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Effect of Temperature on Toxicity of Deltamethrin and Oxygen Consumption by *Porcellio scaber* Latr (Isopoda)

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Abstract This study describes the toxicity of deltamethrin, in relation to its LD50 value, as well as variation in respiratory metabolism of the isopod species *Porcellio scaber* Latr kept at 3 temperature values (10, 22 and 30°C). The low LD50 values obtained indicate that deltamethrin is a highly toxic pyrethroid for the crustacean tested, particularly at 10°C. We also observed that, in all the 3 experimental temperatures, the deltamethrin toxicity was lower in females than in males. Particularly distinct differences between both sexes were visible at 10 and 30°C, i.e. temperatures that are too low and too high for the species studied. Oxygen uptake measurement showed an increase in respiratory metabolism directly after intoxication. The most substantial increase, 64 % in males and 80 % in females, was observed at the temperature 10°C, whereas at the other temperatures, it did not exceed 20 %. During the successive experimental days, the respiratory consumption in *P. scaber* had a tendency to decrease, which was more visible at 10 and 30°C, compared to the optimal temperature 22°C.

Keywords Deltamethrin · Oxygen consumption · Temperature · *Porcellio scaber*

The need to supply food to the constantly growing human population necessitates pesticide use (Walker 2002). For this reason, living indicators of soil eco-toxicity are being investigated. The following indicators have been mentioned so far: earthworms, springtails, snails and bees. Lately, there have been more attempts to use terrestrial crustaceans (*Isopoda*) in assessment of subsoil pollution (Cortet et al. 1999; Paoletti and Hassall 1999). These animals arouse interest owing to their widespread distribution and the essential role they fulfil in decomposition of organic matter, both of plant and animal origin, and humification thereof (Gromysz-Kałkowska et al. 1994). Representatives of this group inhabit various environments: forests, parks, gardens, cemeteries, cellars and glasshouses, that is to say, places where they can find humidity and organic matter, discarded by man. As a result of life activity of these animals, there appear conditions for soil microorganisms to live and take part in the final mineralization of organic remains contained in excrements, which contributes to humus formation. Also, the role which the crustaceans play in agricultural activity reveals their sensitivity to insecticides and demonstrates their respiratory metabolism, which is the basis for all metabolic processes responsible for animal survival in a given environment. Scientists attention is drawn to the fact that the *Isopoda* representatives are able to differentiate the types of subsoil (Eijsakers 1991), and to distinguish between contaminated and non-contaminated environments (Unkiewicz et al. 1998; Unkiewicz-Winiarczyk et al. 2003).

The aim of this study was determination of deltamethrin toxicity for the *Porcellio scaber* crustacean depending on the ambient temperature and based on the LD_{50} values and the level of oxygen consumption.

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Materials and Methods

The animals examined in the experiment were obtained from an unpolluted area near Lublin and then kept in glass containers filled with sand and birch litter. The isopods were divided according to gender into 3 control and 3 experimental groups within each gender group. The control animals were kept on birch leaf litter at 10, 22 and 30°C. The experimental crustaceans were poisoned with deltamethrin, the active ingredient of the synthetic pyrethroid "Decis", as an acetone solution in the doses of 0.625; 1; 2; 5 ad 10 µg/g of body weight and kept for 2 weeks on the birch leaf litter at the temperature 10°C (I experimental group); 22°C (II experimental group) and 30°C (III experimental group). The proportion of dead animals at each tested concentration was counted within 2 weeks. LD_{50} values were determined with the method of Lichtfield and Wilcoxon (1949). The significance of differences in sensitivity in males and females was analysed with the χ^2 test.

Oxygen consumption was tested both among the control animals and those intoxicated with deltamethrin doses that caused ≈25 % lethality (½ LD_{50}) in each experimental group. The measurements of oxygen consumption were conducted daily for 2 weeks at the temperature the animals were kept in. The oxygen consumption measurement was conducted with Drastich volumetric respirometer modified by Klekowski (1975) and expressed in mm^3/g/weight/h; in the figure, it is presented as percentage changes with the initial value of 100 %. The significance of differences in the respiratory metabolism was analysed with the Anova test, the value $p < 0.05$ was statistically significant.

