Received: 24 April 2023, Accepted: 21 June 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 5, 827-832

In vitro and *in vivo* Evaluation of the Efficacy of Phoxim and Deltamethrin against Life Stages of *Rhipicephalus sanguineus* (Brown Dog Tick)

Aliaa A. Abdel aziz[1], Abdelfattah M. Abdelfattah[1], Waleed F. Khalil[1], Ahmed E. Mahmoud[2], Eman M. Abouelhassan[3*]

[1]Department of Pharmacology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

[2]Department of Animal Medicine, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

[3]Department of Parasitology, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

***Correspondence**
Corresponding author: Eman M. Abouelhassan
E-mail address: eman_abouelhassan@vet.suez.edu.eg

Abstract

Infestations of brown dog ticks (*Rhipicephalus sanguineus*) are difficult to control. This study aimed to evaluate the efficacy of phoxim and deltamethrin against *R. sanguineus In vitro* and *in vivo*. *In vitro* studies, the evaluation involved the exposure of *R. sanguineus* adults, nymphs, and larvae to phoxim (1 ml/1000 ml) and deltamethrin (1 ml/1000 ml) observations were recorded for 24 hours, and the eggs were assessed and compared with a negative control tick group that had been exposed to water for 14 days, *In vivo* studies, twenty local dogs have been divided into four groups of five each: The 1st group was non-infested dogs; the 2nd group is infested untreated dogs; the 3rd group was infested dogs and treated with a therapeutic dose of phoxim; and 4th group was infested dogs and treated with a therapeutic dose of deltamethrin. Results revealed that phoxim was more effective than deltamethrin on adult of *R. sanguineus*, while deltamethrin appeared to be more effective than phoxim on nymphs and larvae of *R. sanguineus*. Both acaricides significantly inhibited egg hatchability of *R. sanguineus* with the same potency.

KEYWORDS
Phoxim, Deltamethrin, *Rhipicephalus sanguineus*, Dog tick

INTRODUCTION

The Ixodidae is the largest tick family which infests animals, including dogs (Mihalca *et al*., 2011). Their importance is related to their ability to transfer several pathogens to their hosts (Kiss *et al*. 2012). *Rhipicephalus sanguineus*, generally known as the brown dog tick, is the most widespread tick in the world (Dantas-Torres, 2008). It can produce debilitating effects due to blood loss in affected animals and transmits numerous pathogens which cause tick-borne diseases in animals, as well as certain zoonoses in humans. These include *Ehrlichia canis*, *Babesia canis*, *Haemobartonella canis*, and *Hepatozoon canis* (Borges *et al*., 2007; Adenubi *et al*., 2016).

R. sanguineus infestation is associated with tropical and semi-tropical climates. Epidemiological studies of dog infestations in Egypt found *R. sanguineus* accounted for 89.8% of the study subjects (Abdulaziz *et al*., 2019; El Kammah *et al*., 2001). This prevalence rate makes the search for an effective control strategy to minimize the damage caused by these parasites essential. Acaricides are the current agents used to control tick populations in dogs. Tick control is based primarily on the repeated use of acaricides, which are chemicals produced to efficiently control tick populations (Mencke *et al*., 2003). Acaricides can be categorized by their chemical structure and mechanism of action into arsenical preparations, chlorinated hydrocarbons, organophosphorus compounds, carbamates, formamidines, pyrethroids, macrocyclic lactones, phenylpyrazoles, insect growth regulators, and isoxazolines (Nicholson *et al*., 2019).

An ideal acaricide should be effective not only against adult ticks, but also against their eggs, larvae, and nymphs. The ovicidal property of an acaricide is very important for killing or interfering with the hatchability of eggs, which is necessary to ensure proper tick control (Rohdich *et al*., 2014). The main chemical groupings of common acaricides are the organophosphates and synthetic pyrethroids, which act on tick nervous systems. These acaricides act either systemically, or by direct contact with ticks following external application (Mencke *et al*., 2003).

Inappropriate acaricide use, such as with incorrect concentrations, and the high intensity of their use in tick management, has likely contributed to the development of resistance in ticks. This leads to tick control program failures and the development of acaricide resistance. Tick resistance to various insecticides and acaricides has been reported as a huge problem facing veterinarians worldwide (Marchiondo *et al*., 2007; Akande *et al*., 2020).

Both phoxim, an organophosphorus insecticide, and deltamethrin, a synthetic pyrethroid (Worthing and Hance., 1991; Abdel-Daim *et al*., 2013) are used to control ectoparasites. They are applied topically as a wash, spray, or pour-on, at concentrations according to the manufacturer's instructions. Several previous studies have found that pyrethroid compounds are ineffective at controlling tick infestations, which may be due to the development of resistance (Mendes *et al*., 2011). Therefore, this study was designed to evaluate the efficacy of phoxim and deltamethrin against *R. sanguineus In vitro* and *in vivo*.

