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Article

Susceptibility level of *Ornithodoros tholozani* (Acari: Argasidae) to some pesticides in north west of Iran

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Abstract

The cave tick, *Ornithodoros tholozani* is the most important soft tick in central Asia and the Middle East. The populations of this species live in human buildings and animal shelters and are responsible for transmission of relapsing fever in different areas. Residual spraying of pesticides is a common control measure against this species, and vast regions of north west of Iran are sprayed annually with pyrethroids by the local residents. Application of limited range of acaricides and similarity in their mode of actions might contribute to emergenence of resistance, and decline of their efficacy. Thus, this research was conducted to identify the susceptibility level of different populations of this tick to some pesticides in old, well-known foci of relapsing fever. Populations of this tick were collected from animal shelters of Mahneshan, Khodabandeh, Tekab and Bijar districts in north west of Iran during 2012–2013. Adult ticks were exposed to different doses of permethrin, lambdacyhalothrin and carbaryl through topical and residual methods and mortality rate was measured after 24 hours. The bioassay revealed different levels of tolerance to permethrin in various populations of *O. tholozani* in the study area. The Bijar strain was susceptible and the LD_{50} and LC_{50} values for permethrin were 48.38 ng/tick and 96.98 $\mu g/cm^2$ respectively. Tekab, Khodabandeh and Mahneshan populations were resistant and resistance value in this group was 2. The Bijar strain was also susceptible to lambdacyhalothrin and LD_{50} value for this compound was 8.37 ng/tick. In spite of the susceptibility to pyrethroids in Bijar strain, there was high resistance level to carbaryl and the LD_{50} value was 5.45 mg/tick. Since different levels of resistance to permethrin were observed in various populations of *O. tholozani*, reviewing and refining the control measures against ticks, applying new formulations of acaricide mixtures and monitoring the susceptibility level of acaricides seem necessary in these regions. Moreover, future studies to identify the molecular characteristics of resistance to acaricides and determining the effective acaricide are highly recommended in these areas.

Key words: Soft ticks, *Ornithodoros tholozani,* permethrin, lambdacyhalothrin, carbaryl, Bioassay.

Introduction

The cave tick, *Ornithodoros tholozani* Laboulbène & Mégnin, 1882 (Acari: Argasidae) is one of the most important soft tick species in central Asia and the Middle East (Cunha 2000; Sonenshine 2005; Rahbari *et al*. 2007). It is responsible for transmission of relapsing fever, *Borrelia persica*, and is a nidicolous species whose populations live in or in the vicinity of human settlements and animal shelters inhabiting all kinds of crevices and cracks (Cunha 2000; Sonenshine 2005; Cutler 2010).

North west of Iran particularly Zanjan, west Azerbaijan, and Kurdistan Provinces are the most significant infested areas with *O. tholozani* and over 75% of reported cases of Tick Borne Relapsing Fever are from these regions (Ghavami *et al*. 2002; Banafshi *et al*. 2003; Aghighi *et al*. 2007; Mahram & Ghavami 2009; Masoumi Asl *et al*. 2009; Moemenbellah-Fard *et al*. 2009; Barmaki *et al*. 2010).

Several studies have been carried out on biology, infection rate and behavior of *O. tholozani* in different areas of the world (Arshi *et al*. 2002; Vatandoost *et al*. 2003; Salarilak *et al*. 2008; Assous & Wilamowski 2009; Safdie *et al*. 2010; Rafinejad *et al*. 2011). However, the studies concerning susceptibility level of this species are few and restricted to last decades (Uspenskiy 1982). In the north west of Iran indoor residual spraying of pesticides has been one of the most important control measures against this species and other significant livestock ticks which is performed routinely every year (Vatandoost *et al*. 2012). On the other hand, there has been a history of organochlorinate insecticides application such as DDT in these regions which was part of malaria eradication plane during previous years.

Over the recent years, pyrethroids have been widely used throughout the world due to high toxicity to vectors, low mammalian toxicity and negligible residues (Quelennec 1988; Khamby & Rothamsted 2010). However, similarity in mode of action of pyrethroids to organochlorinate pesticides, their low diversity and persistent usage, could diminish their efficacy and develop resistance in ticks (Georghiou 1986; Miller 1988; WHO 1992; Sangster 2001; Soderlund 2008).

