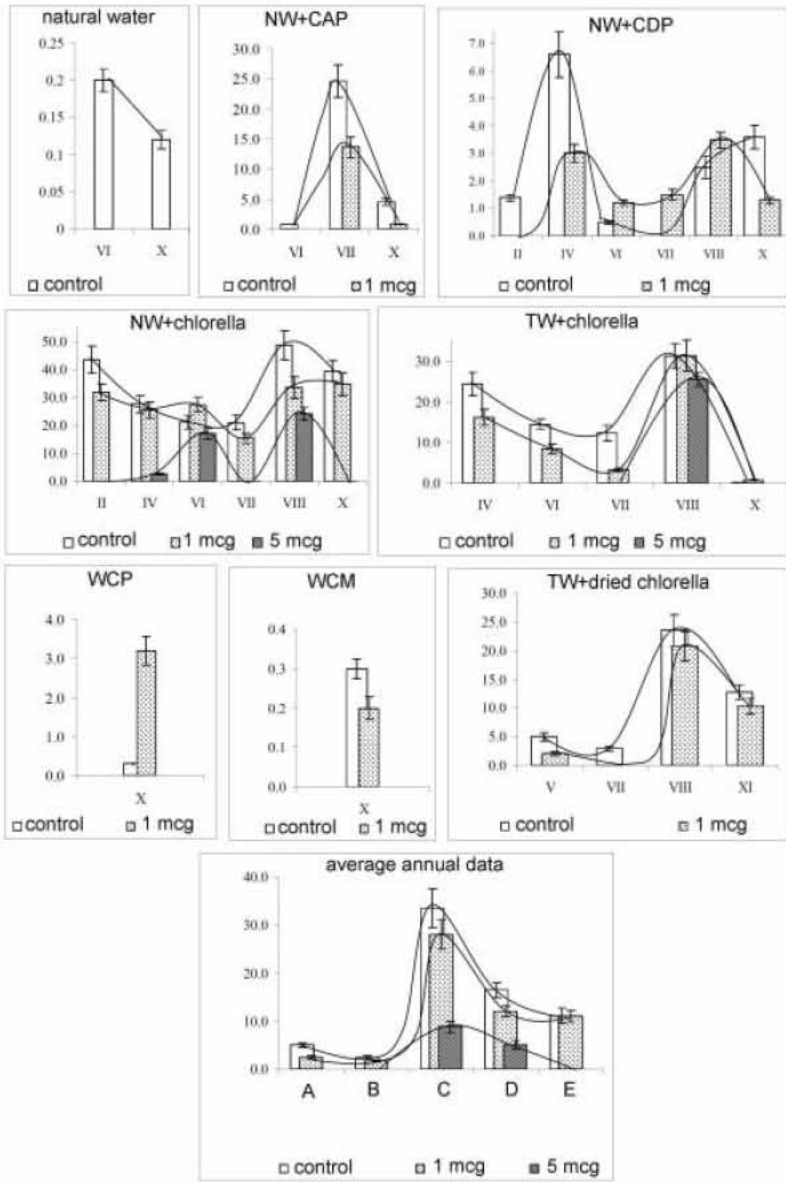

Fig. 2: The influence of deltamethrin on *D. magna* reproduction at various microcosm. Note: NW=natural water; CAP=concentrate of alive plankton; CDP=concentrate of dried plankton; WCM=water from consortium of macrophytes; WCP=water from consortium of plankton; TW=tap water; A=natural water plus CAP; B=natural water plus CDP; C=natural water plus chlorella; D=TW plus chlorella; E=TW plus dried chlorella. Roman numerals mean month of observation. Axis Y presents *D. magna* reproduction (a number of offsprings per one female).

