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Insecticide efficacy on ticks (*Dermacentor spp.*) – Case study from an infested territory in Transylvania, Romania

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Abstract: Ticks can be a major concern for humans and animals alike through the transmission of various viral and bacterial diseases. Ticks have also developed tolerance to several active compounds due to intensive insecticide treatments. The excess use of insecticides against ticks worldwide is mostly environmental contamination rather than effective control. Thus, information about the useful chemicals is essential. *Dermacentor marginatus* and *Dermacentor reticulatus* individuals were tested, being collected in Transylvania, near the city of Târgu-Mureş, Romania. The experiment was performed under laboratory conditions in two replicates, using a total of 420 ticks and 19 insecticides. The best results were achieved with alpha-cypermethrin and pyrethrin.

Keywords: infestation, tolerance, pyrethroids, organophosphates, disease

1. Introduction

Ticks are highly specialized, obligate, ectoparasitic arthropods [1]. Ticks feed on the blood of terrestrial mammals, birds, and reptiles, and they can introduce pathogens into the host. The adult female begins to lay eggs after several days of feeding (6–12 days). Depending on the species, it lays 1,000–18,000 eggs [2]. They can travel several kilometres on their host and can reach other continents too on migratory bird species [3].

Many new diseases are transmitted by arthropod vectors. Mosquitoes spread malaria, dengue-dengue fever, and yellow fever [4], [5], sand flies transmit leishmaniasis [6], ticks transmit Lyme disease, ehrlichiosis [7], [8], babesiosis, or anaplasmosis [9]. Tick-borne diseases are an important cause of losses to the livestock industry. Worldwide losses due to ticks and controlling them have been estimated at several billion dollars annually [10]. The main problem is the transmission of a wide spectrum of pathogenic microorganisms [10]. In some regions, *Dermacentor* spp. are important vectors of bovine anaplasmosis and babesiosis. There are several species that play an important role in human medicine as vectors of rickettsial diseases such as the European canine babesiosis [10]. The first case was reported in Croatia in 1957 [11]. The most common symptoms are high fever, headache, vomiting, and diarrhoea. Older people may also have more severe symptoms such as kidney failure and, ultimately, death [12].

Insecticide tolerance has been detected in many major vector species, and the list is constantly expanding. Resistant or tolerant populations have also been reported in several tick species [13]. Insecticide tolerance has a biochemical basis, and two main forms are known: target-site tolerance, in which case the insecticide is no longer able to bind to the binding spot because it changes, and enzyme-based (metabolic) tolerance, which occurs when oxidases or esterases show increased activity and prevent the activation of the active substances [13]. These esterases contain six families of proteins belonging to the α/β-hydrolase superfamily [14]. The term multiple tolerance is used when pests can tolerate two or more insecticides with significantly different modes of action [15].

Experiments in Mexico have shown that the *Rhipicephalus microplus* tick has developed multiple tolerance to widely used organophosphates and pyrethroids [15]. A study in Queensland, Australia, showed that 5 treatments per season is already a risk factor for acaricide tolerance [16]. Researchers in Argentina reported similar results. Cattle ticks have developed tolerance to pyrethroids and organophosphates [17].

It is also important to mention the mode of action of insecticides. Organophosphates belonged to the first group of chemical compounds, which were also used against arachnids. They are inhibiting the enzyme acetylcholinesterase [18]. Decreases in sensitivity to the organophosphates used were recorded in the early 1950s, and today ticks have developed tolerance against 30 organophosphates in 40 countries [19]. Pyrethrins are produced from *Chrysanthemum cinerariaefolium* and *C. coccineum,* and a limitation in their application was that they decomposed to UV light and oxidation [20]. As a result, synthetic but more stable pyrethroids were produced. Their mode of action is exerted by blocking the permeability of Na^+-ion channels, creating a permanent stimulus [20]. In the case of *neonicotinoids*, the mechanism of action is mediated by the inhibition of acetylcholine binding to the receptor [21]. Excess use of insecticide against ticks is widely practised worldwide and is mostly environmental contamination rather than effective control. Thus, information about the useful chemicals is essential to reduce environmental pollution.