Results and Discussion

The research conducted has shown that deltamethrin is highly toxic to the crustacean *P. scaber*, which is confirmed by the low LD_{50} value (Table 1). The data obtained are in agreement with our previous research on other macrosaprophages (Gromysz-Kałkowska et al. 1995, 2000).

Relationships between pesticide toxicity for insects and the degree of permeability of the body cover, i.e. the thickness and degree of sclerotisation, were reported by Wigglesworth (1942), Skworcow (1946) and Nowosichow et al. (1975). Moreover, these authors found that pesticides in the form of solutions in organic solvents penetrate into the insect body more easily than aqueous solutions. *P. scaber*, a terrestrial crustacean living in the humid environment, has less chitinized and mineralized exoskeleton than insects, especially in the spaces between tergites; therefore deltamethrin administered in an organic solution penetrated into the body quickly. The poor chitinization of the *P. scaber* skeleton was also observed while the animals were being transferred to rearing containers and measurement vessels cups with tweezers and while held during deltamethrin application on the dorsal part of the animals' bodies. The high permeability of the body shells in this crustacean was further confirmed by rapid disappearance of the acetone solution of pyrethroid applied on the shell during the experiment.

Due to the fact that body shell does not constitute an effective barrier against toxins, this crustacean has a different protective mechanism based on chemoreception (Migula 1991). *P. scaber* is capable of distinguishing the degree of environment contamination, which is convincingly elucidated in the research of Unkiewicz-Winiarczyk (1999). Namely, in the conditions of free choice, *P. scaber,* unlike the other related macrosaprophages, choses sites with non- or little-contaminated litter.

Literature data demonstrate unequivocally that crustaceans are highly sensitive to pyrethroids. Laboratory research of Zitko et al. (1979) showed that the representatives of this group of arthropods reveal high mortality at even low concentrations of permethrin and decamethrin, at which insects display only slight behavioural symptoms.

The LD_{50} values obtained in this research indicated a clear dependence of deltamethrin toxicity on ambient temperature; this pyrethroid turned out to be much more toxic at 10°C than at 22°C, or, in the case of females, at 30°C (Table 1) (Fig. 1). Based on the LD_{50} values obtained, it can be assumed that at the temperature 22°C, which is in the range of the optimal values for *P. scaber* (Gromysz-Kałkowska and Oder 1983), this crustacean readily tolerated the pyrethroid used.

A similar temperature dependence was observed by Delabie et al. (1985) in bees in the case of cypermethrin.

Table 1 Toxicity of deltamethrin for *P. scaber* Latr. at different temperatures

Temperature	Gender	n	LD_{50} value	
			Mean	Range
10°C	♂	240	0.566	0.403–0.796
	♀	240	0.925	0.728–1.175
22°C	♂	297	0.941	0.659–1.342
	♀	291	1.003	0.666–1.511
30°C	♂	257	0.646	0.472–0.885
	♀	261	1.196	0.958–1.494

