PSM Veterinary Research ISSN(Online): 2518-2714

Research Article 2019 | Volume 4 | Issue 1 | 1-12

Comparative In-vitro Efficacy of Different Acaricides for Controlling Ticks (Acari: Ixodidae) of Veterinary and Public Health Importance

Amer Ragheb Abdulaziz[1]*, Heba S. El-Mahallawy[2], Abdulaziz M. Almuzaini[3], Amal A. Hassan[4]

[1]Department of Parasitology, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt.
[2]Department of Animal Hygiene, Zoonoses and Animal Behaviour and Management, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.
[3]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia.
[4]Department of Zoology, Faculty of Science, Damanhour University, Behera, Egypt.

Article Info

Open Access

Citation: Abdulaziz, A.R., El-Mahallawy, H.S., Almuzaini, A.M., Hassan, A.A., 2019. Comparative In-vitro Efficacy of Different Acaricides for Controlling Ticks (Acari: Ixodidae) of Veterinary and Public Health Importance. PSM Vet. Res., 4(1): 1-12.

Received: December 23, 2018

Accepted: March 12, 2019

Online first: March 19, 2019

Published: March 31, 2019

Corresponding Author:
Amer Ragheb Adel Aziz

Email:
amerragheb36@yahoo.com

Abstract:
Ticks are hematophagous ectoparasites parasitizing farm animals causing great economic losses worldwide, they can also transmit several zoonotic diseases to humans. Tick infestation is a big problem faced by livestock breeders especially the smallholders in Egypt. The efficacy of six commercial acaricides; Cyfluthrin, Deltamethrin, Lambodcyhalothrin, Esbiothrin, Chlorpyrifos, and Malathion were tested in-vitro for controlling the most prevalent tick species in Egypt namely; *Boophilus annulatus, Hyalomma dromedarii,* and *Ripicephalus sanguineus*. Ticks were collected from cattle, camels, and dogs, and tested with the targeted acaricides through the larval packet test (LPT) and the disposable pipette method using the recommended concentration and dose by the manufacturer. Resistance level against acaricides was categorized as I, II, III and IV, based on the resistance factor calculated from probit analysis of the obtained data. Cyfluthrin was the most efficacious acaricide against adult and larval stages of all tick species, however, the other five acaricides showed level II of resistance for the larval stage, and level III of resistance for adult ticks ($P \leq 0.05$). All the used acaricides used showed a better effect on the larval stages than on the adult ticks, and generally the effect was higher with longer exposure time ($P \leq 0.05$). The obtained data in this study proved the excellent efficacy of Cyfluthrin as an acaricide and pay the attention for its use by the governmental authorities and the veterinarians in their clinics to control *Boophilus annulatus, Hyalomma dromedarii*, and *Ripicephalus sangueneus*, in cattle, camels and dogs in Egypt.
Keywords: Resistance, Acaricides, Cyfluthrin, Deltamethrin, Chlorpyrifos, Malathion, *Boophilus annulatus, Hyalomma dromedarii*, and *Ripicephalus sangueneus.*

INTRODUCTION

Ticks are among the important ectoparasites parasitizing farm animals causing great economic losses in tropics and sub-tropics worldwide. Also they can transmit several tick-borne diseases (TBD) to animals and some zoonotic diseases to humans. The problem is most commonly elucidated in areas with tropical and semitropical climates, especially Egypt, due to warm and humid environment suitable for propagation of ticks. *Boophilus annulatus*, *Hyalomma dromedarii*, and *Ripicephalus sangueneus* are the most prevalent Ixodid tick species distributed in Egypt (Abdel-Shafy *et al.,* 2012). In Egypt, *Boophilus annulatus* is the main cattle tick's species responsible for transmission of *Babesia bovis*, *B. bigemina*, and *Anaplasma spp*. that causes great economic losses in beef and dairy production (Soulsby 1982; Ram *et al.,* 2004). *Hyalomma dromedarii*, the main hard tick species of camels in Egypt, reported to be the main vector of Coxiella burnetii, the causative agent of zoonotic Q fever worldwide (Abdullah *et al.,* 2018). Moreover, this tick spp. transmits *Theileria annulata* the causative agent of tropical theileriosis and the Dhori virus (DHOV) that was originally reported from camels in India, Egypt, Portugal, Russia, and Transcaucasia; causing death of the affected animals and eventually resulted in economic losses (Coetzer and Tustin, 2004). *Ripicephalus sangueneus* ticks of dogs are vectors for *Babesia canis* and *Ehrlichia canis*. Additionally, it's an important reservoir for many human pathogens; *Ehrlichia chaffeensis*, *E. ewingii*, *E. phagocytophila* group and *Rickettsia conorii* in areas with temperate climates and in urban environments resulted from expansion of tick range during movement of sub-clinically infected dogs into non-endemic areas (Shaw *et al.,* 2001).