Model species: The most common species in Romania belong to the *Ixodidae* and *Argasidae* families [22]. By integrating the literature data with those derived from personal investigations, the authors present the distribution of the 27 tick species (25 ixodids and 2 argasids). In our experiment, two members of the *Ixodidae* family, namely *Dermacentor marginatus* and *Dermacentor reticulatus*, were used. The Eurasian *Dermacentor* tick species, *Dermacentor marginatus* (Sulzer, 1776) and *Dermacentor reticulatus* (Fabricius, 1794), are extremely common in Europe [23]. *Dermacentor reticulatus* is the second most common tick in Central Europe after *Ixodes* [24]. It is more present in the cooler zones. Its geographical location shows almost the same range as *Dermacentor marginatus*, from northern Portugal through Kazakhstan to western Siberia, but generally further north than *Dermacentor marginatus* [25]. In Hungary, they occur on xerophilic plants located near oak forests [26]. *Dermacentor marginatus* lives on steppes, alpine pastures, forest clearings, and semi-desert areas. In Germany, it particularly prefers open meadows, while in Italy it prefers open oak forests and dry meadows. It is found in the south of France from sea level up to 960 meters above sea level [27].

Objectives: Our first aim was to get a more accurate picture of the insecticide tolerance of tick populations (*Dermacentor marginatus* and *Dermacentor reticulatus*) collected from one of the most infected regions in Transylvania, near Târgu-Mureş city, Romania. We consider that the tick population from this region can reasonably characterize the potential insecticide tolerance of the entire tick population in the Carpathian Basin. The major objectives were therefore to detect the most efficient chemicals and provide an effective control method to reduce excess chemical use in the environment.

2. Materials and methods

2.1. Study area

The sampling procedure took place in an area of more than 10 hectares of agricultural land, orchards, herb, vegetable garden as well as ornamental garden, all belonging to the university campus. The area is bounded on the west by forests and is located near the main international road.

2.2. Field collections of ticks and insecticide tolerance experiment

The collected individuals were *Dermacentor marginatus* and *Dermacentor reticulatus* adults from different points of the infested territory. During the collection, approximately 420 individuals were collected. The GPS coordinates are 46° 31' 19.4988" N 24° 36' 0.4428" E. The sample collection point is marked with a red arrow.

The planned concentration for each insecticide was measured, as shown in *Table 1*. Two replicates were followed for each treatment. We used a total of 42 Petri dishes, two of which were used as control, where the treatment was pure water. We put randomly 10 individuals in each Petri dish and sprayed them with insecticides from the same distance. As no significant differences in mortality rate between the two species were detected, we presented the results together. Treatments were added until the substance formed a film surface on the tick bodies. This is a similar effect as when a normal in-field treatment is planned.

Table 1. Insecticides and concentration levels used in treatments

Mode of action	**Active substance**	**Used conc. mg/l (act. subs.)**	**Commercial name**
Acetylcholinesterase (AChE) inhibitors	*dimethoate*		Danadim Progress
	pirimiphos-methyl	1,000	Actellic 50 EC
Sodium channel modulators	*alpha-cypermethrin*	50	Fastac 10 EC
	delthametrin	25	Decis Mega 50 EW
	lambda-cyhalothrin	50	Karate Zeon 50 CS
	pyrethrin	20	Pestanal
Nicotinic acetylcholine receptor (nAChR) competitive modulators	*acetamiprid*	100	Mospilan 20 SG
	clotiniadin	250	Dantop 50 WG
	imidacloprid	200	Nuprid 200 SC
	thiacloprid	240	Calypso 480 SC
	thiamethoxam	125	Actara 25 WG
Glutamate-gated chloride channel (GluCl) allosteric modulators	*abamectin*	9	Vertimec 1.8 EC
	milbemectin	10	Milbeknock EC
Voltage-dependent sodium channel blockers	*indoxacarb*	150	Steward 30 DF
Mite growth inhibitors affecting CHS1	*hexythiazox*	50	Nissorun 10 WP
Mitochondrial complex III electron transport inhibitors	*bifenazate*	120	Floramite 240 SC
Mitochondrial complex I electron transport inhibitors	*fenazaquin*	200	Magus 200 SC
	fenpyroximate	100	Ortus 5 SC
	pyridaben	300	Sanmite 20 WP

One minute after the treatment, the individuals were examined separately, and this was repeated in every 15 minutes for one hour, and after that in every hour. Observations were made once after 24 hours and again after 1 week. It was previously detected concerning this mechanism that the gnathostome remained open in a V-shaped form for all dead individuals in all cases. We believe that this can be a method to detect mortality; however, no parallel action was detected for these individuals either – they all were considered dead.