Permethrin has been proven to be effective against various arthropod vectors including soft and hard ticks (WHO 1990; Burridge *et al*. 2003; WHO 2006; Nodari *et al*. 2012; Roma *et al*. 2009, 2012). It demonstrates a neurotoxic effect by binding to the membranous proteins of sodium channels and inhibiting the deactivation and thus, stabilizing the open configuration of the channels (Dong 2007). Structural changes in sodium channels due to mutation and metabolic detoxification can reduce the sensitivity of ticks to pyrethroids (Hernandez *et al*. 2000; Miller *et al.* 2007; Morgan *et al*. 2009; Domingues *et al*. 2012; Kumar *et al.* 2012). Therefore, determination of resistance status of tick population to permethrin could clearly express resistance in next generation of pyrethroids. Moreover, Carbaryl is another suitable pesticide for indoor residual treatment (WHO 2006). However, the mode of action of this compound differs from pyrethroids and application of its mixture with pyrethroids could affect the management of resistant vectors.

Regarding the significance of pyrethroid application to control *O. tholozani* and other livestock ticks, persistent use of pesticides, cross reaction of pyrethroids with organochlorinates, and prolonged longevity of soft ticks, it seems necessary to investigate the susceptibility level of the cave tick populations to permethrin, lambdacyhalothrin and carbaryl in the most significant known focal points of Tick Borne Relapsing Fever in Iran.

Material & methods

This study was conducted from March 2012 to November 2013 in Zanjan, West Azarbayjan and Kordestan Provinces of Iran. These Provinces are located in north west of Iran, at latitudes, longitudes and altitudes of 35°15′–36° 59′ N, 46° 46′- 48° 59′ E and 1450–2050 m a.s.l., respectively. Annual ambient temperature and rainfall of the study areas were in the range of −28.6 °C through 43 °C and 252–642 mm, respectively.

Tick collection & maintenance

Ticks were collected using the standard method described by World Health Organization, Food and Agriculture Organization (WHO 1964, 1992; FAO 2004). The following equipment was used for tick collection: flash light, forceps, thermometer & hygrometer, overalls, test tubes, boots, latex gloves, disposable masks, labels, marker, cell culture flasks, awl, screw driver, tray and 10X magnifying glass. Ticks were handpicked under close scrutiny from all kinds of surfaces including cervices and cracks on the walls of human dwellings, animal shelters and indoor caves.

The collected ticks were transferred into Petri dishes and counted. The frequency of each species was determined following identification of the species through morphological characters and the standard key (Nuttal *et al*. 1908).

The identified specimens of *O. tholozani* collected from particular areas were designated as strains and kept at 28 °C , 70% RH and photoperiod of 12:12 (L:D) hours in growth chamber. The ticks were fed on neonate mice and engorged adults were used in bioassay survey.

Chemical compounds

Technical grade of permethrin (India) with 96% active ingredient, 42:58 cis:trans ratio; carbaryl (Carbaryl 80WP, Mainland China), and lambdacyhalothrin (Icon 12 WP, England) were used for this study.

Preparation of solutions

Stock solutions of acaricides were made by dissolving selected compounds in acetone (propanone). The top concentration was provided by adding a volume of the stock solution to acetone (for topical application) and a mixture of acetone and olive oil with a final ratio of 3:1 (V/V) (for paper impregnation) was prepared. Serial dilutions from the top concentration were made using acetone and dilution of olive oil in acetone.

A 0.5 ml volume (at least 30 drops) of each dilution was pipetted to Watman filter papers of 8 cm diameter, fitted in plastic Petri dish and was allowed to dry in room temperature. Control groups were also treated with mixture of acetone and olive oil.

Bioassay

Five to seven graded concentrations of each insecticide leading to > 5% and < 100% mortality were used. In residual method, five engorged adult ticks were released to each impregnated Petri dish and six replicates were run for each applied concentration.

Topical application was conducted by micro applicator equipped with a 25 µl ILS glass micro-syringe fitted with a 31G needle. A 0.5µl of solution was applied to the dorsum of engorged ticks and treated ticks were kept in Petri dish. Twenty five to 36 ticks were subjected to each concentration.

Treated specimens were kept in an incubator at 28 °C, 70% RH and the number of dead ticks in each group was recorded after 24 hours. Death of ticks was assessed by stimulation independent motion assay. The ticks in each Petri dish were examined under stereo-microscope with magnification of 40X. If the legs were extended and then retracted tightly or moved back and forth independently, the tick was considered alive. If the legs were not extended or extended under haemostatic pressure, but not retracted fully, the tick was considered dead.