ISSN: 2090-6277/2090-6269/

MATERIALS AND METHODS

Ethical approval

This study was approved by the Ethics Committee of the Suez Canal University, following the guidelines of the Guide for the Care and Use of Laboratory Animals, Faculty of Veterinary Medicine Science, Suez Canal University, Egypt.

Drugs

The phoxim used in this study was purchased from Bayer Animal Health GmbH, Germany, under the trade name Sebacil®, and the deltamethrin was purchased from MSD Animal Health, France, under the trade name Butox®.

Vitro studies

Tick collection and identification

One hundred and eighty *R. sanguineus* (adults and nymphs) were collected via untoothed forceps from infested dogs in Ismailia, Egypt. The collected ticks were then transported directly to the laboratory of the Parasitology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. Tick identification was then carried out according to the identification keys of Walker (2003).

Larvae and egg production

Engorged female ticks were randomly selected and transferred into different flasks and covered with a thin net to allow them to lay eggs. The eggs were then incubated at 27°C –28°C and 85%–90% relative humidity, according to the method described by Ibelli *et al*. (2012) to produce larvae.

Immersion test

The test was performed according to the method described by Zaman *et al*. (2012). Briefly, the tick (adult/nymph) specimens were selected and divided into three homogenous groups, each containing three replications, with each one containing ten ticks (adult/nymph). The first group of ticks was treated with phoxim diluted with tap water at 1 ml/1000 ml. The second group was treated with deltamethrin diluted with tap water at 1 ml/1000 ml. Water (3 ml) was used as a negative control in the third group.

The tick adults and nymphs were immersed in 3 ml of each drug for 5 min in clean Petri dishes. Following the immersion, the ticks were placed in clean Petri dishes with filter paper and incubated at 28°C, with 80% relative humidity. The ticks were kept under observation using a stereomicroscope at 30 min, 1, 2, 3, 6, 12, and 24 h time intervals (Du *et al*., 2008). The viability of the ticks was checked regularly, and death was recorded when the tick did not show any reaction to stimulation with a needle.

Larval packet test

Larvae were divided into three groups; each group contained one hundred larvae (12–14 day old) and was placed on Whatman filter paper which had been previously soaked with the drugs being tested. Additionally, these had been diluted with tap water at 1 ml/1000 ml in labeled plastic Petri dishes. In the control group, the paper was soaked in water (Abdulaziz *et al*., 2019). The Petri dishes were incubated at 28°C, with 80% relative humidity, and each larva was closely observed for any death using a stereomicroscope at 30 min, 1, 2, 3, 6, 12, and 24 h time intervals (Du *et al*., 2008). The viability of larvae was checked regularly by stimulation with a needle.

The mortality percentage was calculated for each acaricidal compound on adults, nymphs, and larvae of *R. sanguineus* using the following equation:

Mortality percentage = Number of dead ticks/Total number of ticks ×100 (Krishnaveni and Venkatalakshmi, 2014).

Resistance percentage was calculated for each acaricidal compound on adults, nymphs, and larvae of *R. sanguineus* using the following equation:

Resistance percentage: (Nt/Nw) × 100; where Nt = Number of living treated ticks, and Nw = Number of untreated ticks (Shyma *et al*., 2013).

Egg hatchability test

Three groups (three replicates) of approximately 200 *R. sanguineus* embryonated eggs (~10 mg) were placed in glass tubes and immersed for 5 min in 0.5 ml of the test drug, which had been diluted with tap water at 1 ml/1000 ml. The tubes were closed with muslin cloths and were incubated at a temperature of 27±2°C, and relative humidity of 80±5% for 14 days to evaluate the hatching rate. Water was used as a negative control (Godara *et al*., 2014). The percentage of hatching inhibition was calculated according to the following equation: number of unhatched eggs/number of unhatched eggs + the number of dead or living larvae (Bicalho *et al*., 2001).

Vivo studies

Experimental animal and design

Twenty local dogs at one year old and weighed between 5.5 and 8 kg have been divided into four groups of five each: The 1st group was non infested dogs; the 2nd group is infested untreated dogs; the 3rd group was infested dogs and treated with a therapeutic dose of phoxim; and 4th group was infested dogs and treated with a therapeutic dose of deltamethrin. The dogs were housed in disinfected separated cages under controlled hygienic measures and fed on well-balanced healthy food (Fig. 1).