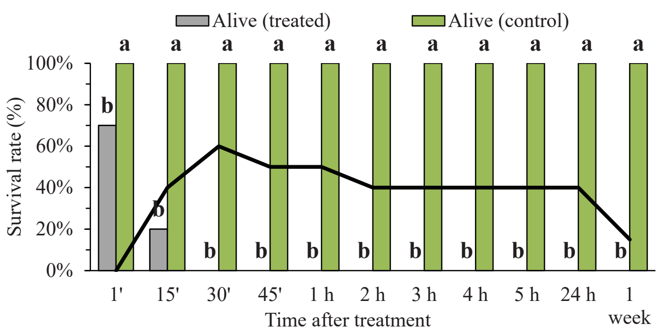
2.3. Data analysis

Data were analysed with PASW Statistics 18, release version 18.0.0 (29 June 2012). The original data normality was not met; therefore, the non-parametric Mann–Whitney U tests was used to compare the treatments. Means with different letters in diagrams represent statistically significant differences. Values were considered to be significantly different at $p < 0.05$.

3. Results and discussions

3.1. Efficacy of pyrethroids

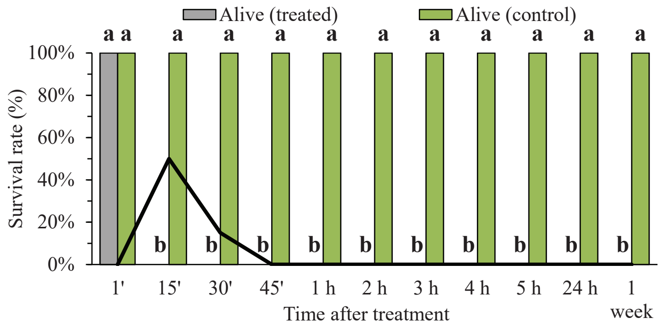
The treatment with the natural pyrethrin had an effective knock-down effect against ticks (*Figure 1*). After one minute, 30% of the individuals were dead, and after 15 minutes the mortality rate reached over 80%.



Note: the different letters mean statistically significant differences.

Figure 1. Efficacy of pyrethrin on tick adults (Mann–Whitney test: $p < 0.05$)

The best effect was obtained with alpha-cypermethrin (*Figure 2*), which killed all individuals after 30 minutes. Deltamethrin and lambda-cyhalothrin treatment produced similarly good results; after 1 hour, the mortality rate was 100%.

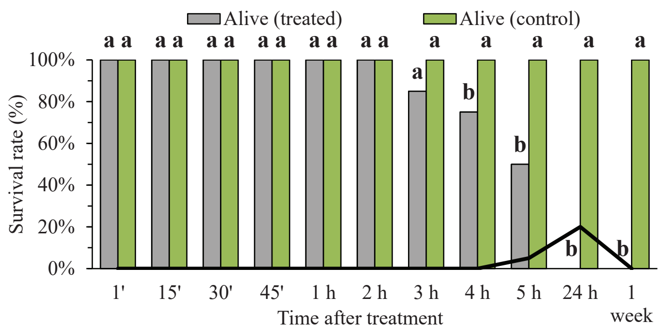


Note: the different letters mean statistically significant differences.

Figure 2. Efficacy of alpha-cypermethrin on tick adults (Mann–Whitney test: $p < 0.05$)

3.2. Efficacy of organophosphates

Significant difference was found between pirimiphos-methyl treatment and the control (*Figure 3*). After 3 hours, 10% of the individuals were dead, in 4 hours the mortality rate reached 20%, and after 5 hours half of the adults were dead. 24 hours after the application of insecticides, the mortality rate was 80% and 20% of the individuals showed weak signs of life. After 1 week, the mortality rate reached 100%. The treatment with dimethoate yielded similar results, with the difference that the first individuals died just after 24 hrs, and no live individuals were found after 1 week.