The average toxicoresistance of daphnia to deltamethrin was as followed (coefficient of survivability was taken into account): tap water (0.23) < water from plankton gregarization (0.32) < natural water (0.56) < natural water plus concentrate of alive plankton (0.59) < tap water plus chlorella (0.61) < natural water plus concentrate of dried plankton (0.67) = water from macrophyte consortium (0.67) < tap water plus dried chlorella (0.89) < natural water plus chlorella (0.91). Concerning fertility, the following dependencies were detected (copy per 1 daphnia): natural water plus concentrate of dried plankton (1.75) < natural water plus concentrate of alive plankton (2.4) < tap water plus dried chlorella (11.02) < tap water plus chlorella (16.0) < natural water plus chlorella (28.0).

Table 1: Number and biomass of phytoplankton of Kazanka river in a seasonal dynamics

Organism	February	April	June	August	October	November
Number (x1000 cells per L)						
Cyanophyta	absent	absent	99.6±10.2	2789.3±195.6	absent	absent
Dynophyta	absent	absent	absent	154.7±14.8	absent	absent
Euglenophyta	absent	3.0±0.32	16.2±2.3	229.3±18.5	110.9±9.4	absent
Chrysophyta	absent	3.0±0.27	62.8±7.1	58.7±6.3	23.1±2.1	5.3±0.4
Bacillariophyta	absent	3.0±0.34	796.4±55.2	2789.3±223.3	808.9±67.6	206.3±17.6
Chlorophyta	absent	47.6±5.1	4845.2±205.6	810.7±68.1	12124.1±605.9	absent
Biomass (mg/L)						
Cyanophyta	absent	absent	0.005±0.0004	0.421±0.03	absent	absent
Dynophyta	absent	absent	absent	1.036±0.12	absent	absent
Euglenophyta	absent	0.02±0.0015	0.014±0.002	2.231±0.15	0.036±0.0017	absent
Chrysophyta	absent	0.001±0.0003	0.008±0.0005	0.021±0.002	0.002±0.00018	absent
Bacillariophyta	absent	0.002±0.0003	0.691±0.04	5.077±0.43	1.305±0.11	0.8±0.076
Chlorophyta	absent	0.017±0.0024	0.895±0.06	0.276±0.022	0.814±0.058	absent

Table 2: Number and biomass of zooplankton of Kazanka river in a seasonal dynamics. *Note*: ND=not detected

Organism	April	June	July	August	September
Number (x1000 copies per m^3)					
Rotatoria	13.5±1.8	207.0±28.5	0.67±0.08	513.1±62.5	133.98±17.9
Cladocera	ND	1.5±0.23	277.3±35.6	223.53±37.8	4.6±0.75
Copepoda	ND	12.0±2.7	108.31±13.8	124.86±15.2	4.62±0.95
Biomass (mg/m^3)					
Rotatoria	12.0±2.3	247.0±32.9	13.33±1.9	1315.18±152.4	234.0±33.2
Cladocera	ND	22.0±2.8	5498.65±590.3	6003.23±720.8	35.0±4.1
Copepoda	ND	19.0±2.1	4405.78±480.3	3014.97±338.8	2.0±0.22

Table 3: Number of spontaneous microflora (x1000 cells per mL) in various variants with *D. magna* measured in April and August. *Note*: 1-tap water; 2-natural water; 3-natural water plus alive concentrate of plankton; 4-natural water plus dried concentrate of plankton; 5-natural water plus chlorella. 1D-5D-variants with deltamethrin. ND=not detected

	Deltamethrin 1 μg/L, April				Deltamethrin 5 μg/August		
	Day of experiment						
Variants	0	2	4	8	0	2	4
1	60±7.3	50±6.1	0.1±0.016	0.32±0.025	ND	ND	ND
1D	22±5.2	15±1.7	0.64±0.052	3±0.28	ND	ND	ND
2	ND	ND	ND	ND	1000±120	20±1.8	0.5±0.042
2D	ND	ND	ND	ND	320±22	40±4.3	0.5±0.051
3	200±11.4	20±3.2	1.1±0.09	16±2.4	6000±250	60±5.7	400±45
3D	200±16.2	40±5.3	1±0.08	3±0.18	1800±124	120±14	1200±112
4	600±52.4	40±4.9	2.2±0.18	14±1.2	400±33	20±1.7	3.2±0.26
4D	200±21.8	100±8.6	3±0.24	3±0.38	45±3.8	80±8.6	4±0.51
5	ND	ND	ND	ND	400±42	80±7.7	0.5±0.04
5D	ND	ND	ND	ND	400±39	40±5.3	0.5±0.03

