ORIGINAL ARTICLE

The toxic effect of permethrin and cypermethrin on engorged *Ixodes ricinus* females

Alicja Buczek[1], Katarzyna Bartosik[1], Paweł Kuczyński[1,2]

[1] Chair and Department of Biology and Parasitology, Medical University, Lublin, Poland
[2] Chair and Department of Rehabilitation and Orthopaedics, Medical University, Lublin, Poland

▌ Abstract

Introduction. *Ixodes ricinus* tick is of great medical and veterinary importance and has a wide range of geographical distribution. The study presents the effect of permethrin (Per) and cypermethrin (CM) on engorged *I. ricinus* females.
Materials and method. The effect of perythroids studied on engorged *I. ricinus* females was assessed on the basis of the pre-oviposition and oviposition period. Remote effects of Per and CM application were assessed by investigation of the length and course of embryonic development and larval hatching from eggs laid by pyrethroid-treated females. Per (Copex WP) was used at doses of 0.78125–25.0 µg/1 specimen, and CM (Kordon 10WP) was applied at 0.3125–10.0 µg/1 specimen. Immediately after the feeding period, *I. ricinus* females were sprayed with 20 µl of a pyrethroid solution and kept at 28 °C and 75%RH.
Results. The experiments demonstrated that CM exerted a stronger toxic effect on *I. ricinus* females than Per. The lowest doses of CM doubled the length of the pre-oviposition period while its highest doses prolonged the period nearly three times compared with the control. The pyrethroids applied reduced the number and weight of eggs and changed the parameters of the oviposition process. Application of the tested pyrethroid doses led to disturbances in the embryonic development of *I. ricinus*, i.e. the development was prolonged, few normal larvae hatched, numerous eggs and embryos at various developmental stages died, and larval hatch was inhibited.
Conclusions. Knowledge about the sensitivity of engorged females to different doses of the tested pyrethroids and the remote effects of their action can be used in practice for tick control among livestock animals, and the reduction of tick population abundance in the environment.

▌ Key words

Ixodes ricinus, permethrin, cypermethrin, acaricide, tick reproduction, egg development

INTRODUCTION

Ticks cause mechanical damage to host skin with their mouth organs, inflammatory lesions at the site of introduction of salivary secretion, and systemic reactions induced by saliva components. They also transmit a variety of bacterial, rickettsial, viral, and protozoan diseases in humans and animals. *Ixodes ricinus* has a predominant epidemiological importance in Europe and is also the most common species in its distribution range.

Tick infestations can be prevented in various ways, i.e. using personal protection agents (repellents) or by applying chemical [1, 2, 3] and, less frequently, biological methods for reduction of tick populations in the environment. Chemical tick control relies on chemical substances with a different structure, activity, and toxicity. Given their positive effects and toxicological parameters, synthetic pyrethroids have been introduced into practical use.

Based on their chemical structure and activity, pyrethroids have been divided into two groups; substances from the first group contain a-cyano-3-phenoxybenzyl alcohol or other alcohols in their molecule and affect primarily the cell membrane of peripheral parts of the axon. Substances classified into the second group contain cyjano-3-phenoxybenzyl alcohol and depolarise the cell membrane in the central part of the axon. Pyrethroids from the first group cause hyperactivity and convulsions in arthropods, while those from the second group disturb motor coordination. Pyrethroids are neurotoxins whose high lipophilicity facilitates distribution in the organism. The action of pyrethroid changes ion fluxes in the sodium channel, which results in a constant influx of sodium ions into the cell and disturbances in conduction of stimuli. The Na+ ion influx into the neutron near the presynaptic membrane of the motor end-plate causes release of glutamate, i.e. an excitatory neurotransmitter. Binding of glutamate with the receptor opens Na+ and Ca2+ channels and causes influx of these ions into the muscle cell and, consequently, generation of an excitatory postsynaptic potential. By changing the level of calcium, pyrethroids disturb calcium homeostasis in nervous cells. Additionally, they inhibit the activity of pyrophosphatase, Ca2+- and Mg2+-dependent ATPases, phosphodiesterase, and adenyl cyclase [4, 5].

The current study presents the effect of two pyrethroids, i.e. permethrin and cypermethrin, on engorged *I. ricinus* females from a Polish population and their doses that reduce the numbers of tick offspring.