Controlling these tick species is the cornerstone in combating tick borne diseases of veterinary and public health importance worldwide. Generally, several types of organophosphate (OP), and synthetic pyrethroid (SP) have been used in eradication and elimination of hard ticks of cattle, camels, and dogs worldwide (Jyoti *et al.,* 2016). Unfortunately, there are reports on development of different resistance levels by *Boophilus spp*, *Hyalomma spp* and *Ripicephalus spp* against these acaricides in several countries worldwide (Vatsya and Yadav, 2011; Sharma *et al.,* 2012; Kumar *et al.,* 2013; Singh and Rath, 2014, Sudha *et al.,* 2018).

Sohag Governorate is situated in the Upper Egypt occupying around 125 km long from the Nile Valley with about 16 to 20 km width. Based on complains from animal owners', farmers and veterinarians in the study area; a lot of animals were showing repeated attack of tick infestation despite of frequent treatment with different acaricides, leading to wasting money in treatment and losses in dairy and milk production. To our knowledge, until now; there is no clear data about the resistance and efficacy status of (SP), and (OP) compounds which are commonly used in controlling hard ticks from cattle, camels, and dogs in southern Egypt. In this context, the aim of this study was to evaluate the efficacy and the developed resistance level for six commercial acaricides commonly used in Egypt, namely; Cyfluthrin, Deltamethrin, Lambodcyhalothrin, Esbiothrin, Chlorpyrifos, and Malathion on adult and larval stages of *Boophilus annulatus*, *Hyalomma dromedarii*, and *Ripicephalus sangueneus* in the study area.

MATERIALS AND METHODS

Ethical consideration

The study followed the institutional ethical and animal care guidelines. All methods were conducted in accordance with the Guide for the Care and Use of Laboratory Animals at Sohag University on October 14, 2015, Egypt.

Acaricides

The following selected acaricides that are used in the veterinary and agricultural fields in Egypt were chosen and used the commercial concentration as follow: Cyfluthrin (Challenge-pure® 5% E.C., Mam pure for medical and chemical comp., Egypt), Lambodcyhalothrin (Lambada® 5% E.C. Greater Kalash, New Delhi), Chlorpyrifos (PESTBAN®48%, Watanya for Agricultural chemicals and investment company, El Behera, Egypt), Deltamethrin (Butox® 50 EC, Intervet production, France), Esbiothrin (Hi-Sol®0.1%, New Masr comp. for insect., Egypt), and Malathion (Malathion 57% EC, KFZ. comp. Egypt).

Collection and preparation of adult ticks and larvae

Fully engorged females of *Boophilus annulatus, Hyalomma dromedarii, and Ripicephalus sangueneus* were collected manually by blunt forceps from the infested cattle, camels, and dogs, respectively, in Sohag, Egypt. The collected ticks were from animals that were not previously exposed to any acaricides based on the farm history. These ticks were used to produce larvae to be tested in this study.

On the other hand, another group of male and female ticks were collected from infested animals in farms and veterinary clinics that were previously treated with one or more of the commercial acaricides used in this study. The collected ticks were placed into clean labeled plastic bags and transferred directly to the laboratory of parasitology department, Faculty of Veterinary Medicine, Sohag University, for tick identification and experimental procedures.

At the laboratory, ticks were identified based on the morphological characters (Walker *et al.,* 2003). The identified tick spp. was washed thoroughly with water, air dried, and incubated at 28-30˚C and 85-90% relative humidity in sterile labeled 15cm petri-dish for oviposition. The laid eggs could hatch into larvae under the same conditions. Larvae of 18 days old were collected by paint brush for larval packet test (LPT). Each one hundred larvae were collected separately to be tested with the acaricide (Shyma *et al.,* 2013).

Testing acaricide efficacy on larvae

This was performed using larval packet test on triplicates. Each one hundred 18 days old larvae were placed onto 11cm diameter Whatman filter paper (No.1) previously soaked with the tested acaricide in labelled plastic petri dishes, however, for the control group the paper was soaked in water. Three petri dishes were prepared of each acaricide product and for the control group for each of the tick species under study (Koch *et al.,* 1984). Four sets of the previously described petridishes were prepared to report larval death rate at 3, 9, 12 and 24-hour intervals. For each time interval, the death rate of larvae was calculated by dividing the number of dead larvae by the total number of larvae in each packet; where larvae that still move their legs were considered alive. Resistance percentage to the tested acaricide was calculated from the following equation: Resistance (%) = (Nt/Nw) × 100; where Nt = Number of alive treated ticks' larvae and Nw = Number of untreated ticks larvae (Shyma *et al.,* 2013).