Fig. 1. Experimental design

Tick infestations and count

Fully engorged females and male of *Rhipicephalus sanguineus* were collected by hand via untoothed forceps from the infested dogs by visited pet clinic in Ismailia government, Egypt. Collected ticks were then put into clean vials covered with muslin cloth to allow air and moisture exchange and brought to the laboratory of parasitology department, Faculty of Veterinary Medicine, Suez Canal University- in Ismailia, Egypt, for further tick examination, characterization, and experimental procedures. These ticks were utilized in dogs' infestation, following the treatments according to the manufacturers' instructions, phoxim and deltamethrin were applied topically as a wash. They were particularly recommended for dogs using a single treatment of 1 ml of each drug added to 1 liter of water. Dogs were sedated with xylazine hydrochloride for tick count. By examination of all body parts through cautious palpation and hair was pushed manually against its natural lie. Ticks were alive if their legs responded to a physical stimulus, and they were dead if there was no response. Ticks were counted on 0, 2nd, 4th and 7th days post treatment where zero is pretreatment day (Cavalleri *et al*., 2017).

The antiparasitic efficacy % was calculated according to the following equation:

Antiparasitic efficacy % = (Mean number of surviving ticks control - mean number of surviving ticks treated)/ (Mean number of surviving tick control) ×100) (Wang *et al*., 2009).

Statistical analysis

In the present study, all results were expressed as the mean + the standard error of the mean. Data were analyzed by one way analysis of variance using the Statistical Package for the Social Sciences program, version 14 (IBM Corp., Armonk, NY, USA), followed by the Fisher's least significant difference test to compare significance between groups (Arrnitage and Berry,1987). The difference was considered significant when p<0.001.

RESULTS

In-vitro efficacy

Adult immersion test

The *In vitro* application of phoxim to *R. sanguineus* adults induced a significant (p <0.001) elevation in the mortality rate while simultaneously reducing the resistance percentages of *R. sanguineus* adults compared to the mortality rates and resistance percentages seen for deltamethrin (Figs. 2 and 3).

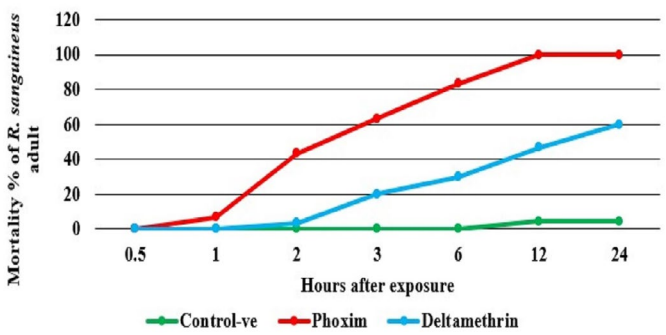

Fig. 2. Mortality percentage of *R. sanguineus* adults exposed to phoxim and deltamethrin *In vitro*.
Data are expressed as mean ± SE. Different superscripts on bars of the same time interval denoted significant difference at P<0.05.

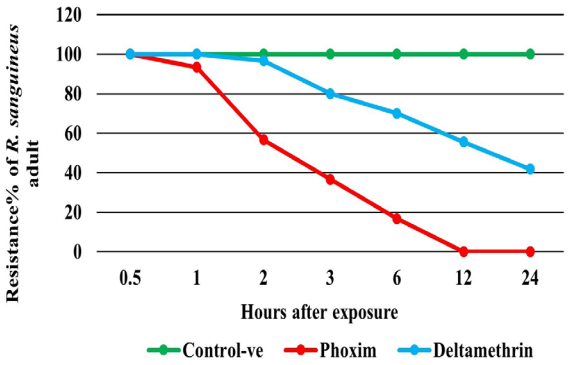

Fig. 3. Resistance percentage of *R. sanguineus* adults exposed to phoxim and deltamethrin *In vitro*.
Data arevexpressed as mean ± SE. Different superscripts on bars of the same time interval denoted significant difference at P<0.05.

Nymphal immersion test

The *In vitro* application of deltamethrin to *R. sanguineus* nymphs induced a significant (p <0.001) elevation in the mortality rate while reducing the resistance percentage of *R. sanguineus* nymphs compared to the rates seen with phoxim (Figs. 4 and 5).

