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RESEARCH ARTICLE

Comparative Efficacy of Synthetic Acaricides Against Tick Infestations in Goats

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Abstract

Four commercial synthetic compounds, pyrethroid, organophosphates, macrocyclic lactones and phenylpyrazole have been used for tick control worldwide. However, periodic monitoring of the effectiveness of acaricides has not been fully explored, although such information could contribute to a more effective application, economic analysis and harmful impact on other organisms and environmental contamination. This study investigates the effect of cypermethrine (CYM), deltamethrin, trichlorphon + dimethylester, ivermectin (IVM) and fipronil on natural infestations of ticks in goats. The *in vivo* quantitative assessment of four tick genera i.e. *Hyalomma, Rhipicephalus, Ixodes* and *Haemaphysalis* revealed that both CYM and IVM treated groups resulted in significantly lower ($P<0.05$) tick counts relative to other compounds and controls on all post-treated days. The maximum reduction in the mean number of ticks in the CYM and IVM treated group was recorded from days 3 to 4, followed by complete shedding of ticks on day 5. However, deltamethrin, trichlorphon + dimethylester and fipronil showed 100% efficacy on the sixth day. *In-vitro* efficacy trials showed a 100% tick's mortality based upon the use of fipronil (0.25 g/100 mL) within the 18th h in the post-treated group, while deltamethrin, trichlorphon + dimethylester and CYM were ranked 2nd, 3rd and 5th based on their 100% efficacy within 24-33 h, 33-42 h and 39-48 h, respectively. The investigation has shown that tested acaricides varied in their efficacy to reduce the tick infestation and further experiments on different formulations of the other members of the major acaricidal classes need to be standardized.

Keywords: *Acaricides, Efficacy trials, Goats, Tick*

Keçilerde Kene Enfestasyonuna Karşı Sentetik Akarisitlerin Karşılaştırmalı Etkinliği

Öz

Dünya genelinde kene kontrolü için piretroid, organofosfatlar, makrosiklik laktonlar ve fenilpirazol olmak üzere dört ticari sentetik bileşik kullanılmıştır. Fakat, akarisitlerin etkinliği periyodik olarak tam olarak izlenmemiştir ki bu tür bilgiler daha etkili bir uygulamaya, ekonomik analize ve diğer organizmalar ve çevresel kontaminasyon üzerindeki zararlı etkilere katkıda bulunabilir. Bu çalışmada, sipermetrin (CYM), deltametrin, triklorfon + dimetilester, ivermektin (IVM) ve fipronil'in keçilerde doğal kene enfestasyonu üzerine etkisi araştırılmıştır. *Hyalomma, Rhipicephalus, Ixodes* ve *Haemaphysalis* gibi dört kene cinsi üzerinde yapılan *in vivo* kantitatif değerlendirmede hem CYM hem de IVM ile sağaltılan grupların, tedavi sonrası tüm günlerde diğer bileşiklere ve kontrollere oranla önemli ölçüde daha düşük kene popülasyonuna sahip olduğu saptanmıştır ($P<0.05$). CYM ve IVM ile tedavi edilen gruplardaki ortalama kene sayısındaki maksimum azalma, 3 ile 4. günler arasında kaydedilmiş, takiben 5. günde kenelerin tamamen döküldüğü izlenmiştir. Bununla birlikte, deltametrin, triklorfon + dimetilester ve fipronil, uygulamanın 6. gününde %100 etkinlik göstermiştir. *In vitro* etkinlik denemelerinde, tedavi sonrası grupta fipronil'in (0.25 /100 mL) oranında kullanımına bağlı olarak 18. saatte %100 kene ölüm oranı saptanırken, deltametrin, triklorfon + dimetilester ve sipermetrin'in %100 etkinlikleri sırasıyla 24-33. saat, 33-42. saat ve 39-48. saatler içerisinde saptanmış ve buna göre etkinlik açısından 2., 3. ve 5. sıralarda yer almışlardır. Bu çalışma, test edilen akarisitlerin kene enfestasyonunu azaltmada etkinliklerinde farklılıklar olduğunu ve temel akarisit sınıflarının diğer üyelerinin farklı formülasyonları üzerinde daha fazla deneylerin standartlaştırılması gerektiğini göstermiştir.