Testing acaricide efficacy on adult ticks

The acaricides evaluation procedure for adult ticks was performed using the disposable pipette method on triplicates (Barnard *et al.,* 1981). Briefly, a volume of 0.5 ml from each acaricide product was transferred by pipette into 16ml size glass vials. Each vial was sealed with cap and rotated to allow complete distribution of the acaricide on the inner surface of the vial. Finally, the excess fluid was poured, and the vial left to air dry. For each coated glass vial; 100 adult ticks (50 male and 50 female) were added and mixed by moving them inside the tube, then closed with perforated plastic lid and incubated at 28-29°C, and 85-90% relative humidity. A negative control group using water was prepared for each acaricide product and for each of the tick species. Four sets of the previously described tubes were prepared to report tick death rate at 3, 9, 12 and 24 hour intervals. For each time interval, the death rate of ticks was assessed by physical pressure on the tick underside with a pair of forceps; if legs not extended, or retracted, the tick was considered dead. The ideal criterion for each acaricides efficacy was identified as the rapid killing within 24 hrs.

Statistical analysis

Reading of each test was recorded as the average of each triplicate. The death rate of larvae and adult ticks was given as percentages. Comparison and significant correlation between different groups was performed using the Paired Samples T test using the IBM-SPSS Statistics software (SPSS Inc. 2007). P value ≤0.05 was considered statistically significant at 95%.

Calculation of Resistance level

The obtained data were analyzed by SPSS software to detect the resistance factor (RF), and the resistance level (RL) in both larvae and the adult tick population and it was classified as Susceptible (S) when (RF≤1.4), resistant level I if (RF=1.5-5), resistant level II at (RF=5.1-25), resistant level III at (RF=25.1-40) and resistant level IV (RF>40) according (Shyma *et al.,* 2013).

RESULTS

The collected and identified tick species used in this study were *Boophilus annulatus* from cattle and buffaloes*, Hyalomma dromedarii* from camel*,* and *Ripicephalus sangueneus* ticks from dogs. Results of in-vitro larval packet test showed susceptibility of all tick species' larvae to Cyfluthrin, however, they showed level II resistance against the other five tested acaricides, as shown in Figure 1, and Table 1.

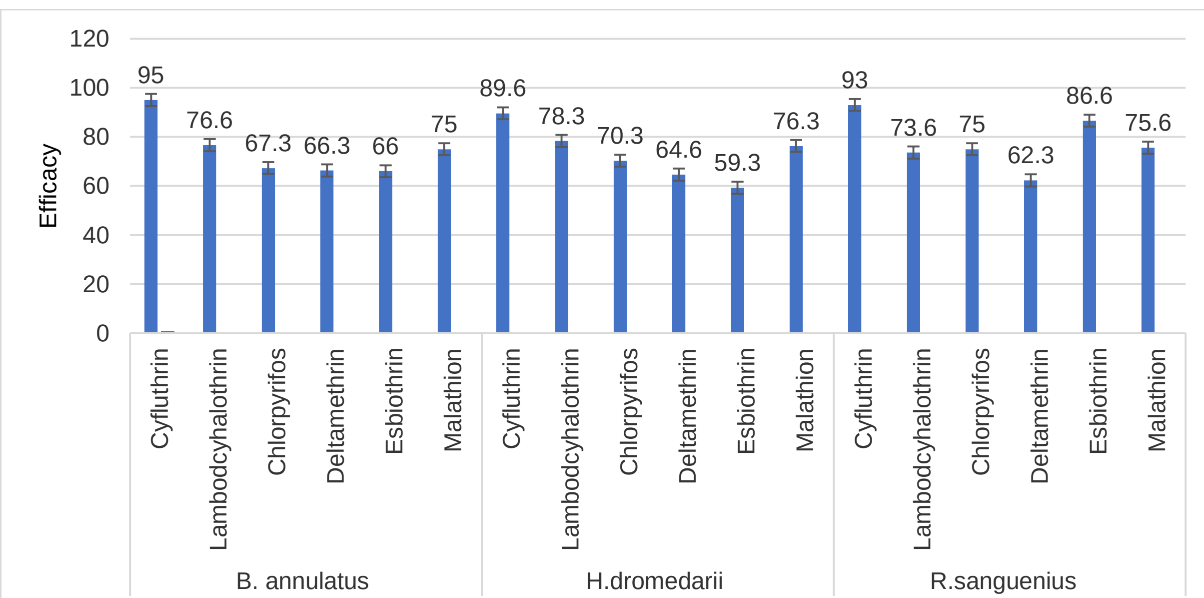

Fig. 1. Comparative Efficacy of different acaricides by larval packet test on field tick larvae after 24 hrs. exposure time.