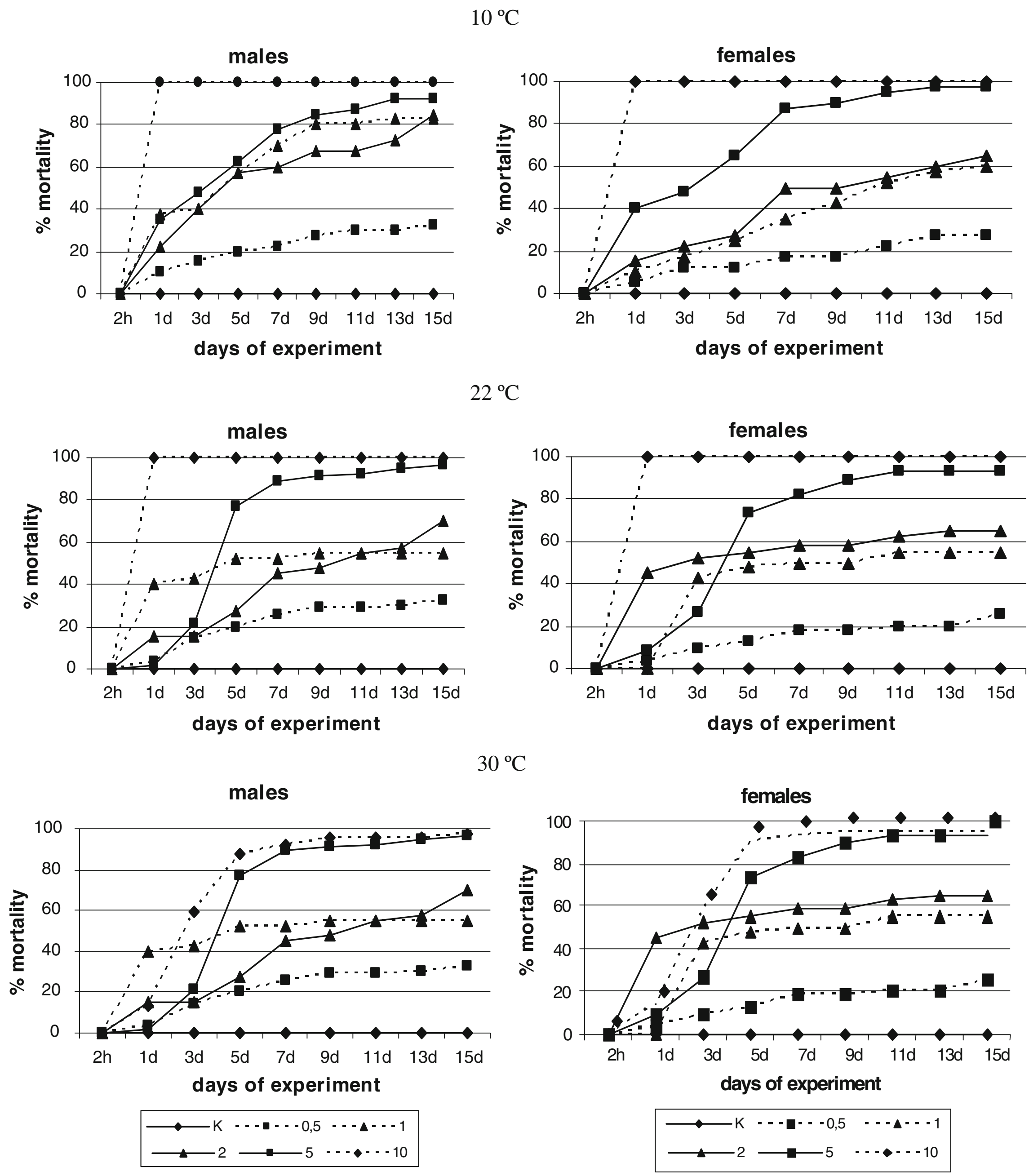

Fig. 1 Mortality of *P. scaber* Latr. in relation to doses and time

The toxic dose of this pyrethroid applied to the dorsal part of the thorax in bees was lower in the range of 12–20°C, compared to the amount required to elicit the same effect at the temperature of 32°C. The research of Malinowski (1986) showed that pyrethroid toxicity for Colorado beetle was higher in the lower temperatures from the range of 10–30°C.

In the present study, we have observed that in all the 3 experimental temperatures the toxicity of deltamethrin in females of *P. scaber* was lower than in males, and particularly distinct differences between both genders were observed at 10 and 30°C, which are too low and too high temperatures for the species studied (Table 1) (Gromysz-Kałkowska and Oder 1983).

The differences in response to pesticide poisoning between the genders, with greater resistance in females, display general regularity typical of many species of animals (Rusiecki 1973; Gromysz-Kałkowska et al. 1995).