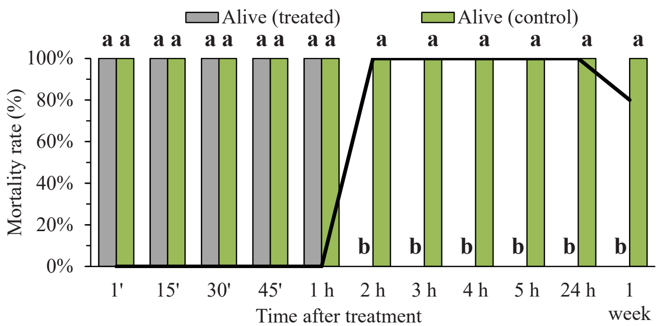


Note: the different letters mean statistically significant differences.

Figure 3. Efficacy of *pirimiphos-methyl* on tick adults (Mann–Whitney test: $p < 0.05$)

3.3. Efficacy of neonicotinoids

Imidacloprid and acetamiprid had no effect on ticks, all individuals were alive after one week. The active substance clothianidin was also ineffective against ticks – after 1 week, a single death was observed. The third member in this chemical group was thiamethoxam, which proved to be completely ineffective, and no dead individuals were found after 1 week, and the last member of the group was thiacloprid. In this one case, the treatment was effective against ticks (*Figure 4*). After 1 hour of treatment, 100% of the individuals showed weak signs of life, and after 1 week the mortality rate reached 20%.



Note: the different letters mean statistically significant differences.

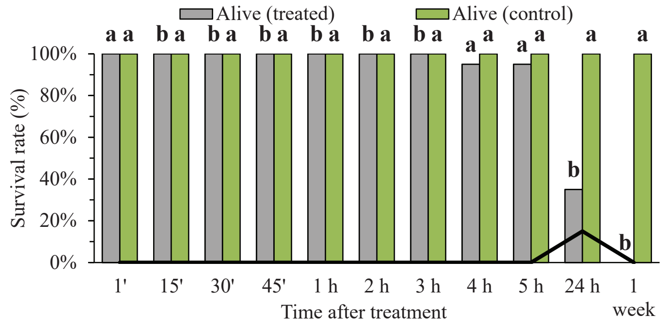
Figure 4. Efficacy of thiacloprid on tick adults (Mann–Whitney test: $p < 0.05$)

3.4. The efficacy of avermectins

Both abamectin and milbemectin treatments have been shown to be ineffective against ticks. In the case of abamectin treatment, all individuals were alive after 1 week, and in the case of milbemectin treatment, the mortality rate was around 10% after 1 week.

3.5. The efficacy of acaricides

The acaricides used in the experiment were not effective enough either. The active substances fenazaquin and hexithiazox were ineffective on ticks, and we did not find any dead individual after 1 week. The treatment with piridaben was not effective, as the individuals were alive after a week. In the case of bifenazate and fenpyroximate (*Figure 5*) treatments, dead individuals were found after 24 hours, but the mortality rate was around 60%, and all of the individuals died just after a week.



Note: the different letters mean statistically significant differences.

Figure 5. Efficacy of fenpyroximate on tick adults (Mann–Whitney test: $p < 0.05$)

3.6. The efficacy of oxadiazine

The treatment with indoxacarb had no effect against ticks: the mortality rate remained 0% after a week.

Tick control in the infected area has been performed with pirimiphos-methyl for the last 3 years. In the first two years, population control was relatively successful, but in the third year the treatment proved unsuccessful, so we decided to collect individuals and investigate the effects of marketed insecticides on the collected individuals. According to these, a specific substance commercialized against ticks (pirimiphos-methyl) was less effective with time, generating a low mortality rate of ticks. As several other insecticides are commercialized as having acaricide effects too, and other acaricides might also have weaker effect, testing these substances to give a clear picture about their effects is extremely important.