Data analysis

Probit analysis of mortality and doses data was performed using Priprobit 1996–2000, Masayuki SAKUMA Version 1.63 software. Differences between study groups were assessed by comparison of point estimation of toxicity values (in 95% confidence interval), slopes and intercepts on different regression lines. The resistance factor (RF) was calculated by dividing the LC_{50} (μg/cm^2) or LD_{50} (*n*g /tick) of a particular tick strain by the LD_{50} values of susceptible strain.

Results

A total of 5076 soft ticks were collected with *O. tholozani* comprising 30% of the species. The species of soft ticks constituted *O. tholozani*; *O. lahorensis* Neumann, 1908; *O. canestrinii* (Birula) and *Argas persicus* (Oken). Out of 1880 collected *O. tholozani*, 1610 specimens were assayed by acaricides.

Data analysis showed that the field populations of *O. tholozani* in the study area were heterogeneous in response to permethrin (Fig. 1).The strains of Khodabandeh, Mahneshan and Tekab showed similar response with no significant difference in either slope or intercept of dose-mortality regression lines. However, slope and intercept of Bijar strain differed from those strains.

In topical application, LD_{50} values of the susceptible (Bijar) strain and tolerant (Khodabandeh, Mahneshan & Tekab) strains were determined as 48.38 and 97.87 *n*g/ tick, respectively. While the LD_{90} values for these two groups were 187.1 and 697.6 *n*g/ tick, respectively (Table 1 & Fig. 2A), in tolerant group the value of RF was 2.

The data on LC_{50} and LC_{90} values in the susceptible and tolerant strains taken from exposure of specimens to impregnated papers with permethrin are shown in Table 2 and Fig. 2B. Similar to topical application, all tolerant populations had 2 fold resistance in residual method compared to the susceptible strain.

Probit analysis of regression lines indicated that tolerant populations exposed through both methods, responded similarly and regression line in two application methods were parallel. In spite of equal slope in regression lines of tolerant populations, the response of susceptible strain differed in two treated methods (Fig. 2). The regression lines of susceptible and tolerant populations are neither equal nor parallel.

The Bijar strain was also susceptible to lambdacyhalothrin in topical application method and the LD_{50} and LD_{90} values for this compound were 8.37 and 28.4 *ng*/tick, respectively (Table 2, Fig. 3). In spite of the susceptibility to pyrethroids in Bijar strain, there was high resistance level to carbaryl in this strain and the LD_{50} and LD_{90} values were 5.45 and 12.63 *mg*/tick, respectively (Table 2, Fig. 3).

Discussion

Selection for resistance in tick populations is a major consequence of extensive application of pesticides and a major threat to the efficacy of control programs against tick borne diseases. The present study revealed different levels of susceptibility to permethrin in various populations of *O. tholozani* in study areas. Resistance rate in tolerant populations was about two times higher than that of susceptible strain. The results of this study showed that permethrin and lambdacyhalothrin have higher toxicity than carbaryl, whereas LD_{50} and LD_{90} values of carbaryl were approximately 100 times higher than those acaricides.

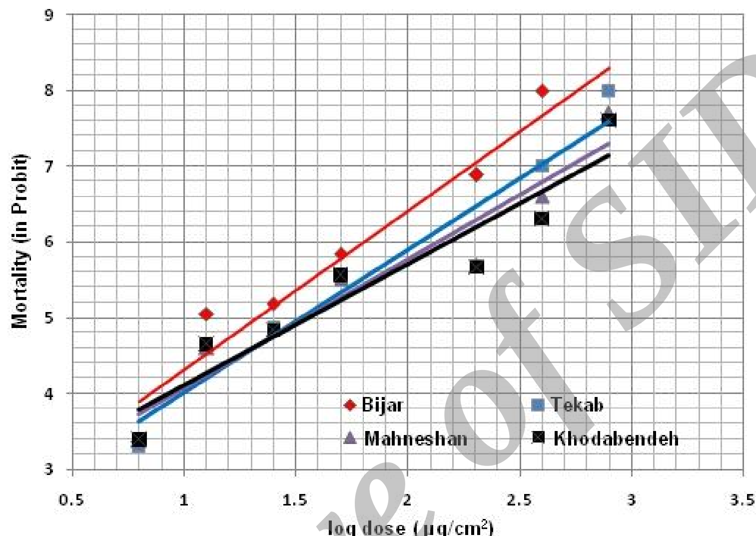

Figure 1. Toxocity of permethrin in residual method on different populations of *Ornithodoros tholozani* in north west of Iran.

