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Journal of Agronomy, Technology and Engineering Management

Article

Repellent Efficacy of Diethyltoluamide on *Dermacentor marginatus* Ticks (Acari: Ixodidae)

Aleksandra Petrović [1]*, Vojislava Bursić [1], Ivana Ivanović [1], Dušan Marinković [1], Tijana Stojanović [1], Gorica Vuković [2] and Snežana Tanasković [3]

[1] Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia; aleksandra.petrovic@polj.uns.ac.rs

[2] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Serbia; goricavukovic@yahoo.com

[3] Faculty of Agronomy in Čačak, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Serbia stanasko@kg.ac.rs

* Correspondence: aleksandra.petrovic@polj.uns.ac.rs

Received: 19 July 2021; Accepted: 25 December 2021

Abstract: Ticks can parasitize on a large number of hosts such as wild and domestic mammals, reptiles, birds, and humans. By changing hosts in order to take a blood meal, various pathogens could be transmitted from ticks to humans and animals. One of the preventive methods widely used to protect from tick bites is the use of substances with proven repellent effect. The aim of this study was to test DEET (diethyltoluamide) repellent efficacy as a function of time against *Dermacentor marginatus* ticks in laboratory conditions. The repellent efficacy was determined by ticks' movement away or in the direction of the administrated preparation and expressed in percentages. Repellence decreased as a function of time, due to the evaporation of the active substance. DEET had 100% repellent efficacy on *D. marginatus* ticks in all replicates up to two hours after preparation application. After four hours it was 88.00%, and after eight hours 90.00% on average. In all five replicates the average repellent efficacy was 95.60%. Considering the confirmed ticks' vector potential, studies on the repellent efficacy of certain substances are necessary, all with the aim to find widely available, highly efficient and economically justified substances, harmless to humans and animals.

Keywords: repellents; ticks; efficacy; *Dermacentor marginatus*; acaricides.

1. Introduction

Hard ticks (Acari: Ixodidae) are very effective disease transmitters, pathogen vectors and reservoirs, as they obligatory take a blood meal from a large variety of reptiles, birds, small and large mammals. Tick-borne diseases (TBDs) are zoonoses, diseases that can be transmitted between animals and humans. According to Jones et al. [1] zoonotic diseases endanger not only the health of animals and humans, but also pose a threat to the global health security. An estimated 60% of known and up to 75% of new or emerging infectious diseases are zoonotic [1]. Infectious diseases account for 15.8% of all human deaths and up to 43.7% of deaths in economically developing countries [2]. Close to 70% of emerging infectious diseases are animal borne and responsible for some of the most high profile epidemics, such as Ebola and COVID 19 [3]. Furthermore, up to 80% of all agents that can be used in bioterrorism are zoonotic.

Preventive measures for protection against obligatory haematophagous ectoparasites such as ticks is necessary in order for people to spend more relaxed and free time in nature for rest, various sports, recreational and other outdoor activities. One of the preventive methods is the use of substances that could be applied to the skin or clothing, and which have a proven repellent effect [4, 5]. Every level in the tick-host interaction can be interrupted, but only certain activities and processes could be counted as *sensu stricto* repellency, which implies complete tick rejection from the host and/or its abandonment [6]. Therefore, studies on the efficacy and duration of certain compounds repellent action is of complex importance and requires constant research and improvement of methodology, following trends in the development of new synthetic and natural substances, the application of adequate tests to assess repellency and finally, introduction and application of economically viable and widely available commercial preparations that are not harmful to animal and human health, especially children.

It is very important to emphasize that repellents do not kill arthropods, insects and ticks; rather their principle of efficiency is based on interfering with chemical senses in order to prevent these haematophagous organisms from locating an adequate host. There are two theories that explain the repellents mode of action [7]. The first theory implies that repellents work by confusing insects and ticks, as they mask the attractants emitted by the host, and the second that explains that repellents are registered by arthropods as harmful and dangerous substances. With these theories, progress has been made in understanding the molecular mechanisms by which DEET (diethyltoluamide; N,N-diethyl-meta-toluamide) works and why flies, mosquitoes, and nematodes avoid it, as have been proven in a number of references [8-13]. Recent evidence of how DEET functions as a repellent includes inhibition and detoxification of the enzyme cytochrome P450 [14, 15] and cholinesterase [16]. When it comes to ticks, the mechanisms of the repellent activity of preparations based on DEET are still under investigation. Ticks have a special sensory organ for finding potential hosts, which is called the Haller organ. It is located on the tarsus of the first pair of legs, and its function is related to host questing, infrared detection, body position, and mating [17-19]. Other sensory organs of ticks are hairs located on the pedipalps and in the region of the mouthparts, which have a chemosensory role [20]. Although numerous studies have been conducted, there is still no adequate explanation for how ticks detect repellents, nor the importance of chemoreceptors in this process [4, 17, 21]. The aim of this study was to test the repellent effects of DEET (diethyltoluamide) as a function of time against *Dermacentor marginatus* ticks in laboratory conditions.

