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Comparison of the toxic effect of pyrethroids on *Ixodes ricinus* and *Dermacentor reticulatus* females

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Abstract

Introduction. Despite the increased rates of infestations with *I. ricinus* (Ir) and *D. reticulatus* (Dr) ticks observed over the last decade, no effective control methods have been developed so far. The present study was focused on assessment of the action of pyrethroids on these both tick species.

Materials and method. The different doses of four pyrethroids, i.e. deltamethrin – D (K-Othrine), permethrin – P (Copex WP), cypermethrin – C (Kordon 10WP), and alphacypermethrin – AC (Alfasekt 5SC) were tested. The LD_{50} for each tested compound was also determined for both tick species. Unengorged and engorged (maintained on rabbit skin) tick females were sprayed with 20µl of 0.01563–0.50% solutions of the tested preparations.

Results. The investigations showed that sensitivity of Ir and Dr to the tested pyrethroids, but the effects exerted by the different doses varied between both tick species and between engorged and unengorged females in these species. The strongest toxic effect on unengorged and engorged Ir and Dr females was exerted by D, whereas the effect of AC was weaker. The LD_{50} (in µg/1 g b.w.) of D, AC, C, and P for unengorged Ir and Dr females was, respectively, 55.4 and 25.5, 105.2 and 48.5, 225.9 and 197.7, and 553.8 and 380.8. In the case of engorged Ir and Dr females, the LD_{50} of AC, D, C, and P reached a value of 0.9453 and 0.2310, 1.0428 and 1.3533, 3.489 and 6.5662, and 8.3955 and 7.3940, respectively.

Conclusions. The differences between the effects of the tested pyrethroids and their different doses on Ir and Dr highlight the necessity for development of a strategy for control of the tick species in different regions, based on investigations of their sensitivity to chemical compounds.

Key words

tick, *Ixodes ricinus*, *Dermacentor reticulatus*, deltamethrin, permethrin, cypermethrin, alphacypermethrin

INTRODUCTION

Since they were synthesised based on natural pyrethroids, new compounds characterised by high photostability and enhanced activity have become primary biocides worldwide. Given their selectivity and low, in comparison to other compounds, toxicity to other organisms inhabiting ecosystems, pyrethroids are widely applied in arthropod control, including ticks. Until recently, investigations of synthetic pyrethroids have usually been focused on ixodid tick species from Africa, the Pacific Islands and Australia, South and Central America, and North America. Less extensive research has been conducted on the Eurasian continent [1, 2]. Although the acaricidal effect of pyrethroids on the parasitic stage of *I. ricinus* and *D. reticulatus*, two common European tick species, is well known [3, 4], there are few papers in the literature concerning the effects of these compounds on the other stages of the tick life cycle [5, Buczek et al. unpubl.]. Knowledge about the sensitivity of various *I. ricinus* and *D. reticulatus* populations to pyrethroids is still insufficient and inadequate given the medical and veterinary importance of both species. *I. ricinus* transmits numerous pathogens, primarily of borreliosis, anaplasmosis, viral encephalitis, spotted fevers, and babesiosis. In turn, *D. reticulatus* is a vector of, e.g., canine babesiosis, tick-borne encephalitic virus, rickettsiosis and CC [6]. Additionally, both species can induce skin lesions with varied distribution and appearance [7, 8]. The increased distribution range and abundance of *I. ricinus* and *D. reticulatus* in Europe, as well as the growing incidence of human and animal tick-borne diseases reported over the last years, have aroused interest in methods for prevention of infestations by these arthropods and in chemical methods for reducing their density in the environment.

Objective. The aim of the presented study was to assess the effectiveness of deltamethrin, permethrin, cypermethrin, and alphacypermethrin in their action against *I. ricinus* and *D. reticulatus* females from a population in eastern Poland. Another objective was to specify the dose of the substances that exerts a teratological effect in both tick species and the lethal dose for females before and after host infestation, and to assess the remote effects of the action of these pyrethroids.