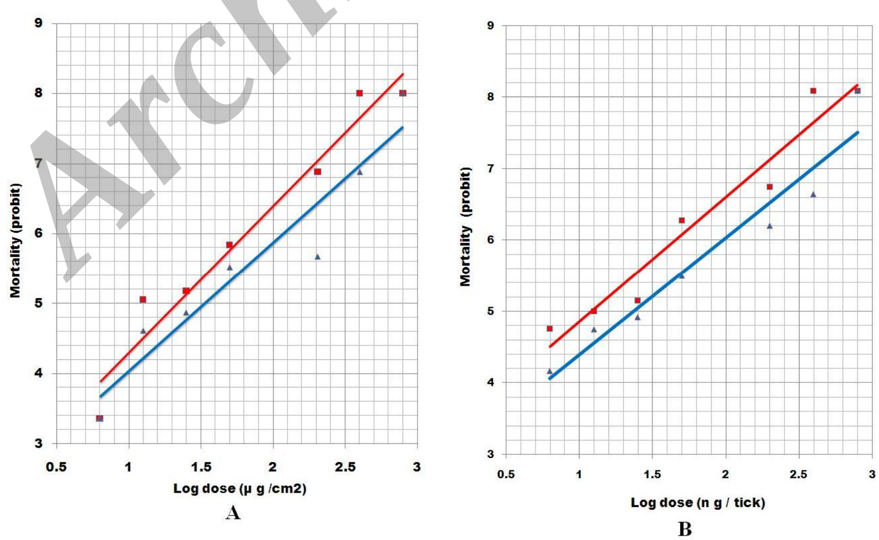

Figure 2. Effect of different doses of permethrin in residual (A) and topical (B) application methods on the susceptible ( ) and tolerant ( ) populations of *Ornithodoros tholozani* in north west of Iran.

Table 1. Results of bioassay through different treated methods of permethrin in two population of *Ornithodoros tholozani*

Study groups	Exposure method	a[1]	b[2]±SE	X^2(df)	*p*-value[3]	n	LD_{50} (95% CI)[4]	LD_{90} (95% CI)
Susceptible[5]	topical	−2.73	2.18 ± 0.28	2.54(4)	0.05	210	48.38 (38.18–60.44)*	187.1 (141.9–276.8)*
	residual	−2.12	1.36 ± 0.26	2.41(5)	0.05	240	96.98 (34.21–217.4)*	838.8 (342.8–6545.3)
Tolerant[6]	topical	−2.34	1.50 ± 0.23	2.41(5)	0.05	205	97.82 (52.02–174.28)*	697.6 (390.7–1842.5)*
	residual	−2.3	1.18 ± 0.19	3.50(5)	0.04	260	240 (107–487.8)*	2926.3 (2132.2–51048.9)*

[1] a = intercept of regression line, [2] b = slope of regression line and its standard error, [3]*p*-value = probability for heterogeneity, [4] CI = confidence interval, [5]Susceptible = Bijar strain, [6] Tolerant = Khodabandeh, Mahneshan & Tekab strains, *= target dose in ng/tick, *= exposure dose in µg/cm

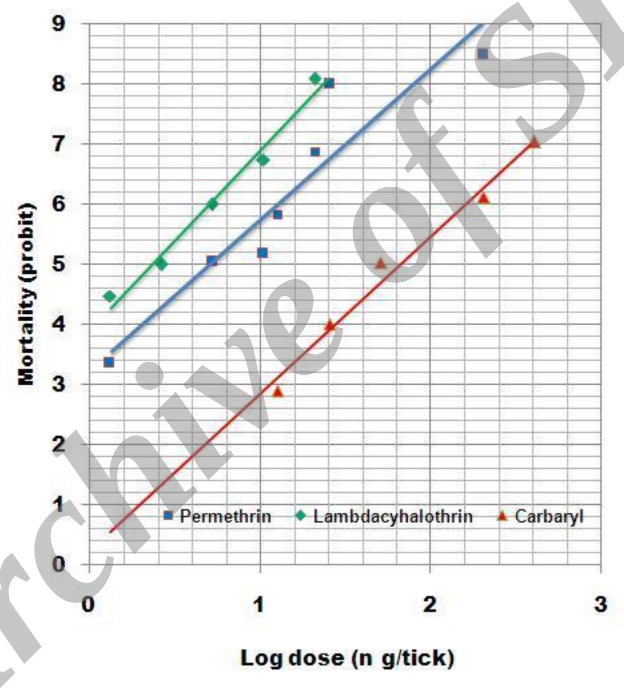

Figure 3. Probit regression lines of lambdacyhalothrin, permethrin and carbaryl in topical method on the Bijar strain of *Ornithodoros tholozani*.