Address for correspondence: Alicja Buczek, Chair and Department of Biology and Parasitology, Medical University, Lublin, Poland
e-mail: alicja.buczek@umlub.pl

260
Annals of Agricultural and Environmental Medicine 2014, Vol 21, No 2
Alicja Buczek, Katarzyna Bartosik, Paweł Kuczyński. The toxic effect of permethrin and cypermethrin on engorged *Ixodes ricinus* females

MATERIALS AND METHOD

Adult *I. ricinus* stages used in the experiments were collected by the flagging method near Lubycza Królewska (23°31'E 50°20'N) in the Lublin province of south-eastern Poland. Before the experiments, the ticks were kept in rearing chambers at 5 °C and ca. 90–100% humidity. In order to obtain engorged specimens, 15 females and 5 males of *I. ricinus* were placed on each host – albino New Zealand rabbits (*Oryctolagus cuniculus*). The course of feeding was assessed daily at the same time and engorged, detached specimens were collected. The experiments were carried out at room temperature ca. 20 °C and 50% humidity. Engorged females collected from the hosts were weighed with an accuracy of 0.01 mg using a WPA 120/C/1 analytical laboratory digital balance (Radwag, Poland). Next, each female was placed on filter paper and transferred to individual rearing chambers. The dorsal side of the body was sprayed with 20 µl of the tested pyrethroid using a 0.2–50 µl micropipette with an accuracy of ± 0.5–2% (Labsystems O.G., Helsinki, Finland). Throughout the egg development and oviposition periods, the females remained in the dark rearing chambers at a temperature of 28 °C and 75% relative humidity, which are favourable conditions for the development of the tick in the laboratory. Each experiment was observed every day under a Zeiss stereoscopic microscope. After the oviposition period, females and eggs laid by each female were weighed. After weighing, the eggs were kept in chambers until larval hatching. Assessment of the course of embryonic development of *I. ricinus* was performed in a similar way as in the investigations of *Dermacentor reticulatus* [6]. The same procedures as in pyrethroid testing were employed in the control experiments, but ticks in this group were sprayed with 20 µl of distilled water only.

Parameters characterising the effect of the different doses of the tested pyrethroids on the development of *I. ricinus* eggs and larvae were determined in each experimental group. They included the pre-oviposition period (PP) – the period between the end of feeding and the onset of oviposition (in days); egg lying frequency (ELF) – the number of engorged females capable of laying eggs in the individual experimental group (in %); female post-oviposition weight (FPW) – weight of female body after completion of oviposition (in grams); female oviposition weight loss (FOWL) – an indicator of the percentage of loss of female body weight during the oviposition period (in %) calculated according to the formula FOWL (%)=FEW – FPW/FEW × 100, where: FEW – female engorged weight; FPW – female post-oviposition weight; EMW – egg mass weight = total weight of eggs laid by one female (in milligrams); ECF – egg conversion factor = weight of engorged female utilised for production of eggs [7]; EA – egg amount = total number of eggs laid by a female; hatching frequency; HF – percentage of egg batches in the tested group with at least one hatched larva (in %); EP – embryogenesis period = the period between onset of oviposition and hatching of the first larva (in days), and hatching success; HS – the proportion of larvae hatched from eggs laid by one female (in %).

Embryonic development was assessed based on the number of dead eggs, dead embryos, larvae with hatching disturbances, and normal larvae. The stages in which embryonic death occurred were determined. In embryogenesis stage I (onset of the cleavage stage), the eggs were matt and exhibited granularities. In embryogenesis stage II (blastoderm formation and organogenesis), changes in the structure of the embryo caused by cell divisions and shifts of embryonic material were visible through egg casings, whereas in stage III, elements of the gnathosoma and idiosoma as well as walking legs were visible. Larvae with hatching disturbances had walking legs trapped in egg casings, which impaired their motor skills. Morphological assessment was performed under a Zeiss stereoscopic microscope.

Tested acaricides. The activity of two synthetic pyrethroids, i.e. permethrin (Copex WP, 25% active ingredient (AgrEvo Environmental Health Ltd., Cambridge, UK) and cypermethrin (Kordon 10WP, 10% active ingredient, isomers 40/60 cis: trans (AgrEvo, UK) was tested. The pyrethroid doses used in the experiments were obtained by serial dilution of the preparations. 2 µl of the 0.015625%, 0.03125%, 0.0625%, 0.125%, 0.25%, and 0.5% acaricide solutions contain, respectively; 0.78125; 1.5625; 3.125; 6.25; 12.5, and 25.0 µg of permethrin and 0.3125; 0.625; 1.25; 2.5; 5.0, and 10.0 µg of cypermethrin.

Statistical analysis. Calculations and graphs were generated using the STATISTICA 5 PL and Microsoft Excel XP programmes. The significance of the differences between the parameters of the non-parasitic stage of the developmental cycle in the control and experimental groups, resulting from application of the chemical substance at the specified pyrethroid dose, was analysed with the Mann-Whitney U test. The Kruskal-Wallis H test was used for verification of the hypothesis concerning equality of the parameters for the individual acaricide doses. Probability at p ≤ 0.05 was regarded as significant and as highly significant at p ≤ 0.01.