Table 2 Oxygen use in *P. scaber* Latr. at different temperatures

Day of experiment	Gender	Oxygen consumption (mm^3/g/hour) ± SEM		
		10°C	22°C	30°C
Initial value	♂	86.50 ± 7.24	267.50 ± 8.80	273.96 ± 9.27
	♀	76.34 ± 7.21	232.70 ± 13.09	256.19 ± 6.36
2 h	♂	141.73 ± 8.67[1]	303.22 ± 20.15[2]	309.96 ± 33.00
	♀	137.79 ± 9.65[1]	253.14 ± 15.29[2]	305.82 ± 21.46
1	♂	126.82 ± 7.36[1]	257.06 ± 10.17	211.08 ± 16.07[1]
	♀	123.93 ± 11.08[1]	226.35 ± 9.49	199.30 ± 19.15
3	♂	129.41 ± 8.96[1]	263.14 ± 6.66[2]	183.09 ± 11.50[1,2]
	♀	122.70 ± 6.77[1]	226.35 ± 9.72[2]	241.13 ± 20.68[2]
5	♂	127.26 ± 10.55[1]	259.81 ± 13.86[2]	220.94 ± 24.92[1]
	♀	121.81 ± 10.57[1]	219.70 ± 10.46[2]	253.20 ± 23.74
7	♂	107.17 ± 12.42	243.20 ± 13.70	207.61 ± 19.67[1]
	♀	112.20 ± 9.26[1]	226.36 ± 6.12	175.39 ± 11.99[1]
9	♂	110.73 ± 13.65	225.16 ± 10.15[1]	184.95 ± 15.66[1]
	♀	107.11 ± 11.44	219.55 ± 14.88	214.84 ± 8.05[1]
11	♂	81.59 ± 8.16	210.39 ± 11.06[1]	172.56 ± 15.05[1]
	♀	93.32 ± 6.40	200.55 ± 8.66	193.99 ± 8.93[1]
13	♂	85.64 ± 8.85	217.70 ± 19.12[1]	173.83 ± 4.99[1]
	♀	92.37 ± 9.85	204.79 ± 13.51	196.11 ± 7.60[1]
15	♂	89.47 ± 8.53	214.52 ± 12.59[1]	177.43 ± 6.49[1]
	♀	83.38 ± 6.27	200.55 ± 16.35	198.07 ± 10.49[1]

[1] Differences significant to the control

[2] Differences significant between the genders

Our observations of the behaviour of *P. scaber* individuals intoxicated with deltamethrin provides evidence for its neurotoxic effect. The crustaceans displayed uncoordinated, spasmodic movements, later they became unable to move, which was accompanied by excitation visible in continuous movements of the limbs and antennae. Similar symptoms were reported from water invertebrates poisoned with cypermethrin: larvae of mayfly, water boatman, water beetle, water strider and flower fly (Bennett et al. 1980). The reactions to pyrethroid poisoning described above are regarded as "knock down effect" in the literature. The neurotoxic effect of pyrethroids is associated with their impact on the functioning of ion conduction channels in the neuron membrane (Różański 2002).

In crustaceans, temperature is an essential factor of oxygen requirement. Respirometry tests conducted by Gromysz-Kałkowska and Szubartowska (1984) at 9 temperature levels ranging from 10 to 34°C revealed that the optimal temperature for *P. scaber*, i.e. not increasing oxygen requirement, is within the range of 19–22°C. In the present study, the rate of oxygen consumption changes in intoxicated crustaceans varied in a temperature-dependent manner (Table 2, Fig. 2). At 10°C we noted a considerable increase in respiratory metabolism within the first few