Table 1. Comparative Efficacy of different acaricides by larval packet test on field tick larvae in Egypt

Tick species	Acaricide	Efficacy percentage after exposure times to differing acaricides (%)				Resistance %	Resistance factor	Resistance level
		3hrs	9hrs	12hrs	24hrs			
B. annulatus	Cyfluthrin	73.3	79	86.3	95	5.0	00.99	S
	Lambodcyhalothrin	55.6	63.3	69.6	76.6	23.4	5.03	I
	Chlorpyrifos	41	46.3	61.0	67.3	32.7	9.99	II
	Deltamethrin	45.6	55	56.3	66.3	33.7	9.03	II
	Esbiothrin	43	49.6	59.6	66	34	5.08	II
	Malathion	64.3	70	73	75	25.0	3.82	II
H.dromedarii	Cyfluthrin	63	86	88.6	89.6	10.4	1.30	S
	Lambodcyhalothrin	56.3	66.3	72.3	78.3	22.7	5.01	II
	Chlorpyrifos	43.3	47.3	64.3	70.3	29.7	6.99	II
	Deltamethrin	40.6	53.3	54.3	64.6	35.4	10.3	II
	Esbiothrin	36.6	42.3	49.6	59.3	40.7	14.4	II
	Malathion	67	72	74.3	76.3	23.7	5.06	II
R.sanguenius	Cyfluthrin	71.6	77	83	93	7.0	1.09	S
	Lambodcyhalothrin	52.3	64.3	68	73.6	26.4	6.05	II
	Chlorpyrifos	44.6	53	73	75	25.0	6.99	II
	Deltamethrin	43	52.6	53.6	62.3	37.7	11.5	II
	Esbiothrin	49.6	52.6	74.6	86.6	13.4	1.6	I
	Malathion	65.3	71.3	74	75.6	24.4	5.78	II
Control	Water	0	0	0	0	00		

Note: the resistance level was represented as follow: if the resistance factor (RF≤1.4), the acaricide id considered susceptible, and if R.F is more than 1.4 it is considered resistant and devided into levels as follow: level I of resistance if (RF=1.5-5), level II if (RF=5.1-25), level III if (RF=25.1-40) and level IV if (RF>40)

Results of disposable pipette method, for all adult tick species, revealed their susceptibility only to Cyfluthrin. Deltamethrin and Lambodcyhalothrin showed 3rd level of resistance to *Hyalomma dromedarii*, and *Ripicephalus sangueneus,* and 2nd level resistance for *Boophilus annulatus.* While all other tested acaricides resulted in 3rd level of resistance, figure 2, and Table 2. Overall, larvae and adult stages of all examined tick species were susceptible to Cyfluthrin, however, they showed a variable degree of resistance against the other five acaricides (P≤0.05). At the tick species level, Cyfluthrin showed the highest efficacy (95% and 91.7%) after 24 hrs. on both larvae and adults of *Boophilus annulatus* followed by *Ripicephalus sangueneus* and *Hyalomma dromedarii*, however, Esbiothrin showed the least efficacy on all species of adult ticks and *Boophilus annulatus* and *Hyalomma dromedarii* larvae figure 1, and 2.