Anahtar sözcükler: *Akarisit, Etkinlik denemeleri, Keçi, Kene*

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Introduction

Ticks are one of the leading vectors of diseases of economic importance to the livestock industry in tropical and sub-tropical countries of the world. In tropical country like Pakistan, the warm-humid climate favors perpetuation and propagation of ticks. Tick fauna of Pakistan is rich in number of genera and species [1]. In Pakistan, the overall rate of tick infestation has been detected about 50%. Economic losses creating food insecurity [2], reduced growth and milk production and causes estimated global cost of control and productivity losses of 7 billion USD per year [3]. The adverse effects include paralysis/toxicosis and tick-transmitted haemoparasites that reduce production or cause mortality [4].

In Pakistan tick fauna comprises of at least 40 species belonging to mainly three genera i.e. *Hyalomma*, *Haemaphysalis* and *Rhipicephalus* [5]. The prevalence of tick infestation in small ruminants was estimated as 27.85%. Tick infestation was apparently found higher in goats (30.67%) than sheep (23%). A significant variation in the prevalence (22.2%-70.5%) of bovine ticks i.e. *Hy. anatolicum*, *Hy. hussaini*, *Hy. scupense*, *Rh. annulatus* and *Rh. microplus* was recorded across five agroecological zones of Pakistan [6].

Ticks are the major constraints to small ruminant production, and worldwide its control is based mainly on the repeated use of acaricides [7,8]. Number of methods exists to suppress tick's population i.e. dusting, hand spraying, mechanical spray race, hand dressing, systemic and dipping [9]; However, chemotherapeutic control remains the most extensively applied method in the developing world. Acaricides such as synthetic pyrethroids and organophosphates, macro-cyclic lactones, organochlorines, carbamates, and insect growth regulators have been found with significant efficacy for tick control [10-12]. However, populations of several tick species mainly in tropical and subtropical countries have developed resistance to all major classes of these compounds due to the high intensity of their use in tick management [13,14].

In Pakistan the main tick control methods in small and large ruminants are periodic application of acaricides i.e. macrocyclic lactones, trichlorfon and cypermethrin [1,15,16]. However, studies on assessment of the in vivo efficacy of acaricidal drugs are limited and to date few in vivo efficacy testing studies on coumaphos, cypermethrin, diazinon and ivermectin were performed in both sheep and goats in Pakistan [17,18]. Therefore, it is necessary to undertake periodic monitoring of effectiveness of acaricides to provide updated information on the efficacy of commercial acaricides for effective control against tick infestations on animals. Here, current study presented *in vivo* and *in vitro* efficacy testing to establish the current level of acaricidal resistance for five products representing every major acaricidal class (cypermethrine and deltamethrin representing the synthetic pyrethroid; trichlorphon + dimethyl ester representing organophosphates; ivermectin representing the macrocyclic lactones and fipronil representing phenylpyrazole compound) in controlling natural infestations with ticks of goats.

Material and Methods

Site/Experimental Animal's Selection

The present research was carried out at Livestock Research Station (LRS), National Agricultural Research Centre (NARC), Islamabad (33.6844° N, 73.0479° E) involving female goats between 2-5 years aged naturally and heavily infested with ticks. Ticks were collected from infested goats with the help of forceps avoid damage to mouth parts. Identification of ticks was performed through observation of morphological characteristic under stereomicroscope following the taxonomic keys [19,20].

In vivo Acaricidal Efficacy Trials

The *in vivo* acaricidal efficacy trials were conducted per guideline of WAAVP [21]. Briefly, 60 adult female goats, age between 2-5 years, with semi-intensive management, no history of acaricidal treatment and tick infestation rate of 100-120 ticks per animal, are used. Five compounds were subjected to acaricidal treatment viz., cypermethrine, deltamethrin, trichlorphon + dimethylester, ivermectin and fipronil. These compounds were selected based on their extensive usage in livestock farms for tick control.

The animals were divided into six equal groups named A through F *(Table 1)*. Groups A to E were treated with acaricidal compounds as per manufactory instructions, while group F served as control. After treatment with either of the above mentioned acaricides, the animals were examined quantitatively through "finger counting" [22], the number of ticks shed after the first 24 h and the duration for which the treatment remained effective that calculated from the data. The data were expressed as post-treatment tick burden on days 0, 1, 2, 3, 4, 5, and 6.