RESULTS AND DISCUSSION

Engorged *I. ricinus* females laid eggs after application of 0.78125–3.125 µg permethrin and 0.3125–5.0 µg cypermethrin per each specimen. Together with increasing pyrethroid doses, the proportion of females capable of developing and laying eggs decreased (Tab. 1). After application of a single 5.0-µg dose of cypermethrin per each tick (0.25% preparation solution), the number of ovipositing females decreased to 14.3%, and to 42.9% at 3.125 µg of permethrin (0.0625% solution; control 100%). The number and weight of eggs laid by females decreased statistically significantly after application of both pyrethroids, which reduced the egg conversion factor and increased the female oviposition weight loss (Tab. 1, 2). Cypermethrin inhibited egg development, hence; compared with the control, the preoviposition period was two-fold longer (16.33±5.391 days) after application of its lowest doses and almost three-fold longer (24.0±0 days) at the highest doses (Tab. 2). The H test (p=0.0002) confirmed the statistically significant differences in the length of the preoviposition period in females treated with different cypermethrin doses. A similar effect was exerted by permethrin, but statistically significant differences were found after treatment of engorged females with 1.5625–3.125 µg of the compound per specimen (Tab. 2). Application of the different doses of this pyrethroid caused statistically significant differences in the course of oogenesis (test H; p=0.0009), which was reflected by prolonged oviposition at increasing doses.

Annals of Agricultural and Environmental Medicine 2014, Vol 21, No 2

Alicja Buczek, Katarzyna Bartosik, Paweł Kuczyński. The toxic effect of permethrin and cypermethrin on engorged *Ixodes ricinus* females

261

Table 1. Average values of parameters of oviposition and embryonic development in *I. ricinus* under the influence of different concentration of permethrin and cypermethrin at temp. 28°C and 75% RH

Chemical	Concentration (%)	ELF[1] (%)	EA[2]	HF[3] (%)	EP[4] (days)	HS[5] (%)
Permethrin N=43	0.01562	71.4	1207	100	34	5.79
	0.03125	71.4	1130	100	34.8	0.0
	0.0625	42.9	933	100	38.3	0.0
	0.125	0.0	-	0	-	-
	0.25	0.0	-	0	-	-
	0.5	0.0	-	0	-	-
	1.0	0.0	-	0	-	-
Cypermethrin N=45	0.01562	85.7	1254	100	34.3	0.0
	0.03125	71.4	1274	100	36	0.0
	0.0625	28.5	1218	100	33	-
	0.125	28.5	630	100	30.5	-
	0.25	14.3	-	0	-	-
	0.5	0.0	-	0	-	-
	1.0	0.0	-	0	-	-
Control N=50		100	2615	100	29.5	89.64

[1] egg lying frequency – the number of engorged females capable of laying eggs in the individual experimental group (in %)
[2] female postoviposition weight – egg amount
[3] hatching frequency – percentage of egg batches in the tested group with at least one hatched larva
[4] embryogenesis period – period between beginning of oviposition to hatching of the first larva
[5] hatching success determines the proportion of laid eggs, from which larvae hatched
N – number of ticks used in the experiment

Disturbed egg development induced by pyrethroids was also found in *D. reticulatus*, but in this species, cypermethrin applied at similar doses as in *I. ricinus* caused a five-fold increase in the length of the preoviposition period, compared with the control [6]. Comparison of both results reveals differences in the effects of the same pyrethroids and the same doses on the different tick species. Although similar trends in the action of the pyrethroids were found, the results of both the presented investigations suggest a need to adjust the doses to tick species in the chemical control. The effect produced by the same chemical substances may

also be different in different tick populations [8, 9, 10], probably due to development of resistance or existence of some physiological differences between populations of the same species (own unpublished observations). The toxic effect of cypermethrin and permethrin during oogenesis was confirmed in a study on argasid ticks [11, 12] and ixodid ticks [13, 14]. Yet, the mechanisms of the action of pyrethroids during maturation and development of eggs in various tick species are still little known. By their effect on the nervous system, pyrethroids can affect secretion of hormones regulating the course of oogenesis in ticks. In oocytes of semi-engorged *Rhipicephalus sanguineus* females, permethrin induced morphological changes, such as vacuolisation of the cytoplasm, reduced the amount of yolk, and decreased the size of oocytes, which lead to cell death. Development of ovaries in engorged adult stages of *Ornithodoros moubata* and release of 20-hydroxyecdysone, i.e. a vitellogenesis hormone, were also inhibited by cypermethrin [12]. Additionally, this pyrethroid disturbed development of oocytes in *Amblyomma hebraeum* [13]. Tick development and reproduction is also inhibited by the toxic effect of another acaricide – fipronil [15, 16].