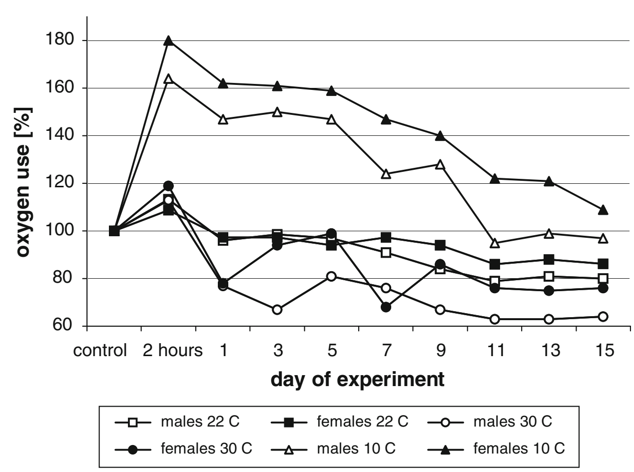

Fig. 2 Oxygen consumption in *P. scaber* Latr. at different temperatures

hours after intoxication. In the next days of the experiment, there was a gradual decrease towards the initial value in males, and a slightly smaller decrease in females. At 22°C, immediately after intoxication, there was only a slight increase in oxygen requirement, which after 24 h displayed somewhat lower values than those before the application of deltamethrin. It equalled 79 %–81 % of the initial value in males and 86 %–88 % in females in the final stage of the measurement. The rate of changes in oxygen consumption in *P. scaber* kept and intoxicated with deltamethrin at 30°C did not show the regularity noted at 10 and 22°C. In the first hours after intoxication, it slightly exceeded the initial value in males and females, but during the first day it was decreased by 23 % and 22 %, respectively. In the last days of the experiment (day 9–15), it maintained the level of 63 %–67 % in males and 76 %–84 % in females with respect to the initial value.

Enhanced respiratory metabolism in the initial stage of deltamethrin effect on *P. scaber* organism could be caused by activation of detoxication processes. Following pyrethroid poisoning, two enzymatic systems are activated: an esterase system of cleaving ester bonds of pyrethroid and oxidases oxidizing hydroxymethyl derivatives to respective aldehydes and carboxyl acids (Witkowski 1984). Experiments conducted on ryanodyne, a toxin obtained from a tropical *Ryania* plant, demonstrated a several-fold increase in oxygen consumption in the case of the ryanodyne-induced paralyse in *Orthoptera*; however, when the nervous system was not excited, there was no increase in oxygen requirement in ants and *Galleria* larvae (Keister and Buck 1964).

In isopods intoxicated with deltamethrin at 10°C, the substantial increase in oxygen consumption followed by a decline, visible in subsequent measurements, seems to have resulted not only from detoxication of pyrethroid, but mainly from strong excitation of the nervous system at this temperature. The measurement values of oxygen consumption in *P. scaber* presented above as well as literature data confirm this fact. At 22°C (optimal for *P. scaber*), the decrease in respiratory metabolism was presumably caused only by the effect of deltamethrin. A similarly slight decline in oxygen consumption persisting several days was observed by Gromysz-Kałkowska and Szubartowska (1994) at 22°C in three species of diplopods intoxicated with cypermethrin. The authors explain the results obtained with slow detoxication of pyrethroid, probably due to the presence of α-cyanide group in its cell structure, which prevents enzymes from quick hydrolysis of ester bonds of pyrethroid. Since deltamethrin is a pyrethroid containing an α-cyanide group, too, it can be assumed that not only at 22°C but also 30°C the same mechanism is responsible for the decreased oxygen consumption persisting several days after intoxication.

The respiratory metabolism in *P. scaber* was lower than the initial value at 22°C and 30°C throughout the study, which seems to result from disturbances in the activity of respiratory enzymes and damaged mitochondria (Beskid et al. 1973; Sitkiewicz and Konecka 1975). Although oxygen consumption did not return to the initial value in the temperatures mentioned, the mortality rate of the crustaceans only slightly increased from day 7 after intoxication with the lowest dose, apparently due to the fact that deltamethrin was not toxic to animals after that time (Fig. 1).

Our research and literature data indicate that the effect of pyrethroids on the animal organism is a complex process, dependent on their toxicodynamic properties, temperature of the environment, time and kind of exposure and also on the species of animals.

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