In vitro Acaricidal Efficacy Trials

The fully engorged ticks (4-5 mm in size) were collected from naturally infected goats managed at livestock research stations. Two different dilutions of each acaricidal compounds were prepared *(Table 2)*, and 30 adult ticks were used in each *in vitro* test dilutions, while one group served as control treated with distilled water. The petri dishes were kept at 25±2°C and 80±5% relative humidity in an incubator for 24 h. The mortality of ticks in all groups was evaluated after different time intervals.

Statistical Analysis

Descriptive analyses were performed according to the

Table 1. *In vivo therapeutic trial against tick's infestation in goats*

Experimental Groups	Composition and Packing	Dilution of Medicines	Dose Rate/Mode of Application
A (10 goats)	Cypermethrine 25%	5 mL /liter	Spray on animals 20 mL/animal
B (10 goats)	Deltamethrin 2.5% W/V (100 mL)	4 mL/liter	Spray on animals 20 mL/animal
C (10 goats)	Trichlorphon 98% W/W, Dimethylester of (2,2,2- trichloro-1 hydroxy-ethyl phosphoric acid) (100gm)	2 g/liter	Spray on animals 20 mL/animal
D (10 goats)	Ivermectin-1gm Vit-A-2500,000U Vit-D-375000U Vit-E-2.5gm	As such	Sub-cut administration 1 mL/50 kg live-body weight
E (10 goats)	Fipronil 0.25g in each 100 mL	As such	Spray on animals 20 mL/animal
F (10 goats)	Control (Water)	-	-

Table 2. *In-vitro acaricide efficacy trial against ticks collected from goats*

Medicine	Petri Dish #	Concentration
Cypermethrine 25%	A1	5 mL/liter (0.125 g/mL)
	A2	4 mL/liter (1 g/m)
Deltamethrin 2.5% W/V (100 mL)	B1	4 mL/liter (0.1 mg/mL)
	B2	3 mL/liter (0.075 mg/mL)
Trichlorphon 98% W/W, Dimethylester of (2,2,2- trichloro-1 hydroxy-ethyl phosphoric acid) (100 gm)	C1	2 mg/mL
	C2	1.5 mg/mL
Fibronil 0.25g in each 100 mL	D 1	0.0025g /100 mL
	D2	0.002 g/100 mL
Control	E	Water

scale of infestation as recommended [23]. The raw data of the ticks count were transformed in a natural logarithm of 10 (count +1). The data were analyzed using the analysis of variance test (ANOVA) followed by least significant difference (LSD) test for means comparisons. The level of significance used was $P \leq 0.05$. The threshold of 90% reduction in the counts of ticks in treated goats compared to untreated ones was considered as of acceptable efficacy for tick control agents, as recommended [23].

The data of five acaricides efficacy in *in vivo* trail were initially analyzed by descriptive statistics (mean, standard error) using Statistix 8.1 program. The efficacy was determined as follows:

Efficacy (%) = C-T/C X 100

Where: C = present mean number of ticks per animal in the control group and T = mean number of ticks per animal in the treatment group.

The data of acaricides efficacy in *in vitro* trail were as follows:

Efficacy (%) = N_0- N/N_0 100*

Where N_0 is the number of ticks prior to acaricidal treatment and N is the number of ticks recorded post-treatment [21].

Results

In vivo Experiment

The experimental goats were infested with four tick genera i.e. *Hyalomma*, *Rhipicephalus*, *Ixodes* and *Haemaphysalis*. The *in vivo* post-treatment quantitative assessment of tick burden revealed that both cypermethrine and ivermectin treated groups resulted in significantly lower ($P<0.05$) tick counts relative to other medicines and controls on all post-treated days. The finger counts were significantly higher ($P=0.00$) in group A (cypermethrine -treated group) than in group D (ivermectin treated), as shown in *Table 3*. From day 0 (pre-treatment) to day 1 (post-treatment), the reduction in the mean number of ticks was not significant ($P>0.05$) in all treatments. The maximum reduction in mean number of ticks in the CYM and IVM treated group was recorded from day 3 to 4, followed by complete shedding of ticks on day 5. However, deltamethrin, trichlorphon-dimethylester and fipronil showed 100% efficacy on the sixth day. The ticks in control group almost remained the same with no significant ($P>0.05$) changes during the experimental period.