I. ricinus larval hatching was observed in all egg batches oviposited by females after application of 0.3125–2.5 µg of cypermethrin (0.015626% – 0.125% solutions of the preparation) and 0.78125–3.125 µg of permethrin per each specimen (0.015625–0.0625% solutions). In comparison with *D. reticulatus* [6], hatching frequency in *I. ricinus* was higher at the same doses of cypermethrin. After application of the pyrethroids, the length of the embryonic development period in *I. ricinus* changed in comparison with the control (Tab. 1), which implies remote effects of permethrin and cypermethrin in ticks. This effect can significantly reduce the abundance of tick populations in the environment. Statistically significant differences were found between groups treated with different doses of the active substance. The course of embryonic development was also disturbed; hence, normal larvae hatched only after application of lower doses of tested pyrethroids. The largest number of eggs with inhibited development was found at the onset of embryogenesis. 0.01% cis-cypermethrin, 0.015%

Table 2. Parameters of eggs maturation and oviposition course in *I. ricinus* females under the influence of different concentration of permethrin and cypermethrin at temp. 28°C and 75% RH

Chemical	Concentration (%)	Preoviposition (days)			FPW[1] (g)			FOWL[2] (%)			EMW[3] (mg)			ECF[4]		
		M	SD	p	M	SD	p	M	SD	p	M	SD	p	M	SD	p
Permethrin N=43	0.01562	12.8	6.340	0.3961	0.125	0.024	0.2782	55.42	10.65	0.0184	0.091	0.022	0.0004	0.321	0.107	0.0015
	0.03125	15	2.739	0.0004	0.104	0.035	0.5716	66.90	8.05	0.8875	0.083	0.020	0.0004	0.271	0.061	0.0006
	0.0625	15.3	3.05	0.0048	0.119	0.021	0.5730	66.11	3.31	0.4155	0.078	0.018	0.0048	0.219	0.023	0.0048
Cypermethrin N=45	0.01562	16.3	5.39	0.0022	0.094	0.028	0.1612	9.41	9.41	0.1743	0.078	0.030	0.0002	0.314	0.173	0.0074
	0.03125	18.4	9.58	0.0088	0.112	0.011	0.9436	5.90	5.90	0.3961	0.100	0.018	0.0006	0.312	0.064	0.0013
	0.0625	19.5	3.53	0.0195	0.121	0.010	0.5334	5.26	5.26	0.0195	0.065	0.020	0.0165	0.172	0.012	0.7554
	0.125	22.5	0.70	0.0195	0.167	0.028	0.0292	21.37	21.37	0.0516	0.036	0.017	0.0195	0.120	0.082	0.0195
	0.250	24	0.00	1.0000	-	-	-	-	-	-	-	-	-	-	-	-
Control N=50		8.4	1.19	x	0.115	0.020	x	67.11	6.36	x	0.187	0.031	x	0.537	0.109	x

[1] female post-oviposition weight – weight of female body after oviposition
[2] female oviposition weight loss – an index of the percentage of weight loss during oviposition calculated as the ratio of the difference between engorged female weight and post-oviposition weight/engorged female weight
[3] egg mass weight – the total weight of eggs laid by a female
[4] egg conversion factor – engorged female weight used for egg production
N – number of ticks used in the experiment

262

Annals of Agricultural and Environmental Medicine 2014, Vol 21, No 2

Alicja Buczek, Katarzyna Bartosik, Paweł Kuczyński. The toxic effect of permethrin and cypermethrin on engorged *Ixodes ricinus* females

cypermethrin, and 0.0025% deltamethrin inhibited larval hatch in *Rhipicephalus sanguineus* in 72.1, 67.3, and 42.0%, respectively [17].

CONCLUSIONS

In *I. ricinus*, the tested acaricides did not induce teratological changes as those induced by various chemical substances in argasid and ixodid ticks [6, 18, 19, 20]. Morphological anomalies such as oligomely (lack of legs), symely (change of location of the structure), schistomely (fusion of legs), and deformations of the body were caused by deltamethrin in *D. reticulatus* [6].

In many regions of the world, some tick populations have developed resistance to chemical compounds applied in tick control [9, 21, 22, 23, 24]. This fact highlights the need for deliberate action taking into account appropriate preparations and doses against individual species that, as shown in the presented on *I. ricinus* and *D. reticulatus*, may exhibit varied sensitivity to chemical substances.

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