MATERIALS AND METHOD

Adult stages of *Ixodes ricinus* and *Dermacentor reticulatus* were collected by the flagging method in their habitats near Lubycza Królewska and Ostrów Lubelski (23°31'E, 50°20'N) in the Lublin Province in south-eastern Poland. Prior to the start of the experiments, the ticks were kept in laboratory conditions at a temperature of 4–6 °C and relative 90–100% humidity. The collected ticks were divided into two groups;

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one group was used for investigations of the effect of the tested substances on unengorged specimens, and the other group was designed for similar investigations to be performed on engorged tick females. In order to obtain engorged specimens, 15 unengorged females and 5 males of one tick species were placed on each albino New Zealand rabbit (*Oryctolagus cuniculus*). The course of feeding was assessed daily at the same time in order to collect engorged females immediately after detachment from host skin. Directly after feeding, each engorged female, as well as each unengorged female of *I. ricinus* and *D. reticulates,* were weighed with an accuracy of 0.1 mg using a WPA 120/C/1 analytical laboratory balance (Radwag, Poland). Subsequently, each specimen was placed on Whatman filter paper and kept in individual rearing chambers; the females were sprayed with 20 µl of solutions of the tested compounds using a 0.2–50 µl micropipette (Labsystems O. G., Helsinki, Finland) with an accuracy of ±0.5–2.0. The chambers were transferred to thermostats providing constant temperature and humidity, i.e. 28 °C and 75% RH and 25 °C and 90% RH for *I. ricinus* and *D. reticulatus*, respectively. As specified previously, these are optimal conditions for the development of these tick species in the laboratory. Tick rearing was performed in accordance with a method developed by A. Buczek, a member of our research team.

The effect of the tested pyrethroids was assessed 48 h after application of the substances, taking into account the number of dead and active specimens in each experimental group. Observations were performed under a stereoscopic Carl Zeiss microscope. Ticks that neither exhibited signs of movement nor reacted to mechanical stimuli were regarded as dead.

Simultaneously, control experiments were performed for both tick species, in which the procedures and temperature and humidity conditions were the same as in the experiments with pyrethroids. However, in the control group, each tick was sprayed with 20 µl of distilled water instead of the active substance solution.

Based on the results obtained, doses of the tested pyrethroids with acaricidal activity against *I. ricinus* and *D. reticulatus* females were determined. Additionally, the lethal dose (LD_{50} in µg/1 g b.w.) was determined for each of the tested compounds, i.e., the amount of the toxic substance per body weight unit that caused 50% tick mortality after a single application. The LD_{50} with corresponding confidence intervals were calculated using a computer programme based on the method developed by Lichtfield and Wilcoxon [9].

Tested acaricides. The investigations were carried out using formulated deltamethrin (K-Othrine 2,5 flow, 2.5% active ingredient (Roussel Uclaf, France) (Roussel Uclaf was taken over in 1997 by Hoechst AG, Frankfurt-am-Main – MT) permethrin – Copex WP, 25% active ingredient (AgrEvo Environmental Health Ltd., Cambridge, UK), cypermethrin – Kordon 10WP, 10% active ingredient, isomers 40/60 cis: trans (AgrEvo Environmental Health Ltd., UK), and alphacypermethrin – Alfasekt 5SC, 5% active ingredient (ASPRANT s.c., Jaworzno, Poland).

The effect of different pyrethroid concentrations – 0.01563%, 0.03125%, 0.0625%, 0.125%, 0.250%, and 0.50% solutions – and doses per specimen was tested. The solutions were obtained by serial dilution of the agents. The quantity of the active substance contained in 20µl of the solution applied to the dorsal side of each female is presented in Table 1.