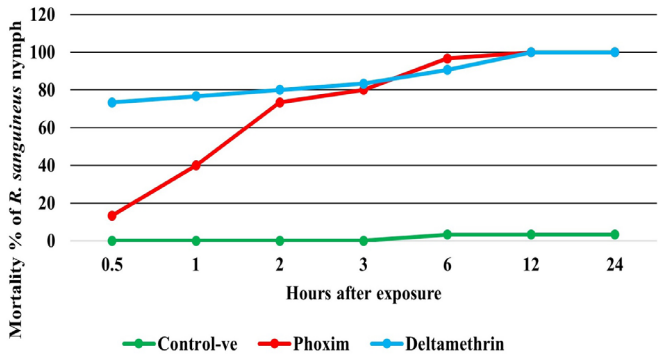

Fig. 4. Mortality percentage of *R. sanguineus* nymphs exposed to phoxim and deltamethrin *In vitro*
Data are expressed as mean ± SE. Different superscripts on bars of the same time interval denoted significant difference at P<.05.

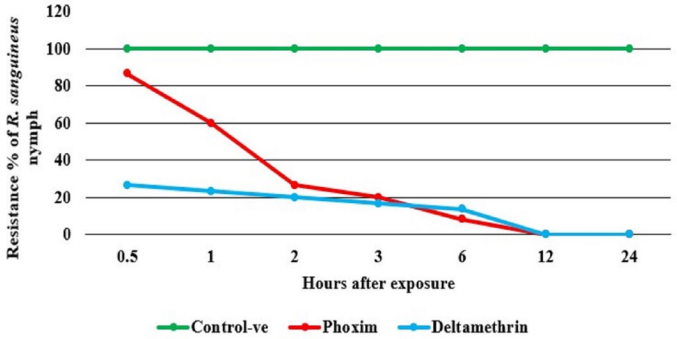

Fig. 5. Resistance percentage of *R. sanguineus* nymphs exposed to phoxim and deltamethrin *In vitro*.
Data expressed as mean ± SE. Different superscripts on bars of the same time interval denoted significant difference at P<0.05.

Larvae packet test

The *In vitro* application of deltamethrin to *R. sanguineus* larvae induced a significant (p <0.001) elevation in the mortality rate while reducing the resistance percentages of *R. sanguineus* larvae compared to the rates seen with phoxim (Figs. 6 and 7).

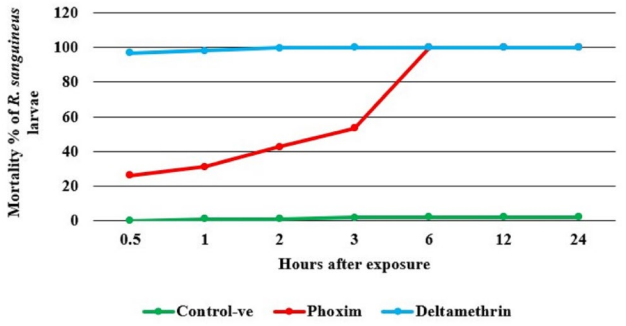

Fig. 6. Mortality percentage of *R. sanguineus* larvae exposed to phoxim and deltamethrin *In vitro*.
Data are expressed as mean ± SE. Different superscripts on bars of the same time interval denoted significant difference at P<0.05.

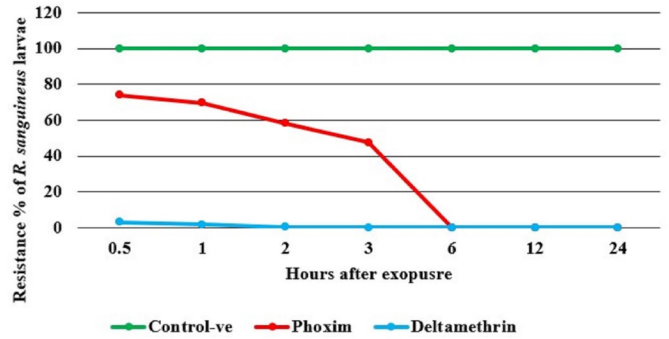

Fig. 7. Resistance percentage of *R. sanguineus* larvae exposed to phoxim and deltamethrin *In vitro*.
Data are expressed as mean ± SE. Different superscripts on bars of the same time interval denoted significant difference at P<0.05.

Egg hatchability test

There was a non-significant difference observed in phoxim and deltamethrin treated groups on the hatching inhibition percentages of R. sanguineus eggs. However, there was a significant (p <0.001) increase in the egg hatching inhibitory effect in the phoxim and deltamethrin treated groups when compared to the control group after 14 days of egg hatching (Fig. 8).