2. Materials and Methods

In order to test the repellent efficacy of DEET, ticks were collected using the flag-hour method [22] in forest ecosystems in the vicinity of Novi Sad. Collected ticks were placed in plastic test tubes with perforated lids to provide sufficient ventilation. To prevent the ticks' desiccation during the transport, a piece of cotton wool moistened with water was added as well. Collected ticks were determined up to species level and life stages according to the identification key [23]. Sixty adult males (30) and females (30) of *Dermacentor marginatus* tick species were included in the experiment. The repellent efficacy of DEET was determined according to the methods proposed by Kröber et al. [24] and Adenubi et al. [25]. DEET was manufactured and provided by BioGenesis.

The bottoms of Petri dishes (90 × 14.5 mm) were covered with filter paper on which three fields were drawn: zone without repellent, neutral - initial zone and the zone with repellent. Each tick was exposed to the repellent for 3 minutes. The repellent efficacy was determined by ticks' movement away or in the direction of the administrated preparation, and calculated according to Thorsell et al. [26] and Tunón et al. [27]. The ticks' behavior was observed immediately after placing the ticks in the Petri dishes, and then after 1, 2, 4 and 8 hours, in five replicates. The following grades were assigned to each tick:

- if they moved away from the zone with repellent, the grade (A) was assigned,
- if they approached the zone with repellent the grade (B) was assigned,
- if they walked along the neutral zone the grade (C) was assigned, and
- if they were not moving, and stayed at the initial zone, the grade (D) was assigned.

The efficacy was calculated only for ticks graded with "A". After each test cycle of 3 minutes, ticks were removed, and Petri dishes were stored in the laboratory, at a constant temperature of 20-22°C, and a relative humidity of 40-43%. The control group was treated with distilled water.

The obtained results were statistically analyzed by ANOVA and Fisher's LSD test using Statistica 14.0.0 (TIBCO, University license).

3. Results

The average repellent efficacy was calculated for all graded ticks, five replicates, and five time intervals and presented in Table 1. DEET had 100% repellent efficacy on *D. marginatus* ticks in all replicates up to two hours after preparation application. Repellence decreased as a function of time, due to the evaporation of the active substance. After four hours it was 88.00%, and after eight hours 90.00% on average (Table 1).

Table 1. Average percentage of repellency depending on time.

Replicates	**Time intervals**					**Mean**
	3 minutes	**1 hour**	**2 hours**	**4 hours**	**8 hours**	
1	100.00%	100.00%	100.00%	90.00%	90.00%	96.00%
2	100.00%	100.00%	100.00%	80.00%	80.00%	92.00%
3	100.00%	100.00%	100.00%	90.00%	90.00%	96.00%
4	100.00%	100.00%	100.00%	90.00%	90.00%	96.00%
5	100.00%	100.00%	100.00%	90.00%	100.00%	98.00%
Average	100.00%	100.00%	100.00%	88.00%	90.00%	95.60%
Control group	20.00%	20.00%	30.00%	30.00%	20.00%	24.00%

The highest percentage of repellency was recorded in the fifth replicate (98.00%). The average repellent efficacy in all five replicates was 95.60%.

Statistical analyses of the obtained results indicated that there was a high statistical significance in the repellency percentage between all replicates and the control group (p=0.000000 for p<0.01), while the time intervals were not statistically significant. The Fisher's LSD test confirmed obtained results (Table 2).

Table 2. Fisher's LSD test results.