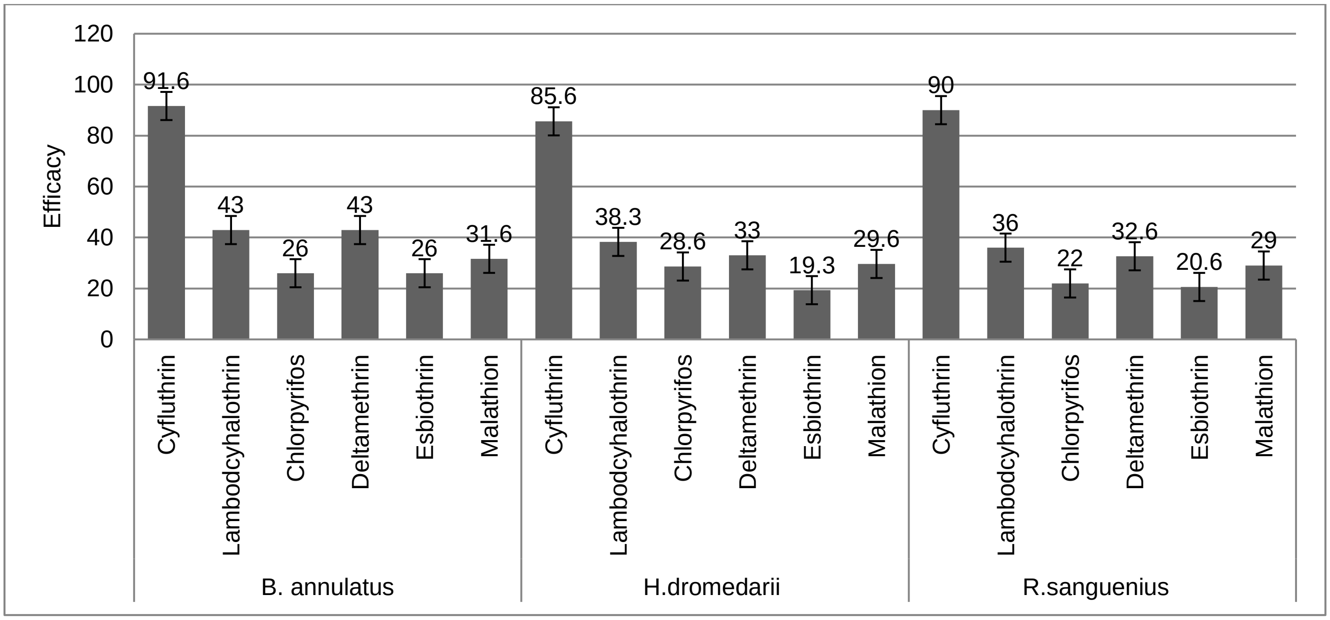

Fig. 2. Comparative Efficacy of different six acaricides on Adult ticks in vitro after 24hrs. exposure time.

Regarding the tick developmental stage, the efficacy of each of the acaricides used was higher on larvae than the adult ticks (P≤0.05) Table 3. Overall, the longer the exposure time to any of the acaricides, the more and more efficacy observed (P≤0.05) (Table 3).

DISCUSSION

Boophilus annulatus, Hyalomma dromedarii, and *Ripicephalus sangueneus* are the most prevalent Ixodid tick species distributed in Egypt (Abdel-Shafy *et al.,* 2012), causing great economic losses in animals and transmitting several zoonotic diseases to humans. Based on complains from animal owners', farmers and veterinarians about acaricide failure, we designed this study to evaluate the efficacy and the developed resistance level to the commonly used acaricides in controlling ticks in the study area.

Results obtained in this study coincided with many previous reports regarding developing different resistance levels in both larvae and adults of *Boophilus annulatus, Hyalomma dromedarii,* and *Ripicephalus sangueneus* against different acaricides. In this study, Deltamethrin 5% showed level II resistance in *Boophilus spp, Hyalomma spp*, and *Ripicephalus sangueneus*. This was noted in reports from earlier studies (Pradeep *et al.,* 2010; Sharma *et al.,* 2012; Shyma *et al.,* 2012; Shyma *et al.,* 2013; Singh *et al.,* 2013; Singh *et al.,* 2015; Kumar *et al.,* 2017; Sudha *et al.,* 2018).

Table 2. Comparative Efficacy of different acaricides on Adult ticks.

Tick species	Acaricide	Efficacy percentage after exposure times to differing acaricides %				Resistance %	Resistance factor	Resistance level
		3hrs	9hrs	12hrs	24hrs			
B. annulatus	Cyfluthrin	51.3	56.3	63.0	91.6	8.4	1.05	S
	Lambodcyhalothrin	22.3	30	33.6	43	57	23.4	II
	Chlorpyrifos	7.6	13	14.3	26	74	29.02	III
	Deltamethrin	19	21.6	33	43	57.7	24	II
	Esbiothrin	9.6	13	16	26	74	32	III
	Malathion	24.3	26.6	28.6	95	68.4	28.02	III
H. dromedarii	Cyfluthrin	44.6	49.3	55.3	85.6	14.4	1.39	S
	Lambodcyhalothrin	23	29.6	32.3	38.3	62.7	25.1	III
	Chlorpyrifos	10	14	17.6	28.6	71.4	30.3	III
	Deltamethrin	14	20	23	33	67	27	III
	Esbiothrin	8.6	9.6	13	19.3	80.7	35	III
	Malathion	21	22	24.3	29.6	70.3	30	III
R. sanguenius	Cyfluthrin	48	52	57.3	90	10	1.19	S
	Lambodcyhalothrin	19	24.3	28	36	64.4	26.2	III
	Chlorpyrifos	11.3	36.3	16.3	22	78.0	33.01	III
	Deltamethrin	16	19.6	21.3	32.6	67.4	27.1	III
	Esbiothrin	10	11.3	15	20.6	79.4	34	III
	Malathion	22	28	28	29	71	30.2	III
Control	Water	0	0	0	0	00		

Note: the resistance level was represented as follow: if the resistance factor (RF≤1.4), the acaricide id considered susceptible, and if R.F is more than 1.4 it is considered resistant and devided into levels as follow: level I of resistance if (RF=1.5-5), level II if (RF=5.1-25), level III if (RF=25.1-40) and level IV if (RF>40)

Table 3. statistical analysis by the paired-samples T test revealed the correlation and significance between the variables used in this study.