Table 4: Correlations between the coefficient of survivability (CS) and various components of natural water and toxicoresistance of *D. magna* at different cocentrations of deltamethrin

1 μg/L of deltamethrin				5 μg/L of deltamethrin	
CS-number of phytoplankton	CS-biomass of phytoplankton	CS-number of *Rotatoria*	CS-biomass of *Rotatoria*	CS-number of *Rotatoria*	CS-biomass of *Rotatoria*
0.62	0.43	0.44	0.35	0.57	0.56

The seasonal dynamics of the coefficient of survivability was as follows (at 1 μg/L concentration of deltamethrin): at natural water-November (0.24) < February (0.27) < July (0.35) < June (0.72) < August (0.73) < April (0.88) < October (0.9). Egg laying was almost absent. At natutal water plus concentrate of alive plankton: February (0.2) < November (0.23) < April (0.64) < July (0.65) < August (0.80) < October (0.88) < June (0.93).

Egg laying was detected in July, August and October. Youngsters were observed in July (14 copies per ♀) and October (0.8 copies per ♀). At natural water plus dried concentrate of plankton: November (0.33) < February (0.36) < July (0.43) < April (0.93) < August (0.95) < June (0.97) < October (1.0). Egg laying was absent in February and November, youngsters were observed in June (1.2 copies per ♀); October (1.3 copies per ♀); July (1.5 copies per ♀); April (3 copies per ♀); August (3.5 copies per ♀).

The longstanding studies showed that toxicoresistance of daphnia was higher in natural water (in the case of the absence of toxic waste) in comparison to tap water. Figure 1 confirms this fact. We detected that at 5 μg/L concentration of deltamethrin adaptation abilities of water crustaceans was highly reduced. The components of *D. magna* microcosm could not compensate the toxic effects of the pollutant. Only chlorella addition (600.000 cells per mL) was able to restore living activity in the organisms.

It was interesting to study the presence of any correlation dependencies between daphnia resistance to a pollutant and biological properties of natural water used as cultivation medium. It is clear from Tables 1 and 2 that there were seasonal changes in qualitative and quantitative content of zooplankton and phytoplankton. It should be noted here that this instability of hydrobiological regimes of natural water seems very important because effect of compensation of xenobiotic toxicity may be noticeable in the case of its good expression.

Table 3 presents data on dynamics of bacteria in the used media. It is known from the literature that daphnia are able to feed with bacterioplankton at the early stage of their existence. Then their feeding preference moves to phytoplankton. In April, a number of saprophiles reached a minimum by 4th day of experiment while in August this minimum was detected by 2nd day. It should be noted that in August a number of bacteria was much higher in comparison to April. This favored to daphnia survival at 5 μg/L concentration of deltamethrin. The obtained results suggest a presence of autoregulation of ecological systems and toxicoresistance of separate populations owing to interaction with bacterioplankton, phytoplankton, zooplankton (Tables 1 and 2) and products of their living activity.

We detected correlation dependencies between the coefficient of survivability and reproduction of *D. magna* on natural water with deltamethrin and its chemical and biological content. The results are presented in Table 4.

The presented data show that resistance of *D. magna* to a pollutant may greatly depend on the environmental microcosm. This is not a surprising fact because biologicals produced by macrophytes and invertebrates may be used by the test organism as nutritionals thus reducing the general stress under deltamethrin action. As in our early report [6], the most prominent antitoxic effect was detected in the cases with chlorella addition. So, environmental microcosm is a vital factor in the resistance of test orhanisms to toxicants. This should be taken into account while toxicological studies.

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