In vitro Experiment

The results of *in vitro* efficacy trail showed that fipronil recorded 100% tick's mortality within 18th h post-treatment

Table 3. *Mean (±SD) and percentage reduction (%) in tick burden against different acaricidal treatments in goats*

Acaricide	Pre-treatment	Post-treatment Days					
		1st	2nd	3rd	4th	5th	6th
Cypermethrine	117.7±4.2 (0.4)	117.3±4.1^{a} (33.6)	75.9±1^{c} (73.3)	18.9±3.4^{c} (81.91)	4.4±1.4^{c} (87.12)	0.9±0.3^{b} (100)	
Deltamethrin	118.9±6.12 (0.7)	118.1±6.16^{a} (21)	92.1±3.2^{b} (67.4)	29.8±4.8^{bc} (66.7)	8.2±0.9^{c} (72.1)	2.1±1.1^{b} (95.8)	0.1±0.0^{b} (100)
Trichlorphon Dimethylester	116.7±1.81 (0.3)	116.3±1.94^{a} (22.1)	91±9.2^{bc} (65.6)	29.4±2.3^{bc} (69.2)	8.5±0.83^{c} (78.6)	1.9±0.6^{b} (95.5)	0.2±0.0^{b} (100)
Ivermectin	113.9±2.67 (0.3)	113.5±2.3^{a} (22.3)	87.9±6.6^{b} (70.8)	25.5±4.2^{bc} (73.3)	6±1^{c} (96.2)	0.2±0.6^{b} (100)	
Fibronil	118±3.46 (0.8)	117±3.17^{a} (14.6)	99.9±4^{b} (63.6)	36±5.5^{b} (59.4)	15.6±8.5^{b} (90.8)	1.3±0.4^{b} (56.7)	0.5±0.4^{b} (100)
Control	118.1±0.63 (0.9)	117.2±1.13^{a} (0.1)	116.3±1.42^{a} (-0.6)	116.9±1.4^{a} (0.2)	117.6±0.8^{a} (0.2)	117.5±0.8^{a} (-1.4)	118.8±0.4^{a} (0.2)
P- Value		0.90	0.00	0.00	0.00	0.00	0.00

Parenthesis indicates the tick's mortality percentage; Mean with different letters are significantly different (P<0.05)

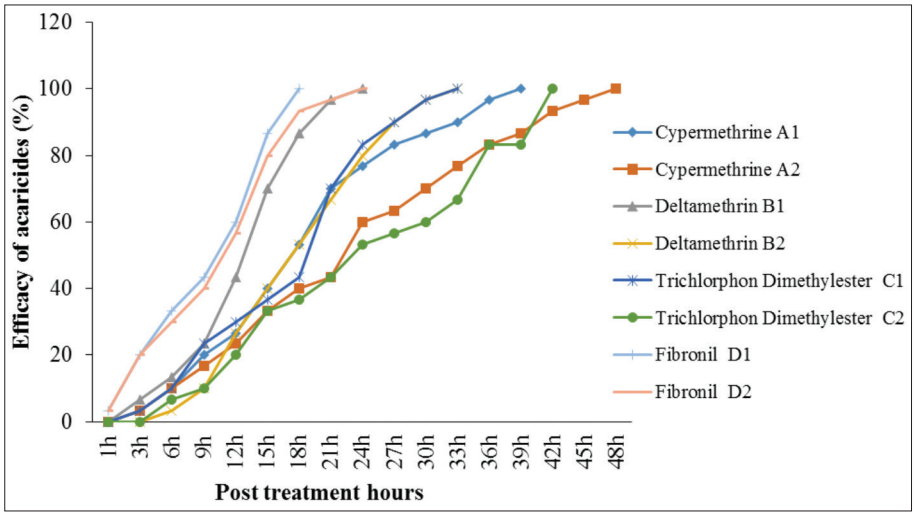

Fig 1. *In-vitro* percentage reduction of ticks on goats against two different concentrations of five commercial acaricides

with higher concentration (0.25 g/100 mL) and 24 h with lower concentration (0.2 g/100 mL). Deltamethrin, trichlorphon + dimethylester and cypermethrine were on 2nd, 3rd and 5th ranked based of their 100% efficacy within 24-33 h, 33-42 h and 39-48 h, respectively *(Fig. 1)*. However, all the four acaricides i.e., trichlorphon+ dimethylester, deltamethrin and cypermethrine showed 100% tick mortality within 48 h of post application. The cypermethrine and trichlorphon+ dimethylester treated group (A2 and C2) showed lowest efficacy, as 83.3% after 36th h.