Variables		Paired Differences: Mean	Std. Dev.	Std. Error Mean	95% Confidence Interval of the Difference: Lower	Upper	T	Correlation	Sig.
Pair 1	Acaricides - Efficacy	-42.88333-	23.86579	1.98882	-46.81461-	-38.95206-	-21.562-	-.293-*	.000*
Pair 2	Acaricides - Exposure	1.00000	2.04837	0.17070	0.66258	1.33742	5.858	0.000	1.000
Pair 3	Tick - Efficacy	-44.38333-	23.32168	1.94347	-48.22498-	-40.54169-	-22.837-	0.000	.995
Pair 4	Tick - Acaricides	-1.50000-	1.89958	0.15830	-1.81291-	-1.18709-	-9.476-	0.000	1.000
Pair 5	Efficacy - Exposure	43.88333	23.00371	1.91698	40.09406	47.67260	22.892	.293*	.000*
Pair 6	Efficacy - Stage	44.88333	23.70064	1.97505	40.97926	48.78741	22.725	-.779-*	.000*

This table indicates the correlation between efficacy and type of acaricide and it seens to be significantly correlated also there is a significant correlation between efficacy and exposure time as the time of exposure increase the efficacy increased significantly, also there is a significant correlation between the efficacy and stage of ticks as larvae more sensitive that adult to diffenet acaricides. But there is no any correlation between efficacy and tick spp, no correlation between acaricides type and exposure time, also no correlation between tick spp. and acaricide type at 95% significance level.

In the present study, results of larval packet test revealed development of level I resistance against Esbiothrin in *R. sangueneus* and level II resistance against Lambodcyhalothrin, Chlorpyrifos, Deltamethrin and Malathion in all tick species larvae. However, larvae from all tick species were susceptible to Cyfluthirn (Table 1). The same pattern was recently observed in Egypt, where *B. annulatus* was resistant to Deltamethrin (Aboelhadid *et al.,* 2018). Also resistance to pyrethroid acaricides has been detected worldwide. In Kerala, South India, *B. annulatus* were showing level I resistance to deltamethrin (Jyothimol *et al.,* 2014). Moreover, resistance to cypermethrin has been detected in *B. annulatus* from Mazandaran Province, Northern Iran (Ziapour *et al.,* 2017). Also, tick control failure using Alpha cypermethrin has been detected in *B. geigyi* from Burkina Faso (Adakal *et al.,* 2013).

Ticks revealed level II of resistance against Lambodcyhalothrin 5% and level III of resistance against Chlorpyrifos 48%, Esbiothrin 1.0%, and Malathion 57%. Similar results were obtained in a previous report (Dantas-Torres, 2008) where synthetic pyrethroids such as Deltamethrin, Lambodcyhalothrin, and Esbiothrin developed different levels (I-II-II-IV) of resistance that varies with the population of *Rhipicephalus sanguineus*. This may be due to the presence of esterases enzymes which are involved in the resistance of tick species to pyrethroid acaricides (Miller *et al.,* 2001).

Chlorpyrifos and Malathion is an organophosphate insecticide which acts as an acetylcholinesterase inhibitor, our study results agreed with Dutta et al. (2017) in the fact of resistance but with a different level; they studied the resistance levels against malathion in *Rhipicephalus* and *Boophilus microplus* collected from four districts of Jammu region (India) using the adult immersion test, and with Jyoti et al. (2014), who studied the resistance level in *Rhipicephalus (Boophilus) microplus* and Jyoti et al. (2015), studied the resistance in *Hyalomma anatolicum anatolicum* from Punjab, and the resistance factor (RF) was 3.97 which indicated level I resistance status to malathion, they found that; Resistance to malathion was detected at level I, but resistance level of local Egyptian isolate reach III.

Overall, this high level of resistance in the study area may be due to prolonged, intensive and widespread use of such compounds as both acaricide and an agricultural pesticide in the investigated area. Also, the inadvertent selection of the acaricide product which is mainly depending on farmers' suggestions to each other's (Mendes *et al.,* 2011), leading to acaricide failure. Moreover, haphazard purchasing of acaricides from local drug sources without previuos veterinary prescription is commonly practiced in Egypt.

Generally, Cyfluthrin 5% was the only product showing high degree of susceptibility against larvae and adults of all the studied tick species in this study. Similar findings proved by (Sudha, 2018). This may be because of uncommon use of such acaricide compound routinely in tick control strategy by local and governmental authorities in Egypt.