Table 1. Quantity of active substance in 20 µl of a deltamethrin, permethrin, cypermethrin and alphacypermethrin solutions applied as a single dose (in µg)

Concentration of the solution (%)	D (µg)	P (µg)	C (µg)	AC (µg)
0.01562	0.0781	0.781	0.312	0.1562
0.03125	0.1562	1.562	0.625	0.3125
0.0625	0.3125	3.125	1.250	0.625
0.125	0.625	6.250	2.500	1.25
0.250	1.25	12.50	5.00	2.50
0.500	2.5	25.00	10.00	5.00
1.00	5.0	50.00	20.00	1.00

D – deltamethrin, P – permethrin, C – cypermethrin, AC – alphacypermethrin

RESULTS AND DISCUSSION

All the pyrethroids tested, i.e., permethrin, deltamethrin, cypermethrin, and alphacypermethrin, caused the death of *D. reticulatus* and *I. ricinus* females, but their effects differed between both tick species (Tab. 2). Small, single deltamethrin and alphacypermethrin doses of 0.15625 µg/1 specimen

Table 2. Mortality of Dr and Ir females 48h post exposure to different concentration of certain pyrethroids (in %)

Pyrethroid	Concentration (%)	*Dermacentor reticulatus*		*Ixodes ricinus*	
		unengorged	engorged	unengorged	engorged
Deltamethrin	0.01563	20.0	8.3	40.0	14.3
	0.03125	60.0	25.0	80.0	28.6
	0.0625	100	33.3	100	57.1
	0.125	100	50.0	100	57.1
	0.25	100	100	100	100
	0.5	100	100	100	100
	1.0	100	100	100	100
Permethrin	0.01563	40.0	16.7	60.0	28.6
	0.03125	80.0	33.3	80.0	28.6
	0.0625	80.0	41.2	100	57.1
	0.125	80.0	75.0	100	100
	0.25	100	83.3	100	100
	0.5	100	100	100	100
	1.0	100	100	100	100
Cypermethrin	0.01563	60.0	33.3	20.0	14.3
	0.03125	60.0	41.7	60.0	28.6
	0.0625	80.0	58.3	80.0	71.4
	0.125	100	50.0	100	71.4
	0.25	100	100	100	85.7
	0.5	100	100	100	100
	1.0	100	100	100	100
Alphacypermethrin	0.01563	80.0	66.7	80.0	28.6
	0.03125	80.0	50.0	80.0	42.9
	0.0625	100	75.0	100	85.7
	0.125	100	83.3	100	100
	0.25	100	100	100	100
	0.5	100	100	100	100
	1.0	100	100	100	100
Control		0	0	0	0

(0.03125% deltamethrin and 0.015625% alphacypermethrin solutions) induced a considerable mortality rate in unengorged *D. reticulatus* females, reaching 60% and 80%, respectively, for both substances (control 0%). 100% mortality was found upon application of 0.3125 μg/1 specimen of deltamethrin and 0.625 μg/1 specimen of alphacypermethrin (0.0625% solutions of the tested acaricides). A lower acaricidal effect was produced by cypermethrin, which induced 60% mortality at the dose of 0.3125 μg/1 specimen contained in 20 μl of the 0.015625% solution tested. The weakest effect was induced by permethrin. 48 h after application at the doses of 0.78125 μg/1 specimen and 1.5625 μg/1 specimen (0.015625 and 0.03125% solutions), 40% and 80% of unengorged *D. reticulatus* females, respectively, died. At the same doses of alphacypermethrin, unengorged *I. ricinus* females exhibited the same sensitivity as *D. reticulatus* females (80–100% mortality after application of 0.01563–0.0625% preparation solutions) (Tab. 2). Different mortality dynamics in unengorged *I. ricinus* females than in *D. reticulatus* females was observed after application of the other pyrethroids, but the tendency towards increasing acaricidal activity, together with increasing doses of the agents, was observed in both tick species.

The different effect of the same pyrethroid doses on unengorged females of both species may be caused by the differences in their body weight (Tab. 3) and size [10] and different absorption of the substances from the body surface. Both tick species differ in the degree of chitinisation of the body and physiological traits, e.g., preferences for abiotic factors [11] and the survival rates in various environmental conditions [12] (own unpublished observations).