Discussion

The application of acaricides may significantly reduce the abundance of the tick species [24] and help to mitigate the risk of tick-borne diseases [25]. However, application of acaricides may lead to development of tick resistance to several chemical compounds [26], which needs regular monitoring of acaricides. The present investigation was designed to measure the comparative efficacy of five different formulations of acaricides. The current *in vivo* trials showed 100% tick mortality with cypermethrin and ivermectin on the 5th day of post-treatment. Similar findings were recorded on larval stages of different species of ticks *Hyalomma*, *Haemaphysalis* and *Rhipicephalus* with cypermethrin [27]. *Ixodes ricinus* showed 100% mortality at the 9th day of ivermectin post-treatment [28], while another study reported even longer period of 21 days against *R. microplus* [29]. The resistance of ivermectin against *I. ricinus* was also reported [30,31]. Comparative to the present study, a higher efficacy of cypermethrine as 50% tick's mortality was recorded within 10 min and 100% in 30 min with the dose rate of 1.0 mg/mL or 10.0 mg/m [32]. In contrast to these results, lower mortality (92% and 96.7%) was recorded with cypermethrin application on unfed female of *R. sanguineus* and engorged females, respectively [33]. The differences among the mortality rates may dependent on the dose formulation, mode of application and the type of tick species.

In current research, trichlorphon showed complete reduction of ticks on the 6th day of post-treatment. Several studies recorded lower efficacy, resistance, and reinfection to tick populations after trichlorphon treatment [29,34,35]. *In vitro* trichlorphon concentrations 2 mg/mL and 1.5 mg/mL resulted 50% ticks' mortality within 9th and 24th h and 100% at 18th and 24th h, respectively [36].

The post treatment efficacy of deltamethrin was 100% at the 6th day in the present investigation, which is not consistent with the previous findings [35,37]. Lower efficacy of 13.2%, 12.3% and 16.2% was observed at 3, 7, and 14 days of post-treatment for immature ticks, respectively [38]. Deltamethrin produced about 52.8% reduction of semi-engorged females at 3 days post-treatment and lower percentages were observed at 7 and 14-days post-treatment. The present deltamethrin trials with two concentrations i.e., 0.1 mg/mL and 0.075 mg/mL caused 50% tick's mortality in 12th and 18th h and 100% in 24th and 33rd h, respectively. A previous study on *R. microplus* and *H. anatolicum* ticks showed both susceptibility and resistance to deltamethrin [39]. The deltamethrin (0.0025) tested for *R. sanguineus* engorged female showed low sensitivity [40]. However, resistance with deltamethrin concentration of 0.1 mg/mL was 86.7% (26/30) [34] and for commercial preparation of 1.25% against *R. microplus* was 63% [41]. The possible reasons for differences in results are inconsistent experimental conditions, route of administration, formulation, sampling and analytical methods.

The fipronil *in vivo* formulation presented acaricidal efficacy of 90.8% and 100% on day 4 and 6, respectively in the current study, which agrees with the study recorded maximum efficacy (99.39%) against *R. microplus* female after nine days post-treatment [41]. The mean efficacy of fipronil at a dose of 1 mg/kg in cattle on adults, nymphs and larvae of *R. microplus* female was 74.96%, 92.24% and 80.13%, respectively [41]. Longer period of 17 days of 100% effectiveness was also recorded for fipronil against *R. sanguineus* [42]. However, a study on tick's counts of dogs calculated efficacy of fipronil on weekly basis (2nd, 4th, 8th, 12th) and were 97.6%, 93.8%, 100% respectively [43]. These difference in the effectiveness of fipronil may be dose dependent, as higher dosage caused mortality of both adults and larva of *R. microplus* [42].

The study concluded that application of the tested compounds can reduce the abundance of successive generations of four tick genera namely: *Hyalomma, Rhipicephalus, Ixodes and Haemaphysalis*, which may contribute to reduction of population of tick species. Further-more, experimentation on acaricidal efficacy testing with different formulations of other members of major acaricidal classes needs to be standardized.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's contribution

K.A. and A.R. designed the study. A.M. performed the experiment. A.R., Z.F., M.H. advised on methods, experimentation and interpretation of findings. A.M., K.A., S.F. and A.R. conducted literature search, data analysis and manuscript preparation. K.A. and S.F. reviewed the manuscript. All authors participated in the study and concur with the submission and subsequent revisions submitted by the corresponding author.

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