In this study, there was a significant correlation between the efficacy of the used acaricide and the tick developmental stage in all the studied tick spp. (Table 1, 2), where larvae showed higher susceptibility than adult ticks. This may be because larvae have differences in enzymatic groups responsible for cellular resistance mechanisms and detoxification that present in adult ticks. Moreover, development of several mutations that increase tolerance to chemical compounds are less developed in larvae (Daniela, 2005). This finding may be helpful in designing a successful control and eradication program of ticks in the study area in Egypt.

Results obtained in the present study show development of resistance to commonly used acaricides in Egypt and recommend the use of Cyfluthrin in tick control and eradication programs by the governmental authorities and veterinarians in their clinics against *Boophilus annulatus, Hyalomma dromedarii,* and *Ripicephalus sangueneus* in cattle, camels and dogs. Farmers and livestock breeders should be informed about proper selection and use of acaricides and relying on the manufacturer instructions to avoid development of resistance on the long run.

ACKNOWLEDGMENT

I would like to thank Professor Dr. Ibrahim Kafrawy, Assistant professor of Biostatistics, Faculty of veterinary medicine, Sadat city University, for his smart cooperation in the processing and analysis of obtained data, and to all veterinarian and staff members of parasitology department in faculty of veterinary medicine, Sohag university that help in sample collection, labelling, and processing, and for companies that provides us with free samples of acaricides for testing.

Financial Support and Sponsorship

Nil.

CONFLICT OF INTEREST

All the authors have declared that no conflict of interest exists.

REFERENCES

Aboelhadid, S.M., Arafaa, W.M. Mahrousa, L.N., Fahmy, M.M., Kamela, A.A., 2018. Molecular detection of *Rhipicephalus (Boophilus) annulatus* resistance against deltamethrin in middle Egypt. Vet. Parasitol.: Reg. Stud. Rep., (13): 198-204.

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 Zoonotic Dis.;12(5): 346-59.

Abdullah, H.H.A.M., El-Shanawany, E.E., Abdel-Shafy, S., Abou-Zeina, H.A.A., Abdel-Rahman, E.H., 2018. Molecular and immunological characterization of *Hyalomma dromedarii* and *Hyalomma excavatum* (Acari: Ixodidae) vectors of Q fever in camels. Vet. World., 11(8): 1109-1119.

Adakal, H., Stachurski, F., Chevillon, C., 2013. Tick control practices in Burkina Faso and acaricide resistance survey in *Rhipicephalus (Boophilus) geigyi* (Acari: Ixodidae), Exp. Appl. Acarol. 59: 483–491, http://dx.doi.org/10.1007/s10493-012-9610-5.

Barnard, D.R., Jones, B.G., Rogers, G.D., Mount, G.A., 1981. Acaricide susceptibility in the lone star tick: assay techniques and baseline data. J. Econ. Entomol., 74: 466–469.

Coetzer, J.A.W., Tustin, R.C., 2004. Infectious diseases of Livestock (2nd edition). Oxford University Press**.**

Dantas-Torres, F., 2008. The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae): from taxonomy to control. Vet. Parasitol., 152:173–185. DOI: 10.1007/s12639-013-0322-5.

Dutta, S., Godara, R., Katoch, R., Yadav, A., Katoch, M., Singh, N.K., 2017. Detection of amitraz and malathion resistance in field populations of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in Jammu region of India. Exp. Appl. Acarol., 71(3): 291-301. doi: 10.1007/s10493-017-0111-4.

Freitas, D. R. J. de, Pohl, P. C., Vaz Junior, I. da S., 2005. Characterization of acaricide resistance in *Boophilus microplus*. Acta Scientiae Veterinariae. 33(2): 109-117, 2005.

Jyoti, Singh, N.K., Prerna, M., Singh, H., Rath, S.S., 2015. Detection of Malathion Resistance in *Hyalomma anatolicum anatolicum* from Bathinda District, Punjab. Toxicol. Int.. 22(1): 125-9.

Jyothimol, G., Ravindran, R., Juliet, S., Ajithkumar, K.G., Suresh, N.N., Vimalkumar, M.B., Lenka, D.R., Varghese, S., Ghosh, S., 2014. Low level deltamethrin resistance in ticks from cattle of Kerala, a south Indian state. Vet. Parasitol., 204(3-4):433-438.

Jyoti, Singh, N.K., Singh, H., Rath, S.S., 2016. Multiple mutation in the acetylcholinesterase 3 gene associated with organophosphate resistance in *Rhipicephalus (Boophilus) microplus* tick from Punjab, India. Vet. Parasitol., 216: 108–117.