MS = 0.36667, df = 24.000						
Replicates	{1}	{2}	{3}	{4}	{5}	{6}
	98.000	94.000	98.000	96.000	100.00	22.000
1		0.306677	1.000000	0.606295	0.606295	0.000000*
2	0.306677		0.306677	0.606295	0.130277	0.000000*
3	1.000000	0.306677		0.606295	0.606295	0.000000*
4	0.606295	0.606295	0.606295		0.306677	0.000000*
5	0.606295	0.130277	0.606295	0.306677		0.000000*
Control group	0.000000*	0.000000*	0.000000*	0.000000*	0.000000*	
highly statistically significant differences were marked with *						

4. Discussion

Similar results were obtained by Ogawa et al. [28], who proved that DEET had high repellent efficacy, although the experiment was conducted on different tick species (*Ixodes persulcatus* and *I. ovatus*). The authors tested the repellent efficacy of 12% DEET products on human skin and by pulling the cloth treated with DEET through the vegetation cover. The percentage of repellent efficacy was

84% which confirmed previously published results by Solberg et al. [29] on *Amblyomma americanum* ticks when 25% DEET in ethanol solution was applied in a layer 0.5 mg/cm^2.

The first experiments on DEET repellent efficacy on ticks were performed in the 1980s by Mount and Snoddy [30] and Schreck et al. [31]. They found that application of 20% DEET spray on *A. americanum*, *Dermacentor variabilis* and *I. dammini* had a repellent percentage of 84%, 94% and 86% respectively, while 30% DEET had repellent efficacy of 92% on *I. dammini*. The same authors therefore stated that if the percentage of DEET in the preparations increases, it does not mean conditionally an increase in its efficiency and the percentage of repellency. Quite the reverse, Ferreira et al. [32] showed that DEET and β-citronellol have a repellent effect on *R. sanguineus* s.l. and *A. sculptum*, but that repellency still depends on the preparation concentrations. Their experiment showed that *A. sculptum* is more sensitive to DEET than *R. sanguineus* s.l., while β-citronellol showed higher repellency in both species compared to DEET. The results of this experiment confirmed the results of *in vitro* experiments published by Borges et al. [33] for *R. sanguineus* s.l. and Soares et al. [34] for nymphs of *A. cajennense* s.l. originating from the same geographical climate. According to Dorman et al. [35], when applying repellents, the safety of the individual to whom the preparation is applied is crucially important, so it is not recommended to use preparations containing DEET on animals that maintain hair and fur hygiene by licking.

Koloski et al. [7] emphasized that DEET is an oily synthetic substance that can be applied to the skin in order to effectively repel arthropods, mosquitoes and ticks from adopting a blood meal. The formulation of DEET has undergone many changes in the last few years in order to enhance its basic characteristics: odor, duration of repellency, and potency. Although numerous studies have been conducted confirming the efficacy of DEET, the safety and duration of repellent action, yet, the mode of action and molecular mechanisms have not been fully elucidated [7].

European Chemicals Agency (ECHA) and United States Environmental Protection Agency (USEPA) have published guidelines for biocide products tests recommending the use of nymphs or/and adults as a test organisms to assess the effectiveness of tick repellents, as different stages have been found to behave differently [36]. Tolerance to desiccation and loss of necessary moisture incorporated into the ticks' cuticle increases during the life cycle, which therefore causes a different behavior. Adult ticks can survive on higher vegetation than nymphs during longer period of time [37]. Conditioned by meteorological parameters (temperature, relative air humidity, dew, atmospheric pressure, strong wind) their activity peak also varies [38]. Furthermore, nymphs can feed on several different host species, while adults most often take a blood meal by parasitizing on large mammals [39]. For this reason, Kulma et al. [36] suggest that developmental stages significantly affect the results of experiments when repellents are tested, because nymphs have proven to be significantly more sensitive. According to Kulma et al. [36], female ticks show greater tolerance to low concentrations of repellents compared to the nymphs. From this point of view, a repellent used against nymphs would not be as effective against female ticks. Also, ticks from laboratory colonies have been shown to be more sensitive than ticks collected from nature, which may also lead to protocol changes in efficacy tests [36]. Laboratory tests are expected to inform users about the approximate duration of the repellent effect, because such experiments can never fully simulate real conditions in nature.

5. Conclusions

Considering the confirmed vector potential of ticks as obligatory haematophagous organisms, studies on the repellent efficacy of synthetic and natural substances applied to repel ticks and other arthropods are necessary and required, all with the aim to find widely available, highly efficient and economically cost-effective preparations, harmless to humans and animals.

Funding: This research was supported by the Ministry for Education, Science and Technological Development of the Republic of Serbia.

Acknowledgments: Authors acknowledge BioGenesis for providing the DEET preparation.

Conflicts of Interest: The authors declare no conflict of interest.

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