Previous similar studies from Mexico and Argentina indicated that natural populations of *Rhipicephalus microplus* (Canestrini, 1888) were found to be tolerant to a number of pyrethroids [15], [17]. Another study from Brazil showed that 578 tick larvae were tested for pyrethroids, and 97.44% of them were tolerant to the treatment [18]. However, in our region, we got the best results with pyrethroids. Alpha-cypermethrin and pyrethrin (after 1 h: 100% death or weak signs of life) yielded the best results. 80% of the individuals died after the *pyrethrin* treatment within 15 minutes. We found significant differences between the treatments and the control. The mortality rate reached 100% after 15 minutes with the alpha-cypermethrin treatment. *Deltamethrin* also had a good effect because all the ticks were dead after 1 hour of the application.

In the case of organophosphates, there are several studies where the tolerance has been well described. Two different strains of *Bophilus* were used in the U.S.A. [28]. In the first case, a large number of female adults survived the dip treatment and produced viable offspring. In the second case, similar results were obtained, but the tolerance ratio was higher, and the female adults were able to produce viable offspring [28]. The treatments with dimethoate were not effective because the individuals were dead just after 1 day of treatment. In the case of pirimiphos-methyl, 10% of the individuals were dead after 3 hours, the mortality rate reached 20% in 4 hours, and half of the adults were dead after 5 hours. After 24 hours, the mortality rate was 80%, and 20% of the individuals showed weak signs of life.

After nearly two decades of use, several target pests of neonicotinoids have begun to develop tolerance [29]. In our experiment, the treatments with neonicotinoids, with the exception of *clothianidin*, were totally ineffective against the ticks. The mortality rate was near 60% upon the *clothianidin* treatment, but only after 1 week of the treatment.

Because of the intensive use of the macrocyclic lactones, partial tolerance has been reported in *R. microplus* in different regions [30]. The exact mechanism is still unknown, but some studies [31] concluded that tolerance in ticks might be due to target-site insensitivity. We obtained almost the same results in our experiment. The avermectins (abamectin, milbemectin) were ineffective because 100% of the individuals were alive after one day of the application.

In the European Union, indoxacarb is approved as a topical spot-on flea control product for dogs and cats, and, in combination with permethrin, as a topical spot-on flea and tick control for dogs [32]. In our experiment, we used Steward 30 DF with indoxacarb as active compound, but it had no effect against ticks – the mortality rate remained 0% after a week.

As ticks age, they become increasingly susceptible to acaricides [33]. But populations of several tick species in tropical and subtropical countries have developed high tolerance to compounds due to the high intensity of their use in tick management [34], [35]. The treatments with acaricides contradicted our expectations. Among these, fenpyroximate killed 50% of the individuals, but only 1 day after the treatment; the other compounds were ineffective, and the ticks were highly tolerant to treatments.

Altogether, the tolerance mechanisms seem to have both a genetic and a behavioural background. From a genetic point of view, ticks can develop a tolerance if a compound is widely used, and these mechanisms can be passed on to the next generations too.

From a behavioural point of view (which can be in fact combined with genetic tolerance as well), ticks can move or can be moved artificially and/or accidentally between regions. Several populations with different genetic backgrounds can be mixed in this way, increasing tolerance mechanisms and their spread in the population. Considering these effects, a continuous test of commercialized insecticides and acaricides needs to be made periodically.

Insecticides that showed an effect only after 24 hours or a few days did not prove to be effective because we cannot produce this maximum contact effect under natural conditions but only under laboratory conditions. These wisely used chemicals are not recommended against ticks, as it will only result in high levels of environmental contamination.

Based on our results, the treatments with alpha-cypermethrin, pyrethrin, and other pyrethroids could have a good effect against *Dermacentor* species. We do not recommend other chemicals for tick control.

4. Conclusions

In conclusion, *Dermancentor marginatus* and *Dermancentor reticulatus* species collected from the campus of Sapientia Hungarian University of Transylvania showed a high degree of tolerance to the majority of the 19 insecticides we tested. Considering the treatments, we can conclude that pyrethroid treatments (alpha-cypermethrin, deltamethrin, lambda-cyhalothrin, pyrethrin) were effective against ticks, whereas neonicotinoids, avermectins, and even acaricide treatments were almost completely ineffective. The desired effect was not achieved with the organophosphates either, so it can be concluded that the two *Dermacentor* species involved in the treatments have developed a high degree of tolerance in recent years. We should mention the importance of insecticide rotation, more likely the usage of insecticides with different modes of action to reduce and slow down tolerance development and reduce excessive chemical use.

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