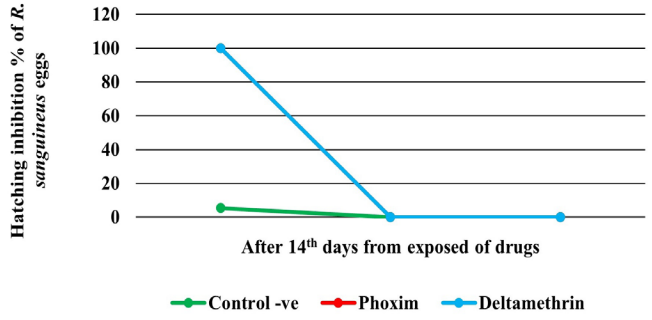

Fig. 8. Hatching inhibition percentage of *R. sanguineus* eggs exposed to phoxim and deltamethrin *In vitro*.
Data are expressed as mean ± SE. Data expressed as mean ± SE. Different superscripts on bars of the same time interval denoted significant difference atP<0.05.

In-vivo efficacy

Tick infestations and count

The administration of phoxim to *R. sanguineus* adult induced significant (p<0.001) elevation in efficacy % and reduction in the mean count of *R. sanguineus* adult than deltamethrin (Figs. 9 and 10).

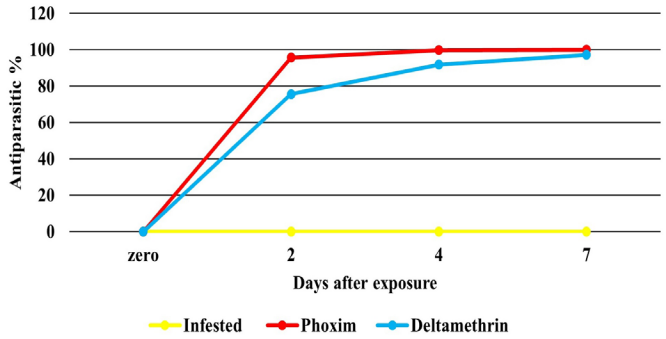

Fig. 9. Efficacy percentage of phoxim and deltamethrin on *Rhipicephalus sanguineus* adult *in vivo*.
Data are expressed as mean ± SE. Different superscripts on bars of the same time interval denoted significant difference at (P<0.05).

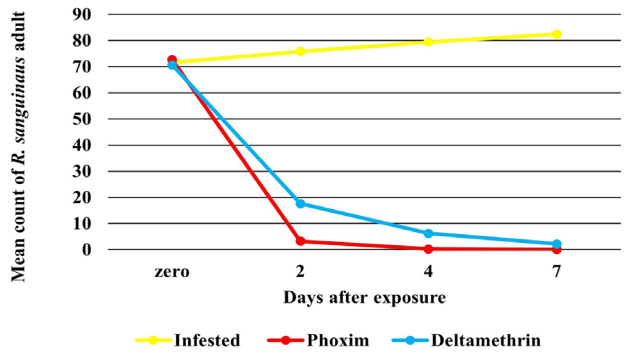

Fig. 10. The mean count of *Rhipicephalus sanguineus* adult *in vivo*.
Data are expressed as mean ± SE. Different superscripts on bars of the same time interval denoted significant difference at (P<0.05).

DISCUSSION

Ticks are considered the most important transporters of several pathogens (Kiss *et al*., 2012). *R. sanguineus* is the most common Ixodid tick species distributed in Egypt (Abdel-Shafy *et al*., 2012). The application of acaricides may significantly reduce the abundance of this tick species, and help to mitigate the risk of tick-borne diseases. However, the application of acaricides may lead to the development of tick resistance to several chemical compounds, which therefore require the regular monitoring of the acaricides in use (Malik *et al*., 2021).. We designed this study to evaluate the *In vitro* and vivo the efficacy of phoxim and deltamethrin against *R. sanguineus* in Ismailia governorate, Egypt.

The present findings indicated that phoxim was more efficacious when used on adult *R. sanguineus* compared to deltamethrin *In vitro* and *in vivo*; while deltamethrin was more efficacious when used on *R. sanguineus* nymphs and larvae, relative to phoxim. In contrast, there were non-significant differences in the efficacy of both acaricide compounds when used on *R. sanguineus* eggs. The results of these efficacy tests agree with the findings of Elbahy *et al*. (2011).

The hatchability of treated eggs showed the same efficacy for both acaricides after 14 days. This result disagreed with the findings reported by Bicalho *et al*. (2001), who recorded that deltamethrin had low efficacy against *R. sanguineus* eggs. The present study's results for phoxim on *R. sanguineus* eggs agreed with the findings of Elbahy *et al*. (2011).

Concerning the mode of action of the two acaricides, phoxim inhibits acetylcholinesterase at the synaptic junction, leading to accumulation of acetylcholine, and the prolongation of the mus-

carinic and nicotinic effects of acetylcholine in the autonomic nervous system, central nervous system, and in neuromuscular junctions (Eddleston, 2008). This differs from deltamethrin, which interacts with sodium channels, causing prolongation of depolarization in neurons (Chrustek *et al*., 2018). The explanation for the efficacy of both drugs in inhibiting egg hatching is due to the penetration of the drugs into the shells of eggs, and their effects on the larvae within the eggs by the same acaricidal mechanisms of action used in larvae and adults (Lima *et al*., 2020).