Jyoti, Singh, N.K., Singh, H., Rath, S.S., 2014. Malathion resistance in *Rhipicephalus (Boophilus) microplus* from Ludhiana district, Punjab. J. Parasit. Dis., 38(4):343-346.

Koch, H. G., Burkwhat, H.E., 1984. Susceptibility of the brown dog tick (Acari: Ixodidae) to fresh residues of acaricides: laboratory assays and comparisons of susceptibility of different life stages of the lone star and American dog tick. J. Econ. Entomol., 77: 670–674.

Kumar, R., Nagar, G., Sharma, A.K., Kumar, S., Ray, D.D., Chaudhuri, P., Ghosh, S., 2013. Survey of pyrethroids resistance in Indian isolates of *Rhipicephalus (Boophilus) microplus*: identification of C190A mutation in the domain II of the para-sodium channel gene. Acta Trop., 125: 237–245.

Kumar, S.S., Rayulu, V.C., Rao, K.S., Kumar, N.V., 2017. Acaricidal resistance in *Rhipicephalus (Boophilus) Microplus* ticks infesting cattle of Andhra Pradesh. J Entomol. Zool. Stud., 5(6): 580-584.

Mendes, M.C., Lima, C.K., Nogueira, A.H., Yoshihara, E., Chiebao, D.P., Gabriel, F.H., Ueno, T.E., Namindome, A., Klafke, G.M., 2011. Resistance to cypermethrin, deltamethrin and chlorpyriphos in populations of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) from small farms of the State of Sao Paulo, Brazil. Vet. Parasitol., 178(3-4):383-388.

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. J. Med. Entomol., 38:298–302.

Pradeep, B.S., Renukaprasad, C., D'souza, P.E., 2010. Evaluation of the commonly used acaricides against different stages of *Rhipicephalus sanguineus* by in vitro tests. J. Vet. Parasitol., 24:185-188.

Ram, H., Yadav, C.L., Banerjee, P.S., Kumar, V., 2004. Tick associated mortality in crossbred cattle calves. Indian Vet. J., 81:1203–1205.

Sharma, A.K., Kumar, R., Kumar, S., Nagar, G., Singh, N.K., Rawat, S.S., Ghosh, S., 2012. Deltamethrin and cypermethrin resistance status of *Rhipicephalus (Boophilus) microplus* collected from six agro-climatic regions of India. Vet. Parasitol., 188(3): 337-345.

Shaw, S.E., Day, M.J., Birtles, R.J., Breitschwerdt, E.B., 2001. Tick-borne infectious diseases of dogs. Trends Parasitol., 17(2): 74-80.

Shyma, K.P., Kumar, S., Sangwan, A.K., Sharma, A.K., Nagar, G., Ray, D., 2013. Acaricide resistance status of *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Haryana. Indian. J. Anim. Sci., 83(6): 591-594.

Shyma, K.P., Kumar, S., Sharma, A.K., Ray, D.D., Ghosh, S., 2012. Acaricide resistance status in Indian isolates of *Hyalomma anatolicum*. Exp. Appl. Acarol., 58: 471-481.

Singh, N.K., Gelot, I.S., Jyoti, Singh, V., Rath, S.S., 2015. Detection of amitraz resistance in *Rhipicephalus (Boophilus) microplus* from North Gujarat, India. J. Parasitic Dis., 39(1): 49-52.

Singh, N.K., Jyoti, Rath, S.S., 2013a. Detection of acaricidal resistance in *Hyalomma anatolicum anatolicum.* India. Vet. J., 90:17-19.

Singh, N.K., Rath, S.S., 2014. Esterase mediated resistance against synthetic pyrethroids in field populations of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in Punjab districts of India. Vet. Parasitol., 204: 330–338.

Soulsby, E.J.L., 1982. Helminths, arthropods and protozoa of domesticated animals, 7th edn. Bailliere Tindal, London, pp 464–465.

SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.

Sudha, R.R., D'Souza, P.E., Byregowda, S.M., Veeregowda, B.M., Sengupta, P.P., Chandranaik, B.M., Thimmareddy, P.M., 2018. In vitro acaricidal efficacy of deltamethrin, cypermethrin and amitraz against sheep ticks in Karnataka. J. Entomol. Zool. Stud., 6(3): 758-762.

Vatsya, S., Yadav, C.L., 2011. Evaluation of acaricide resistance mechanisms in field populations of *Rhipicephalus (Boophilus) microplus* collected from India. Int. J. Acarol., 37: 405–410.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A., Pegram, R.G., Preston, P.M., 2003. Ticks of Domestic Animals in Africa. A Guide to Identification of Species. pp 74-221, Bioscience Reports, London, UK.