Table 2. Toxicity parameters of topical application of permethrin, lambdacyhalothrin and carbaryl in susceptible (Bijar) strain of *Ornithodoros tholozani*

Pesticide	a	b ± SE	χ^2 (df)	*p*-value	n	LD_{50} (95 % CI) (*ng*/tick)	LD_{90} (95 % CI) (*ng*/tick)
lambdacyhalothrin	−1.18	2.41 ± 0.45	2.55 (4)	0.05	180	8.37 (4.62–14.68)	28.4 (15.8–125.6)
permethrin	−2.73	2.18 ± 0.28	2.55 (4)	0.05	210	48.38 (38.18–60.44)	187.1 (141.9–276.8)
carbaryl	−11.61	3.51 ± 0.46	2.65 (3)	0.05	150	5455 (4523.74–6573.74)	12635.2 (10296.9–16853.7)

Studies on the susceptibility of *O. tholozani* to acaricides are restricted and some of which are outdated (Uspenskiy 1982). This study describes a detailed protocol to determine the susceptibility level of *O. tholozani* populations to acaricides for the first time. It can be used for resistance monitoring of the soft tick populations in future studies.

Despite the restricted studies on the susceptibility of this tick, there are several reports on other species of ticks (Khalaj *et al*. 2005; Klafke *et al*. 2006; Jonsson *et al*. 2007; Li *et al*. 2007; Telmadarraiy *et al*. 2007; Roma *et al*. 2009; Hunter *et al*. 2011; Kumar *et al*. 2012). The efficacy of permethrin and carbaryl for the control of ticks has been proved in earlier studies (Gladney *et al*. 1972; Gladney & Dawkins 1976; Koch & Burkwhat 1984; Miller *et al*. 2001; Buridge *et al*. 2003). The LC_{50} values of permethrin in previous studies for susceptible populations ranged between 0.1–10 $\mu g/cm^2$. However, it varied greatly in resistant populations and even reached 3294 $\mu g/cm^2$ in *Rhipicephalus microplus* (Li *et al*. 2007).

Our study confirmed the difference in responses of Bijar population exposed to permethrin in different application methods. This difference may be related to ticks alternative behavior and future studies could confirm this assumption.

The results of our study also showed that susceptible and tolerant populations have different responses when exposed to permethrin. This outcome may mean that detoxification enzymes differ quantitatively and qualitatively in tolerant population. Understanding of biochemical basis and physiological process of detoxification in tolerant population would be an appealing area for future toxicological and biochemical research.

Both the sodium channel mutation and metabolic detoxification mechanisms are known to be responsible for pyrethroids resistance. Based on RF, more populations of *O. tholozani* in study areas are classified as tolerant or level I-II resistant (RF = 1.5–5). It seems that metabolic detoxification plays a major role in this phenomenon. Therefore, the results of future synergist bioassays could suggest the best solution to this issue.

Different strategies including rotation and mixture of acaricides have been reported for resistance management (Curtis 1985; Georghio 1986). However, acaricide rotation may no longer be a good option. Regarding synergism between pyrethroid and other groups of acaricides (such as Amitraz) found in previous studies (Dusbábek *et al*. 1997; Hunter *et al*. 2001; Vincent *et al*. 2006; Li *et al*. 2007; Phyu *et al*. 2011), new formulations of pesticides mixture could be used in future. However, the ratio of each compound in the mixture has to be further tested on the basis of field efficacy trials.

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سطح حساسیت *Ornithodoros tholozani* (Acari: Argasidae) به آفت‌کش‌ها در شمال غرب ایران

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چکیده

کنه اورنیتودوروس تولوزانی، مهم‌ترین گونهٔ کنهٔ نرم است که ناقل و مخزن بیماری تب‌های بازگرد کنه‌ای در مناطق آسیای مرکزی و خاور میانه است. در مطالعه‌ای که در سال‌های۲۰۱۲ و ۲۰۱۳ انجام یافت سطح حساسیت جمعیت‌های مختلف این کنه در مناطق مختلف شمال غرب ایران با ترکیبات پرمترین، لامبداسیالوترین و کارباریل مورد ارزیابی قرار گرفت و مشخص شد که در مناطق گسترده‌ای جمعیت‌های این گونه به پرمترین و کارباریل متحمل هستند.

واژگان کلیدی: کنه‌های نرم، *Ornithodoros tholozani* ، زیست‌سنجی، پرمترین، لامبداسیالوترین، کارباریل.

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