The explanation of the low efficacy of deltamethrin on the adult stage of *R. sanguineus* may be attributed to the development of varying degrees of resistance against deltamethrin. The degree of resistance observed may be due to a mutation of the sodium channel (Miller *et al*., 2001).This is likely because of the long-term administration, extensive use, and prevalent application of these compounds as acaricides and agricultural pesticides in the region (Mendes *et al*., 2011). Meanwhile, the efficacy of phoxim on the larval stage of the ticks seemed to be lower than its efficacy on adults, which could be explained by its correlation to the levels of acetylcholinesterase, which showed an increase in its activities related to the age of the tick (Bicalho *et al*., 2001).

Resistance to pyrethroids has been explained by many researchers, with possible explanations including metabolic detoxication, decreased cuticular penetration, and decreased target site sensitivity (Abbas *et al*., 2014), as well as mutations in voltage-sensitive sodium channels, and increased detoxification mediated by overexpression of cytochrome P450 monooxygenase (Pan *et al*., 2018). Additionally, insects with knockdown resistance have decreased pyrethroid target site (sodium channel) sensitivity due to one or more point mutations in the insect's sodium channel protein. Lastly, another possibility is mutations involving the substitution of a thymine by a cytosine within segment IV of domain III of the sodium channel gene (Yessinou *et al*., 2021). These mutations could confer sodium channel resistance to pyrethroids by reducing the binding of pyrethroids to the sodium channel and/or by counteracting the action of pyrethroids via a binding-independent mechanism (e.g., by altering sodium channel gating). Direct measurement of pyrethroid binding affinity and capacity in insect sodium channels has not yet been achieved. This is due to the high lipophilicity of pyrethroids, which results in extremely high levels of nonspecific binding to membranes and filters, and therefore masks any specific pyrethroid binding (Rossignol, 1988; Pauron *et al*., 1989).

CONCLUSION

Using control strategies to prevent the development of resistance in ticks against acaricides is important. Further studies are required to identify new safe and effective acaricides.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gilleard, J., Iqbal, Z., 2014. Acaricide resistance in cattle ticks and approaches to its management: the state of play. Veterinary parasitology 203, 6-20

Abdel-Daim, M.M., Abuzead, S.M., Halawa, S.M., 2013. Protective role of Spirulina platensis against acute deltamethrin-induced toxicity in rats. Plos One 8, e72991. https://doi.org/10.1371/journal.pone.0072991

Abdel-Shafy, S., Allam, N.A., Mediannikov, O., Parola, P., Raoult, D., 2012. Molecular detection of spotted fever group rickettsiae associated with Ixodid ticks in Egypt. Vector-Borne and zoonotic diseases 12, 346-359

Abdulaziz, A., El-Mahallawy, H., Almuzaini, A., Hassan, A., 2019. Comparative in-vitro efficacy of different acaricides for controlling ticks (Acari: Ixodidae) of veterinary and public health importance. Pakistan Science Mission veterinary research 4, 1-12. https://journals.psmpublishers.org/index.php/vetres/article/view/287

Adenubi, O., Fasina, F., McGaw, L., Eloff, J., Naidoo, V., 2016. Plant extracts to control ticks of veterinary and medical importance: a review. South African Journal of Botany 105, 178-193. https://doi.org/10.1016/j.sajb.2016.03.010

Akande, F.A., Adenubi, O.T., Garba, A.O., 2020. *In vitro* analysis of the efficacy of selected commercial acaricides on the cattle tick *Rhipicephalus* (Boophilus) *annulatus* (Acari: Ixodidae). Egyptian Journal of Veterinary Sciences 51, 153-161. https://dx.doi.org/10.21608/ejvs.2020.21560.1144

Arrnitage, P., Berry, G., 1987. Statistical rnethods in medical research. Blackwell Scientific Publications, (Oxford). https://doi.org/10.1002/sim.4780070711

Bicalho, K.A., Ferreira, F., Borges, L.M.F., Ribeiro, M.F.B., 2001. *In vitro* evaluation of the effects of some acaricides on life stages of *Rhipicephalus sanguineus* (Acari: Ixodidae). Arquivo Brasileiro de Medicina Veterinária e Zootecnia 53, 548-552. https://doi.org/10.1590/S0102-09352001000500006