Table 3. Increase of body mass weight in *D. reticulatus* and *I. ricinus* females

Tick species	Female body mass weight M±SD (g)		Increase of body mass weight (%)	
	unengorged	engorged	range	M
D. reticulatus	0.0048±0.0004	0.407±0.066	65.6–107.5	85
ricinus	0.002±0.0004	0.338±0.062	115.0–250	169

M – mean

Engorged *D. reticulatus* and *I. ricinus* females were more resistant to the tested substances than those that were unengorged (Tab. 2). The same doses of all the active substances yielded a substantially lower mortality rate in engorged than unengorged females of both species (Tab. 2). 100% mortality of engorged *D. reticulatus* females was observed only after application of higher doses of the active substance. The lethal dose of deltamethrin causing 100% mortality of engorged *D. reticulatus* females was 1.25 μg/1 specimen (0.25% solution) and that of alphacypermethrin was 2.5 μg/1 specimen (0.25% solution). In the case of permethrin, all unengorged females of this species died after application of 12.5 μg/1 specimen (0.25% solution), whereas the same mortality in engorged females was reached at a two-fold higher dose, i.e. 25.0 μg/1 specimen (0.50% solution).

The dose of deltamethrin that caused the death of all engorged *I. ricinus* females was four-fold higher (1.25 μg/1 specimen, 0.25% solution), and that of alphacypermethrin and permethrin was two-fold higher, i.e., 1.25 μg/1 specimen (0.125% solution) and 6.25μg/1 specimen (0.125% solution), respectively, than the lethal doses for unengorged females (Tab. 2).

Deltamethrin exhibited the highest toxicity to unengorged *D. reticulatus* and *I. ricinus* females, i.e. 25.5 and 55.4 at LD_{50} (in μg/1 g b. w.), respectively. Lower toxicity was observed for alphacypermethrin, i.e., 48.5 and 105.2 at LD_{50} (Tab. 4). In turn, the highest toxicity for engorged specimens was exhibited by alphacypermethrin (0.2310 at LD_{50} for *D. reticulatus* and 0.9453 at LD_{50} for *I. ricinus*), and lower toxicity was observed for deltamethrin (1.3533 at LD_{50} and1.0428 at LD_{50} for *I. ricinus*). The toxicity of cypermethrin and permethrin was substantially lower in the case of both engorged and unengorged females of both species. The lethal dose was LD_{50} μg/1 specimen for deltamethrin, permethrin, cypermethrin and alphacypermethrin in unengorged and engorged *D. reticulatus* and *I. ricinus* females (Tab. 5).

Table 4. Lethal dose of LD_{50} μg/1g b.w. for deltamethrin, permethrin, cypermetrin and alphacypermethrin in unengorged and engorged *D. reticulatus* and *I. ricinus* females sprayed with 20 μl of perythroid solution

Pyrethroid	*Dermacentor reticulatus*		*Ixodes ricinus*	
	unengorged	engorged	unengorged	engorged
Delta-methrin	25.5 (2.5÷254.8) f LD_{50}= 9.999	1.353 (0.683÷2.682) f LD_{50}=1.928	55.4 (5.5÷553.7) f LD_{50}=9.999	1.042 (0.522÷2.083) f LD_{50}=1.997
Permethrin	380.8 (195.3÷742.8) f LD_{50}=0.513	7.394 (4.401÷12.421) f LD_{50}=1.680	553.8 (55.4÷5537.2) f LD_{50}=9.999	8.395 (2.901÷24.293) f LD_{50}=2.894
Cyper-methrin	197.7 (656.2÷2186) f LD_{50}=0.548	6.566 (1.057÷40.780) f LD_{50}=6.212	225.9 (136.1÷374.9) f LD_{50}=0.602	3.489 (1.894÷6.425) f LD_{50}=1.842
Alpha-cyper-methrin	48.5 (4.8÷484.6) f LD_{50}= 9.999	0.231 (0.070÷0.759) f LD_{50}=3.290	105.2 (10.5÷1051.4) f LD_{50}=9.999	0.945(0.623÷1.433) f LD_{50}=1.516