Borges, L.M.F., Soares, S.F., Fonseca, I.N., Chaves, V.V., Louly, C.C.B., 2007. Resistência acaricida em larvas de *Rhipicephalus sanguineus* (Acari: Ixodidae) de Goiânia-GO, Brasil. 36, 87-95. http://repositorio.bc.ufg.br/handle/ri/166

Cavalleri, D., Murphy, M., Gorbea, R.L., Seewald, W., Drake, J., Nanchen, S., 2017. Laboratory evaluations of the immediate and sustained effectiveness of lotilaner (Credelio™) against three common species of ticks affecting dogs in Europe. Parasites and Vectors 10, 1-7

Chrustek, A., Hołyńska-Iwan, I., Dziembowska, I., Bogusiewicz, J., Wróblewski, M., Cwynar, A., Olszewska-Słonina, D., 2018. Current research on the safety of pyrethroids used as insecticides. Medicina 54, 61. https://doi.org/10.3390/medicina54040061

Dantas-Torres, F., 2008. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. Veterinary Parasitology 152, 173-185. https://doi.org/10.1016/j.vetpar.2007.12.030

Du, Y.-H., Jia, R.-Y., Yin, Z.-Q., Pu, Z.-H., Chen, J., Yang, F., Zhang, Y.-Q., Lu, Y., 2008. Acaricidal activity of extracts of neem (*Azadirachta indica*) oil against the larvae of the rabbit mite *Sarcoptes scabiei* var. cuniculi *In vitro*. Veterinary Parasitology 157, 144-148. https://doi.org/10.1016/j.vetpar.2008.07.011

Eddleston, M., 2008. The pathophysiology of organophosphorus pesticide self-poisoning is not so simple. The Netherlands Journal of Medicine 66, 146-148. https://pubmed.ncbi.nlm.nih.gov/18424860/

El Kammah, K., Oyoun, L., El Kady, G., Shafy, S.A., 2001. Investigation of blood parasites in livestock infested with argasid and ixodid ticks in Egypt. Journal of the Egyptian Society of Parasitology 31, 365-371. https://pubmed.ncbi.nlm.nih.gov/11478436/

Elbahy, N.M., Elbadr, A.-R.M., Abdul-Aziz, A.R., 2011. Identification and elimination of hard ticks infecting ruminants. Master Thesis. Faculty of Veterinary Medicine, Minufiya University, Egypt. https://www.researchgate.net/publication/272088450

Godara, R., Parveen, S., Katoch, R., Yadav, A., Verma, P., Katoch, M., Kaur, D., Ganai, A., Raghuvanshi, P., Singh, N., 2014. Acaricidal activity of extract of *Artemisia absinthium* against *Rhipicephalus sanguineus* of dogs. Parasitology Research 113, 747-754. https://doi.org/10.1007/s00436-013-3704-9

Ibelli, A., Ribeiro, A., Giglioti, R., Regitano, L., Alencar, M., Chagas, A., Paço, A., Oliveira, H.N.d., Duarte, J., Oliveira, M., 2012. Resistance of cattle of various genetic groups to the tick *Rhipicephalus microplus* and the relationship with coat traits. Veterinary Parasitology 186, 425-430. https://doi.org/10.1016/j.vetpar.2011.11.019

Kiss, T., Cadar, D., Spînu, M., 2012. Tick prevention at a crossroad: new and renewed solutions. Veterinary parasitology 187, 357-366.

Krishnaveni, S., Venkatalakshmi, P., 2014. Antimicrobial, larvicidal and acaricidal activities of the ethanolic extract of andrographis paniculata and *Carica papaya* leaves. World Journal of Pharmaceutical Research 3, 660-669. https://www.academia.edu/15465983

Lima, H.G.D., Santos, F.O., Santos, A.C.V., Silva, G.D.D., Santos, R.J.d., Carneiro, K.D.O., Reis, I.M.A., Estrela, I.D.O., Freitas, H.F.D., Bahiense, T.C., 2020. Anti-tick effect and cholinesterase inhibition caused by *Prosopis juliflora* alkaloids: *In vitro* and in silico studies. Revista Brasileira de Parasitologia Veterinária 29, e019819.https://doi.org/10.1590/S1984-29612020036

Malik, A., Afshan, K., Razzaq, A., Fatima, Z., Hussain, M., Firasat, S., 2021. Comparative efficacy of synthetic acaricides against tick infestations in goats. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 27, 159-164. http://DOI: 10.9775/kvfd.2020.24917

Marchiondo, A., Holdsworth, P., Green, P., Blagburn, B., Jacobs, D., 2007. World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines for evaluating the efficacy of parasiticides for the treatment, prevention and control of flea and tick infes-

tation on dogs and cats. Veterinary Parasitology 145, 332-344. https://doi.org/10.1016/j.vetpar.2006.10.028

Mencke, N., Volf, P., Volfova, V., Stanneck, D., 2003. Repellent efficacy of a combination containing imidacloprid and permethrin against sand flies (*Phlebotomus papatasi*) in dogs. Parasitology Research 90, S108-S111.https://doi.org/10.1007/s00436-003-0905-7

Mendes, M., Lima, C., Nogueira, A., Yoshihara, E., Chiebao, D., Gabriel, F., Ueno, T., Namindome, A., Klafke, G., 2011. Resistance to cypermethrin, deltamethrin and chlorpyriphos in populations of *Rhipicephalus* (Boophilus) *microplus* (Acari: Ixodidae) from small farms of the State of São Paulo, Brazil. Veterinary Parasitology 178, 383-388. https://doi.org/10.1016/j.vetpar.2011.01.006

Mihalca, A.D., Gherman, C.M., Cozma, V., 2011. Coendangered hard-ticks: threatened or threatening? Parasites and Vectors 4, 1-7. http://www.parasitesandvectors.com/content/4/1/71

Miller, R.J., George, J.E., Guerrero, F., Carpenter, L., Welch, J.B., 2001. Characterization of acaricide resistance in *Rhipicephalus sanguineus* (Latreille) (Acari: Ixodidae) collected from the Corozal army veterinary quarantine center, Panama. Journal of Medical Entomology 38, 298-302. https://doi.org/10.1603/0022-2585-38.2.298

Nicholson, W.L., Sonenshine, D.E., Noden, B.H., Brown, R.N., 2019. Ticks (ixodida), In: Medical and veterinary entomology. Elsevier: pp. 603-672. https://doi.org/10.1016/B978-0-12-814043-7.00027-3

Pan, J., Yang, C., Liu, Y., Gao, Q., Li, M., Qiu, X., 2018. Novel cytochrome P450 (CYP6D1) and voltage sensitive sodium channel (Vssc) alleles of the house fly (*Musca domestica*) and their roles in pyrethroid resistance. Pest management science 74, 978-986. https://doi.org/10.1002/ps.4798

Pauron, D., Barhanin, J., Amichot, M., Pralavorio, M., Berge, J.B., Lazdunski, M., 1989. Pyrethroid receptor in the insect sodium channel: alteration of its properties in pyrethroid-resistant flies. Biochemistry 28, 1673-1677. https://doi.org/10.1021/bi00430a037

Rohdich, N., Roepke, R.K., Zschiesche, E., 2014. A randomized, blinded, controlled and multi-centered field study comparing the efficacy and safety of Bravecto™(fluralaner) against Frontline™(fipronil) in flea-and tick-infested dogs. Parasites and Vectors 7, 1-5. http://www.parasitesandvectors.com/content/7/1/83

Rossignol, D., 1988. Reduction in number of nerve membrane sodium channels in pyrethroid resistant house flies. Pesticide Biochemistry and Physiology 32, 146-152. https://doi.org/10.1016/0048-3575(88)90007-7

Shyma, K., Kumar, S., Sangwan, A., Sharma, A.K., Nagar, G., Ray, D., Ghosh, S., 2013. Acaricide resistance status of *Rhipicephalus* (Boophilus) *microplus* and *Hyalomma anatolicum* collected from Haryana. Indian J. Anim. Sci. 83, 591-594. https://www.researchgate.net/publication/285530017_

Walker, A.R.,2003.Ticks of domestic animals in Africa: a guide to identification of species. Bioscience Reports Edinburgh, https://www.researchgate.net/publication/265412942_

Wang, G.-x., Han, J., Feng, T.-t., Li, F.-y., Zhu, B., 2009. Bioassay-guided isolation and identification of active compounds from Fructus Arctii against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). Parasitology research 106, 247-255

Worthing, C., Hance, R., 1991. The pesticide manual. A world compendium 9th edition. The British Crop Protection Council

Yessinou, R.E., Adoligbe, C., Atchade, F., Adinci, J., Brahi, H.D., Adehan, S., Mantip, S.E., Farougou, S., 2021. Phenotypic and genotypic characterization of dog tick *Rhipicephalus sanguineus* sensu lato (Acari: Ixodidae) resistance to deltamethrin in Benin. Veterinary Parasitology: Regional Studies and Reports 26, 100638. https://doi.org/10.1016/j.vprsr.2021.100638

Zaman, M.A., Iqbal, Z., Abbas, R.Z., Khan, M.N., Muhammad, G., Younus, M., Ahmed, S., 2012. *In vitro* and *in vivo* acaricidal activity of a herbal extract. Veterinary Parasitology 186, 431-436. https://doi.org/10.1016/j.vetpar.2011.11.018