The differences in the sensitivity to pyrethroids between unengorged and engorged *D. reticulatus* and *I. ricinus* females are associated with the considerable increase (85- and 169-fold, respectively) in the body weight after ingestion of rabbit blood, compared with the body weight of unengorged specimens (Tab. 3), which has an effect on the toxic action of chemical substances. The physiological status of the tick associated with the presence of large nutrient reserves probably changes the metabolism of absorbed pyrethroids and inhibits their action. Other investigations conducted on representatives of the genera *Ixodes*, *Haemaphysalis*, and *Dermacentor* demonstrated higher resistance to acaricides in bigger ticks [13].

Table 5. Lethal dose LD_{50} μg/1 specimen for deltamethrin, permethrin, cypermethrin and alphacypermethrin in unengorged and engorged *D. reticulatus* and *I. ricinus* females

Pyrethroid	*Dermacentor reticulatus*		*Ixodes ricinus*	
	unengorged	engorged	unengorged	engorged
Deltamethrin	0.122	0.501	0.110	0.345
Permethrin	1.831	2.738	1.108	2.798
Cypermethrin	0.950	2.432	0.452	1.163
Alphacypermethrin	0.233	0.086	0.210	0.315

According to Chizyuka and Mulilo [14], tick control is more effective on the host. As shown in the presented study, control of engorged ticks involves application of substantially higher doses of pyrethroids, which should be

taken into account due to the possibility of side- effects in the host. After application of acaricides in various tick species, Uspensky and Ioffe-Uspensky [13] reported a 'slow-death syndrome' and 'overcoming the poisoning', which may raise the risk of infection transmitted by an infected tick that feeds longer on the host, and increase the possibility of transovarial and transstadial transmission within a tick population. Investigations showed that application of synthetic pyrethroids as pour-on formulations in lambs significantly reduced the *I. ricinus* tick infestation rate, but did not affect the prevalence of tick-borne fever [15] or reduce seroprevalence of *Anaplasma phagocytophilum* [16].

Among the pyrethroids tested, the weakest toxic effect on *D. reticulatus* and *I. ricinus* was exerted by permethrin, which contains a 3-phenoxybenzyl moiety in the alcohol residue and chlorine atoms in the acyl group. The enhanced acaricidal activity of deltamethrin was obtained by substitution of the cyano group in the 3-phenoxybenzyl moiety. Besides their structure, the geometry and spatial configuration of molecules are responsible for the toxicity of synthetic pyrethroids. Pyrethroids keep sodium channels open, which results in constant Na+ influx into the neuron leading to disturbed conduction of stimuli and paralysis [17]. Similar to the presented study conducted on adult stages of *D. reticulatus* and *I. ricinus*, alphacypermethrin proved to be a highly toxic pyrethroid in maturation and egg development stages in these species [5, Buczek et al. unpublished data]. Cypermethrin inhibited egg development in *Amblyomma hebraeum* [18], and cis-cypermethrin, cypermethrin, and deltamethrin caused inhibition of larval hatch in *Rhipicephalus sanguineus* [19]. Permethrin induced changes in oocytes in semi-engorged *Rhipicephalus sanguineus* females [20].

Determination of a dose of acaricides that will be effective in the control of adult stages of *D. reticulatus* and *I. ricinus* has practical importance, particularly in areas with sympatric distribution thereof. Although they are allopatric, both these tick species can inhabit the same habitats in some regions (own observation). Precise determination of an effective dose for tick populations is also important due to development of tick resistance to chemical compounds, which is influenced by the duration and frequency of application of pyrethroids for tick control in a given area, as well as the dose of the acaricide used [21, 22]. In recent years, increased resistance to pyrethroids has been reported in some tick populations [23, 24]. In Poland, no chemical methods for tick population control in natural conditions have been employed to date. Successful tick control in a given area can be achieved by devising a strategy based on investigations of sensitivity of tick populations to acaricides applied in different doses and on knowledge of the activity and developmental cycle of various tick species.

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